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مجلة الشمال
للعلوم
الأساسية والتطبيقية

دورية علمية محكمة

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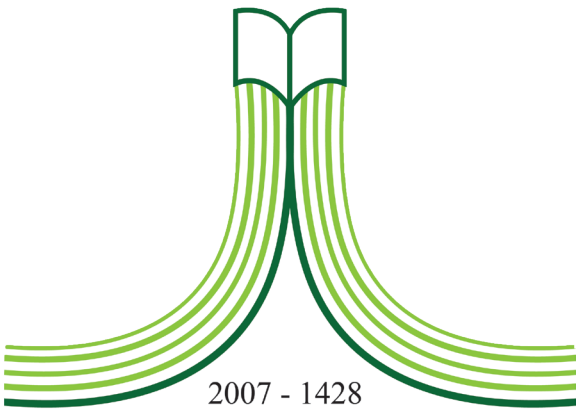

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مجلة الشمال
للعلوم الأساسية والتطبيقية
(JNBAS)

دورية علمية محكمة

تصدر عن

مركز النشر العلمي والتأليف والترجمة

جامعة الحدود الشمالية

المجلد الرابع ـ العدد الثَّاني

نوفمبر 2019م ـ ربيع الأوَّل 1441هـ

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مجلة الشمال للعلوم الأساسية والتطبيقية (JNBAS)

التعريف بالمجلة

تعنى المجلة بنشر البحوث والدراسات العلمية الأصيلة في مجال العلوم الأساسية والتطبيقية، باللغتين العربية والإنجليزية، كما تهتم بنشر جميع ما له علاقة بعرض الكتب ومراجعتها أو ترجمتها، وملخصات الرسائل العلمية، وتقارير المؤتمرات والندوات العلمية، وتصدر مرتين في السنة (مايو ـ نوفمبر).

الرؤية

الريادة في نشر البحوث العلمية المحكمة، وتصنيف المجلة ضمن أشهر الدوريات العلمية العالمية.

الرسالة

نشر البحوث العلمية المحكمة في مجال العلوم الأساسية والتطبيقية وفق معايير عالمية متميزة.

أهداف المجلة

1) أن تكون المجلة مرجعًا علميًا للباحثين في العلوم الأساسية والتطبيقية.
2) تلبية حاجة الباحثين إلى نشر بحوثهم العلمية، وإبراز مجهوداتهم البحثية على المستويات المحلية والإقليمية والعالمية.
3) المشاركة في بناء مجتمع المعرفة بنشر البحوث الرصينة التي تؤدي إلى تنمية المجتمع.
4) تغطية أعمال المؤتمرات العلمية المحكمة.

شروط قبول البحث

1) الأصالة والابتكار وسلامة المنهج والاتجاه.
2) الالتزام بالمناهج والأدوات والوسائل العلمية المتبعة في مجاله.
3) الدقة في التوثيق والمصادر والمراجع والتخريج.
4) سلامة اللغة.
5) أن يكون البحث غير منشور أو مقدم للنشر في أي مكان آخر.
6) أن يكون البحث المستل من الرسائل العلمية غير منشور أو مقدم للنشر، وأن يشير الباحث إلى أنه مستلٌ.

للمراسلة

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شروط النشر

أولاً: ضوابط النص المقدم للنشر

1) ألا تزيد صفحاته عن (35) صفحة من القطع العادي (A4).
2) أن يحتوي على عنوان البحث وملخصه باللغتين العربية والإنجليزية في صفحة واحدة، بحيث لا يزيد عن (250) كلمة للملخص، وأن يتضمن البحث كلمات مفتاحية دالةٍ على التخصص الدقيق للبحث باللغتين، بحيث لا يتجاوز عددها (6) كلمات، توضع بعد نهاية كل ملخص.
3) أن يذكر اسم المؤلف وجهة عمله بعد عنوان البحث مباشرة باللغتين العربية والإنجليزية.
4) أن تقدم البحوث العربية مطبوعة بخط (Simplified Arabic)، بحجم (14) للنصوص في المتن، وبالخط نفسه بحجم (12) للهوامش.
5) أن تقدم البحوث الإنجليزية مطبوعة بخط (Times New Roman) بحجم (12) للنصوص في المتن، وبالخط نفسه بحجم (9) للهوامش.
6) كتابة البحث على وجه واحد من الصفحة، مع ترك مسافة سطر واحد بين السطور، وتكون الحواشي 2.5 سم على الجوانب الأربعة للصفحة، بما يعادل 1.00 إنش (بوصة).
7) التزام الترتيب الموضوعي الآتي:

المقدمة: تكون دالة على موضوع البحث، والهدف منه، ومنسجمة مع ما يَرِدُ في البحث من معلومات وأفكار وحقائق علمية، كما تشير باختصار إلى مشكلة البحث، وأهمية الدراسات السابقة.

العرض: يتضمن التفاصيل الأساسية لمنهجية البحث، والأدوات والطرق التي تخدم الهدف، وترتب المعلومات حسب أولويتها.

النتائج والمناقشة: يجب أن تكون واضحة موجزة، مع بيان دلالاتها دون تكرار.

الخاتمة: تتضمن تلخيصًا موجزًا للموضوع، وما توصل إليه الباحث من نتائج، مع ذكر التوصيات والمقترحات.

8) أن تدرج الرسوم البيانية والأشكال التوضيحية في النص، وترقم ترقيماً متسلسلاً، وتكتب أسماؤها والملاحظات التوضيحية أسفلها.
9) أن تدرج الجداول في النص، وترقم ترقيماً متسلسلاً، وتكتب أسماؤها أعلاها، وأما الملاحظات التوضيحية فتكتب أسفل الجدول.
10) ألا توضع الهوامش أسفل الصفحة إلا عند الضرورة فقط، ويشار إليها برقم أو نجمة، ويكون الخط فيها بحجم (12) للعربي و (9) للإنجليزي.
11) لا تنشر المجلة أدوات البحث والقياس، وتقوم بحذفها عند طباعة المجلة.
12) أن يُراعى في منهج توثيق المصادر والمراجع داخل النص نظام (APA)، وهو نظام يعتمد ذكر الاسم والتاريخ (name/year) داخل المتن، ولا يقبل نظام ترقيم المراجع داخل النص مع وضع الحاشية أسفل الصفحة، وتوضع المصادر والمراجع داخل المتن بين قوسين حسب الأمثلة الآتية: يذكر اسم عائلة المؤلف متبوعا بفاصلة، فسنة النشر، مثلا: (مجاهد، 1988م). وفي حالة الاقتباس المباشر يضاف رقم الصفحة مباشرة بعد تاريخ النشر مثلا: (خيري، 1985م، ص:33). أما إذا كان للمصدر مؤلفان فيذكران مع اتباع الخطوات السابقة مثلا: (الفالح وعياش، 1424هـ). وفي حالة وجود أكثر من مؤلفين فتذكر أسماء عوائلهم أول مرة، مثلا: (مجاهد والعودات والشيخ، 1408هـ)، وإذا تكرر الاقتباس من المصدر نفسه فيشار إلى اسم عائلة المؤلف الأول فقط، ويكتب بعده وآخرون مثل: (مجاهد وآخرون، 1408هـ)، على أن تكتب معلومات النشر كاملة في قائمة المصادر والمراجع.
13) تخرج الأحاديث والآثار على النحو الآتي:
(صحيح البخاري، ج:1، ص: 5، رقم الحديث 511).
14) توضع قائمة المصادر والمراجع في نهاية البحث مرتبة ترتيباً هجائياً حسب اسم العائلة، ووفق نظام جمعية علم النفس الأمريكية (APA) الإصدار السادس، وبحجم (12) للعربي و(9) للإنجليزي، وترتب البيانات الببليوغرافية على النحو الآتي:

- **الاقتباس من كتاب لمؤلف واحد:**

الخوجلي، أحمد. (2004م). ***مبادئ فيزياء الجوامد***. الخرطوم، السودان: عزة للنشر والتوزيع.

- **الاقتباس من كتاب لأكثر من مؤلف:**

نيوباي، تيموثي؛ ستيبتش، دونالد؛ راس، جيمس. (1434هـ/2013م). ***التقنية التعليمية للتعليم والتعلم***. الرياض، المملكة العربية السعودية: دار جامعة الملك سعود للنشر.

- **الاقتباس من دورية:**

النافع، عبداللطيف حمود. (1427هـ). أثر قيادة السيارات خارج الطرق المعبدة في الغطاء النباتي بالمنتزهات البرية: دارسة في حماية البيئة، في وسط المملكة العربية السعودية. ***المجلة السعودية في علوم الحياة***، *14*(1)، 53-72.

- **الاقتباس من رسالة ماجستير أو دكتوراه:**

القاضي، إيمان عبدالله. (1429هـ). ***النباتات الطبيعية للبيئة الساحلية بين رأسي تنورة والملوح بالمنطقة الشرقية: دراسة في الجغرافيا النباتية وحماية البيئة***. رسالة دكتوراه غير منشورة، كلية الآداب للبنات، الدمام، المملكة العربية السعودية: جامعة الملك فيصل.

- **الاقتباس من الشبكة العنكبوتية (الإنترنت):**

- الاقتباس من كتاب:

المزروعي، م.ر. و المدني، م.ف. (2010م). ***تقييم الأداء في مؤسسات التعليم العالي***. المعرِّف الرقمي (DOI:10.xxxx/xxxx-xxxxxxxx-x)، أو برتوكول نقل النصوص التشعبي (http://www...)، أو الرقم المعياري الدولي للكتاب (ISBN : 000-0- 00 - 000000-0)

– الاقتباس من مقالة في دورية:

المدني، م.ف. (2014). مفهوم الحوار في تقريب وجهات النظر. ***المجلة البريطانية لتكنولوجيا التعليم***، *11*(6)، 225-260. المعرِّف الرقمي (DOI:10.xxxx/xxxx-xxxxxxxx-x) أو برتوكول نقل النصوص التشعبي (http://onlinelibrary.wiley.com/journal/10.1111)، أو الرقم المعياري التسلسلي الدولي للمجلة - (ISSN: 1467 - 8535).

15) يلتزم الباحث بترجمة (أو رومنة) أسماء المصادر والمراجع العربية إلى اللغة الإنجليزية في قائمة المصادر والمراجع. وعلى سبيل المثال:

الجبر، سليمان. (1991م). تقويم طرق تدريس الجغرافيا ومدى اختلافها باختلاف خبرات المدرسين وجنسياتهم وتخصصاتهم في المرحلة المتوسطة بالمملكة العربية السعودية. ***مجلة جامعة الملك سعود- العلوم التربوية***، *3*(1)، 143-170.

Al-Gabr, S. (1991). The Evaluation of Geography Instruction and the Variety of its Teaching Concerning the Experience, Nationality, and the Field of Study in Intermediate Schools in Saudi Arabia *(in Arabic). Journal of King Saud University- Educational Sciences, 3*(1), 143-170.

16) تستخدم الأرقام العربية الأصلية (0، 1، 2، 3، ...) في البحث.

17) تؤول جميع حقوق النشر للمجلة في حال إرسال البحث للتحكيم وقبوله للنشر.

ثانياً: الأشياء المطلوب تسليمها

1) نسخة إلكترونية من البحث بصيغتي (WORD) و (PDF)، وترسلان على البريد الإلكتروني الآتي:

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2) السيرة الذاتية للباحث، متضمنة اسمه باللغتين العربية والإنجليزية، وعنوان البريد الإلكتروني الحالي، ورتبته العلمية.

3) تعبئة النماذج الآتية:

أ - نموذج طلب نشر بحث في المجلة.

ب - نموذج تعهد بأن البحث غير منشور أو مقدم للنشر في مكان آخر.

ثالثاً: تنبيهات عامة

1) أصول البحث التي تصل إلى المجلة لا تردّ سواء أُنشِرَتْ أم لم تنشر.

2) الآراء الواردة في البحوث المنشورة تعبر عن وجهة نظر أصحابها.

مجلة الشمال للعلوم الأساسية والتطبيقية (JNBAS)

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المجلد الرابع - العدد الثَّاني نوفمبر 2019م - ربيع الأوَّل 1441هـ

المحتويات

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no significant difference in terms of quality of life between affected and healthy subjects, including desire for marital relation, social activities, desire to learn work and succeed. Because of the non-fatal and life-threat of the majority of dermatological diseases, it does not impair an individual's daily activities, only their psychological well-being. In our study, the majority of subjects reported no effect on those parameters because people in the Northern region of Saudi Arabia, have strong religious beliefs which give them psychological support as they consider it as destiny. Furthermore, we believe a different method, such as WTP (Willing To Pay) reported by Leeyaphal *et.al* (2011) that found that the mean monthly WTP was significantly associated with monthly personal income but was independent of sex, age, occupation, and MASI score. Previous studies reported that the WTP was significantly correlated with the DLQI questions (Leeyaphan, Wanitphakdeedecha, Manuskiatti, & Kulthanan, 2011).

5. CONCLUSION

In this study, we have reported new findings of a high incidence of Melasma among males in the northern region of Saudi Arabia. The disease has no significant impact on quality of life among affected populations.

6. RECOMMENDATION

It is recommend that, further studies be carried out to investigate the genetic predisposition of the disease across the northern region of Saudi Arabia.

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the difference was statistically insignificant (P=0.355).

The relationship between suffering from hyperpigmentation and effect on social communication and participation, self-confidence, marital desire or marital relations, learning, work and success, social relations, sense of fear of people with infections, causing economic problems, need for health education program of the studied population is summarized in table 4.

Half (50%) of the hyperpigmented participants reported no effect on social communication and participation while 25% of hyperpigmented reported occasional effects on their social communication and participation with a statistically insignificant difference (P=0 .706). 47.7% of hyperpigmented participants indicated effect on self-confidence compared with 38.6% that had no effect on self -confidence. 13.6% reported an occasional effect on their self- confidence with a statistically insignificant difference (P=0.033)

47.7% hyperpigmented reported no effect on marital desire or relationship while 34.1% stated an effect on marital desire or relationship compared with 18% that was occasionally affected with a statistically insignificant difference (P=0.221). More than half of hyperpigmented participants (75%) stated that their condition had no effect on their desire to learn, work and being successful compared with 15.9 % of whom reported this ability was reduced due to their condition and 9 % felt their ability was occasionally limited with no statistically significant difference (P=0.487).

4. DISCUSSION

Melasma is a common chronic skin disorder characterized by dark patchy skin discoloration mostly on the cheek, bridge of the nose, chin, above the upper lip and forehead. It is very common among women especially those who are pregnant or take patches or oral contraceptives. In our study, we found significant increase of Melasma and post-inflammatory among subjects between 30-40 years with the condition subsided among subjects above 50 years old, which match the findings (Jobanputra & Bachmann, 2000); Nicolaidou, Antoniou, & Katsambas, 2007). We also found that men were more affected than women which came as a surprise because all previous studies had indicated a high prevalence of the condition among women in different parts of the world, such as USA, Brazil, Nepal, Iran and India (Miot, Miot, Silva, & Marques, 2009; Resnik, 1967; Sacre, Fernandes, Vaisman, & Tendrich, 1996, Sarkar, Jain, & Puri, 2003). However, in this study male vs women were 58.7% vs 41.3% which could be due to the hot dry desert climate of the the Northern Borders Region of Saudi Arabia and because males are mostly exposed to sunlight as they always go camping in the desert and hunt for weeks. The majority of males also work as sheep grazers, the thing which makes them mostly work in open spaces. In addition, 50-60 years ago, the northern region was a desert, ancestors used to live as Badu in tents inside the desert and exposed to sun light at very high temperatures, sometimes reaching 52°C; hence, if hyperpigmentation was present at that time, this could very much support a genetic link for the disease. Our study has also shown that individuals with a family history of the disease are more likely to develop it (61.4%) compared to 31.8% healthy individuals with P-value (0.000), although the disease does not follow the Mendelian segregation patterns (Miot *et al*., 2009). The reported cases among a third of people with a family history in this study suggest a strong genetic predisposition of the condition. Although the genetics of the skin is yet to be understood, multi-factorial inheritance should be considered especially in Saudi Arabia (in particular, the Northern region) where consanguinity and marriage within the same tribe could contribute. We also found significant hyper-pigmentation among university-level subjects compared to individuals less qualified, such as at the secondary, primary school levels and the non-educated. This significant increase may be due to the fact that people with higher education pay more attention to their images. Among females, the use of certain types of chemicals (or radiation) to gain skin tanning may play a role compared to individuals with a lower education level. Finally, we reported

Table 4: Studied determinants of quality of life in the studied population (with and without melasma) in Turaif City, KSA.

Effect on social communication and participation	**Suffering from Melasma**		**Total (n=235)**	**P-value**
	Yes (n=44)	No (n=191)		
Yes	11(25.0%)	37(19.4%)	48(20.4%)	0.706
No	22(50.0%)	103(53.9%)	125(53.2%)	
Sometimes	11(25.0%)	51(26.7%)	62(26.4%)	
Effect on self-confidence				
Yes	21(47.7%)	73(38.2%)	94(40.0%)	0.033
No	17(38.6%)	54(28.3%)	71(30.2%)	
Sometimes	6(13.6%)	64(33.5%)	70(29.8%)	
Effect on marital desire or marital relation				
Yes	15(34.1%)	44(23.0%)	59(25.1%)	0.221
No	21(47.7%)	94(49.2%)	115(48.9%)	
Sometimes	8(18.2%)	53(27.7%)	61(26.0%)	
Effect on learning, work and success				
Yes	7(15.9%)	24(12.6%)	31(13.2%)	0.487
No	33(75.0%)	137(71.7%)	170(72.3%)	
Sometimes	4(9.1%)	30(15.7%)	34(14.5%)	
Effect on social relations				
Yes	9(20.5%)	33(17.3%)	42(17.9%)	0.847
No	22(50.0%)	95(49.7%)	117(49.8%)	
Sometimes	13(29.5%)	63(33.0%)	76(32.3%)	
Sense of fear of people with infections				
Yes	5(11.4%)	24(12.6%)	29(12.3%)	0.632
No	34(77.3%)	135(70.7%)	169(71.9%)	
Sometimes	5(11.4%)	32(16.8%)	37(15.7%)	
Causing economic problems				
Yes	17(38.6%)	83(43.5%)	100(42.6%)	0.812
No	10(22.7%)	37(19.4%)	47(20.0%)	
Sometimes	17(38.6%)	71(37.2%)	88(37.4%)	
Need for health education programs				
Yes	36(81.8%)	152(79.6%)	188(80.0%)	0.319
No	1(2.3%)	16(8.4%)	17(7.2%)	
Sometimes	7(15.9%)	23(12.0%)	30(12.8%)	

Table 3: The relationship between sufferings from melasma and skin color, family history, exposure to sunlight and use of cosmetics of the studied population.

Skin color	**Suffering from Melasma**		**Total (n=235)**	**P value**
	Suffering (n=44)	Non suffering (n=191)		
White	12(27.3%)	61(31.9%)	73(31.1%)	0.656
Dark	10(22.7%)	49(25.7%)	59(25.1%)	
Corny	22(50.0%)	81(42.4%)	103(43.8%)	
Family history				
Yes	27(61.4%)	45(23.6%)	72(30.6%)	0.000
No	14(31.8%)	133(69.6%)	147(62.6%)	
Don't know	3(6.8%)	13(6.8%)	16(6.8%)	
Exposure to sunlight				
Yes	21(47.7%)	61(31.9%)	82(34.9%)	
No	9(20.5%)	46(24.1%)	55(23.4%)	0.134
Sometimes	14(31.8%)	84(44.0%)	98(41.7%)	
Using of cosmetics				
Yes	10(22.7%)	32(16.8%)	42(17.9%)	
No	19(43.2%)	105(55.0%)	124(52.8%)	0.355
Sometimes	15(34.1%)	54(28.3%)	69(29.4%)	

secondary educated were not affected by melasma, with a statistically significant difference (P= 0.01). Half of melasma sufferers (50%) had corny skin; dark-colored skin was less likely to suffer (22.7%) compared with white-colored (27.3%), with a statistically insignificant difference (P=0.65). As regards family history of melasma, people suffering from melasma were more likely to have a positive family history (61.4%) compared with non-sufferers (31.8%), and the difference was statistically significant (P=0.000). People suffering from melasma were more likely to be exposed to sunlight (47.7%) compared to (31.9%), of non-sufferers and the difference was statistically insignificant (P=0.134). 22.7% of participants having melasma were using cosmetics compared to 16.8% in non- suffering participants and

Table 2: Illustrates the relationship between hyperpigmentation and age groups, gender and education level of the studied population.

Age groups	**Suffering from Melasma**		**Total (n=235)**	**P-value**
	Yes (n=44)	No (n=191)		
< 20 years	7(15.9%)	69(36.1%)	76(32.3%)	0.001
20 -	9(20.5%)	61(31.9%)	70(29.8%)	
30 -	15(34.1%)	41(21.5%)	56(23.8%)	
40 -	8(18.2%)	15(7.9%)	23(9.8%)	
50 years or more	5(11.4%)	5(2.6%)	10(4.3%)	
Mean±SD, Minimum, Maximum and Range	28.8±11.3, 14.0, 68.0 and 54.0			
Sex				
Male	25(56.8%)	113(59.2%)	138(58.7%)	0.452
Female	19(43.2%)	78(40.8%)	97(41.3%)	
Educational level				
University or more	24(54.5%)	116(60.7%)	140(59.6%)	0.001
Secondary	0(.0%)	8(4.2%)	8(3.4%)	
Primary or preparatory	13(29.5%)	63(33.0%)	76(32.3%)	
Illiterate	7(15.9%)	4(2.1%)	11(4.7%)	

3. RESULTS

Age group 30 – 40 years was more likely to be affected with hyperpigmentation (34.1%) compared with ages 20-30 (20.5%) and 40-50 years (18.2%) with the least prevalence in the age group 50 years and above, and the difference was statistically significant (P=0.001). Gender wise, males were more likely to suffer from hyperpigmentation (56.8%) compared with females (43.2%), with a statistically insignificant difference (P=0.450). University graduates were more likely to be affected by melasma (54.5%) compared with preparatory (29.5%) and uneducated (15.9%) population, while

(4) the effect on learning; (5) work and success; (6)the effect on social relations; (7) sense of fearing of people from infection and financial difficulties.

2.2. Ethical Considerations

The revision and approval of this study have been made by the Research Ethics Committee of the Faculty of Medicine, the Northern Border University. Candidates were informed that their enrolling in the study was completely optional; written consent was obtained before completing the questionnaire. No personal information was included in the questionnaires for confidentiality purposes.

Statistical analysis

Collected data were coded and analyzed using the statistical package for the social sciences (SPSS, version 15). The w2-test was used as a test of significance and differences were considered significant at P value 0.05 or less.

Table 1: Parameters covered during the study with the mean age of participants.

Parameter	No.	Percentage %
Sex		
Male	138	58.7
Female	97	41.3
Age group		
< 20 years	76	32.3
20 -	70	29.8
30 -	56	23.8
40 -	23	9.8
50 years or more	10	4.3
Mean ± SD, Minimum, Maximum and Range	28.8±11.3, 14.0, 68.0 and 54.0	
Educational level		
University or more	140	59.6
Secondary	8	3.4
Primary or preparatory	76	32.3
Illiterate	11	4.7
Prevalence of melasma		
Yes	44	18.7
No	191	81.3

1. INTRODUCTION

Melasma is a common skin complaint in dermatology clinics characterized by irregular light to dark brown hyperpigmented patches of the face (Moin, Jabery, & Fallah, 2006; Pandya & Guevara, 2000). Factors that may play a role in melasma include ultraviolet light exposure, genetics, pregnancy, contraceptive hormones, hormone replacement therapy, autoimmune thyroid disease, cosmetical preparations, and drug phototoxicity, with exposure to ultraviolet light and genetics being the main players (Goh & Dlova, 1999; Rendon, 2003). However, melasma commonly affects females more than males and is more common among Latinos, Blacks, and Asians than Whites (Goh & Dlova, 1999; Grimes, 1995; Hexsel, Arellano, & Rendon, 2006; Pandya & Guevara, 2000; Rendon, 2003; Sanchez, Pathak, Sato, Fitzpatrick, Sanchez, & Mihm, 1981; Vazquez, Maldonado, Benmamajn, & Sanchez, 1988). The overall prevalence in Latino females varies from 1.5% to 33.3%. A recent study reported a prevalence of 8.8%. The estimated prevalence among pregnant Latina women is between 50% and 80%, and in 33% of them their melasma does not fade out after delivery (Arena, 2005; Bolognia, Jorizzo, & Rapini, 2003; Rendon, 2003; Sanchez, 2003). Melasma is mainly a cosmetic condition; women with melasma report that their social activities, emotional competence, and leisure activities are affected. (Grimes, 1995; Hexsel et al., 2006; Sanchez, 2003; Werlinger, Guevara, Gonzalez, Rincan, & Caetano, Haley, 2007). Melasma has considerable emotional and psychosocial effects on patients. Although melasma is common among Hispanic females, its effect on health-related quality of life (HRQOL) has not been well described. The literature review revealed that melasma has a bad effect on HRQOL. It severely affects social relations, emotional competence, physical health and financial matters in Hispanic women (Pawaskar, Parikh, Markowski, McMichael, Feldman, & Balkrishnan, 2007).

Studies have shown that melasma is common in Latino males. The overall rate of 14.5% is higher than a recently published prevalence of melasma in females 8.8%; (Sanchez, 2003). It was observed that the quality of life is a moderate factor in the incidence of melisma among males. Latinos link melasma with affected health and poor nutrition (Rendon, 2003).

Numerous treatment options are available for melasma. Using cosmetic makeup is a simple and effective option. This measure improves the quality of life in females but is not practical in males (Balkrishnan, McMichael, Hu, Camacho, Kaur, & Bouloc, 2005). The other options may include sun protection, bleaching agents, chemical peeling and laser treatment. Sun protective fedora and sunscreen must be encouraged, but strict sun avoidance may be difficult for outdoors workers (Salas, Mayer, & Hoerster, 2005). Recently topical, oral, and intradermal Tranexamic Acid has been used for the treatment of melasma with encouraging response. (Kaur & Bhalla, 2019.; Grimes, Ijaz, Nashawati, & Kwak, 2019).

2. SUBJECTS AND METHODS

A cross-sectional study was carried out in Turaif City in the Northern Borders Province of Saudi Arabia during the period from January 2015 to January 2016.

2.1. Data Collection Methods

Data were collected by interviews with individuals who fullfiled the selection criteria using a pre-designed questionnaire covering socio-demographic characteristics, including age, gender, educational status, suffering from Melasma, skin color, family history, exposure to sunlight and use of cosmetics. Determinants of the effect of melasma on the quality of life are: (1) the effect on social communication and participation; (2) effect on self-confidence; (3)effect on marital desire or marital relation;

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وبائية الكَلَفْ وتأثيره على جودة الحياة في مدينة طريف في المملكة العربية السعودية

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(قدم للنشر في 1440/10/11 هـ؛ وقبل للنشر في 1441/01/05 هـ)

ملخص: الكلف هو اضطراب فرط تصبغ الجلد، ويعد أكثرشيوعا بين الإناث من الذكور. حيث تعتبر الإناث الحوامل أو من يتعاطين أدوية منع الحمل أكثر عرضة لظهور الكلف. هذا المرض له تأثير على جودة الحياة على الرغم من عدم وجود معايير محددة لقياس التأثير على جودة حياة الشخص المصاب. ويعد المرض أكثر شيوعا بين الذين لهم تاريخ عائلي للمرض. تم إجراء البحث على ما مجموعه 235 فردا (138 من الذكور و 97 من الإناث) وقد تم اختيارهم عشوائيا عبر خمسة من مراكز الرعاية الصحية الأولية في مدينة طريف بالحدود الشمالية من المملكة العربية السعودية، خلال الفترة من كانون الثاني/يناير 2015 إلى كانون الثاني/يناير 2016. وقد خلصت النتائج إلى أن ما نسبته 34.1% من المشاركين تراوحت أعمارهم مابين 30 إلى 40 سنة، وكانت نسبة الذكورالذين يعانون من الكلف مع التصبغ المفرط أعلى من الإناث حيث شكلوا 58.6% و 43.2% بالترتيب. وكان خريجوا الجامعات أعلى تأثرا بالمرض إلى حد كبير حيث بلغت نسبتهم 54.5%، وشكل المصابون الذين لديهم تاريخ عائلي للإصابة بالمرض 61.4%، بينما لم يكن هناك اختلاف واضح في جودة الحياة بين المصابين وغير المصابين بالمرض. وخلصت الدراسة إلى ارتفاع نسبة المصابين الذكور بالمنطقة مع انتشار واسع للمرض بين طبقات الجامعيين والذين لديهم تاريخ عائلي للإصابة بالمرض. وتهدف هذه الدراسة المرجعية إلى تحديد مدى انتشار الكلف وتأثيرة على جودة الحياة للشخص المصاب بين السكان في منطقة طريف بالمملكة العربية السعودية.

كلمات مفتاحية: الكلف، التصبغات.

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Epidemiology of Melasma and its Impact on the Quality of Life in Turaif City, Saudi Arabia

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Abstract: Melasma is a hyperpigmentation disorder of the skin that is more common among females than males. Pregnant females and those using birth control medications are at a higher risk of developing the disease. Melasma has an effect on quality of life despite that no specific tool to determine that it has been established. A total of 235 individuals (138 males and 97 females) attending five randomly selected primary healthcare centers was enrolled for a cross-sectional study carried out in Turaif City in the Northern Borders Province of KSA from January, 2015 to January, 2016. Subjects were selected using a systemic random sampling procedure. Each person was interviewed separately; confidentiality was assured. Subjects within the ages 30-40 years were 34.1%. Males with post-inflammatory hyperpigmentation were higher than females (58.6% for males & 43.2% for females). University graduates were significantly affected (54.5%). Among the subjects, 61.4% were positive for a family history; there was no significant impact on quality of life among the studied population. This work aims to investigate the prevalence of melasma and determine its impact on quality of life among the studied population.

Keywords: Melasma, Hyperpigmentation.

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Sharipova, Mironov, & Chestnova, 2011) and INH-steroid hybrids (Sikharulidze, Nadaraia, & Kakhabrishvili, 2012; Merlani, Kemertelidze, Papadopoulos, & Men'shova, 2004) have not produced effective anti-TB agents in spite of reports that certain glycosides and steroids possess anti-TB activity (Hu *et al*., 2017).

CONCLUSION

Tuberculosis has posed significant challenges to the scientific community. To overcome these challenges, many strategies have been adopted. One of them is the development of newer isoniazid hybrids/derivatives. A good understanding of TB-infectivity and isoniazid metabolism has been fruitful in developing newer anti-TB agents. This is evident from the literature and the progress of LL-3858 in clinical trials. It is expected that in the coming years, safer and effective drugs, including the isoniazid hybrids/derivatives will be approved by drug regulatory authorities globally.

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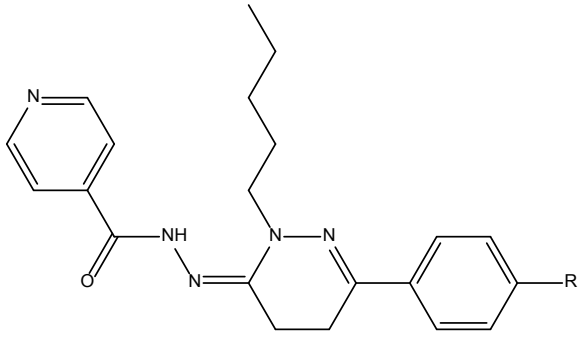
26) and **26b** (Figure 26), exhibited promising anti-TB activity against four different strains of Mycobacterium. However, the activity of these Compounds was less than that of INH.



25a: R=H
25b: R=4-F

Figure 26: Compound 25a and 25b

4. DISCUSSION

A disease such as TB is of great concern to the public and healthcare providers. The mycobacteria grow very slowly both extracellularly and intracellularly. Therefore, a long term treatment is required to eradicate TB effectively and prevent the development of DR-TB (Vasava, Nair, Rathwa, Patel, & Patel, 2018; Gegia, Winters, Benedetti, van Soolingen, & Menzies, 2017). The lipophilic cell-wall of the mycobacteria provide resistance to acid, alkali, alcohol and many disinfectants. It is also reported that this lipid cell wall is responsible for the pathogenicity, development of the drug resistance, immune-reactivity and antigen unresponsiveness of the mycobacteria (Unissa, Subbian, Hanna, & Selvakumar, 2016; Vilchèze & Jacobs, 2019). Further, many of the existing anti-TB agents have associated side effects ; for example, isoniazid in most of the fast acetylators causes hepatotoxicity (Metushi, Uetrecht, & Phillips, 2016; Hassan, Guo, Yousef, Luyong , & Zhenzhou, 2015; Wang, Pradhan, Zhong, & Ma, 2016). A good understanding of the mechanisms of resistance and toxicity and pharmacokinetic and pharmacodynamic profiles of a drug helps scientists to develop a potent, effective and safer medicine that has a rapid anti-TB activity. These types of agents can significantly and effectively eradicate the TB epidemic. Scientists have developed many isoniazid hybrids/derivatives having metabolic stability against the NAT enzyme by protecting the hydrazine unit of INH through incorporating a suitable lipophilic group in it (Hu et al., 2017). This serves dual functions. First, the chances of the generation of acylating species that lead to isoniazid hepatotoxicity are reduced. Second, the incorporated lipophilic group in the structure of INH helps in interfering the abnormal lipophilic cell wall of the Mycobacterium. These two strategies have provided the development of many promising anti-TB agents, including LL-3858 (Martins, Santos, Ventura, Elvas-Leitão, Santos, Vitorino, Reis, Miranda, Correia, Aires-de-Sousa, Kovalishyn, Latino, Ramos, & Viveiros, 2014; Hu et al., 2017) and hybrids of pyrrole, pyrazole, pyrazoline, triazole, triazine, azetidinone, quinoline, quinolone, thiazolidinone, isatin, cinnamic acid, furoxan, as well as Schiff bases of certain aldehydes and ketones. Some of the Compounds reported in this review are isoniazid hydrazone derivatives and cyclized isoniazid derivatives. Based on the existing literature (Metushi, Uetrecht, & Phillips, 2016; Hassan, Guo, Yousef, Luyong, & Zhenzhou, 2015; Wang, Pradhan, Zhong, & Ma, 2016), it can be assumed that isoniazid hydrazone derivatives after metabolism may provide INH as the primary active moiety and these types of derivatives may also be hepatotoxic in the fast acetylators as they can generate the acetyl radicals as mentioned above. On the other hand, cyclized isoniazid derivatives, such as LL-3858 after metabolism may not provide INH as the primary active moiety and these types of derivatives will be safer compared to the isoniazid hydrazones. Further, the development of INH-glycoside hybrids (Sharipova, Strobykina, Mordovskoi, Chestnova, Mironov, & Kataev, 2011; Kataev, Strobykina, Andreeva, Garifullin,

MTB-331/88 (Judge, Narasimhan, Ahuja, Sriram, Yogeeswari, De Clercq, Pannecouque, & Balzarini, 2013). Similarly, INH-N_2-acyl derivatives (Vavříková, Polanc, Kočevar, Košmrlj, Horváti, Bosze, Stolaříková, Imramovský, & Vinšová, 2011) and some hydrazinecarboxamides (Rychtarčíková, Krátký, Gazvoda, Komlóová, Polanc, Kočevar, Stolaříková, & Vinšová, 2014) displayed inferior anti-TB activity on INH.

A study on Schiff bases as anti-TB agents (Hearn, Cynamon, Chen, Coppins, Davis, Joo-On Kang, Noble, Tu-Sekine, Terrot, Trombino, Thai, Webster, & Wilson, 2009) displayed Compounds **22 & 23** (Figure 24); (MIC < 0.025 and 0.03 μg/Ml, respectively) that were better than INH (MIC = 0.06 μg/mL) when compared against the MTB strain Erdman as well as a high therapeutic index. These Compounds were effective against intracellular as well as extracellular organisms.

The present authors have also reported INH-pyridazinone hybrids (Imran, Bawadekji, & Ali, 2018), wherein the Compounds, **26a** (Figure

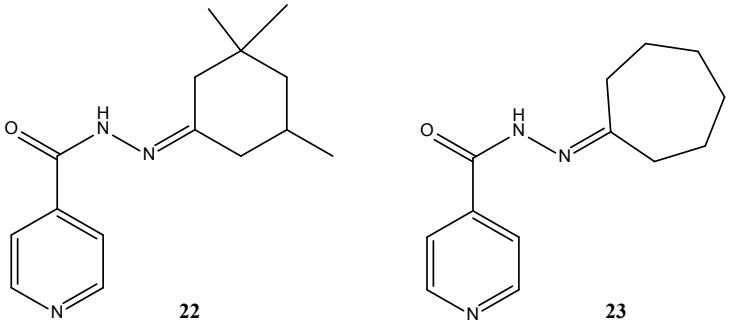


Figure 24: Compounds 22 and 23

A study towards the discovery of a lipophilic anti-TB agent (Pavan, da S Maia, Leite, Deflon, Batista, Sato, Franzblau & Leite, 2010) successfully developed a promising anti-TB Compound **24** (Figure 25) against MTB H37Rv, that has a similar toxicity (IC_{50} = 1250 μg/mL) like INH (IC_{50} = 1250 μg/mL).

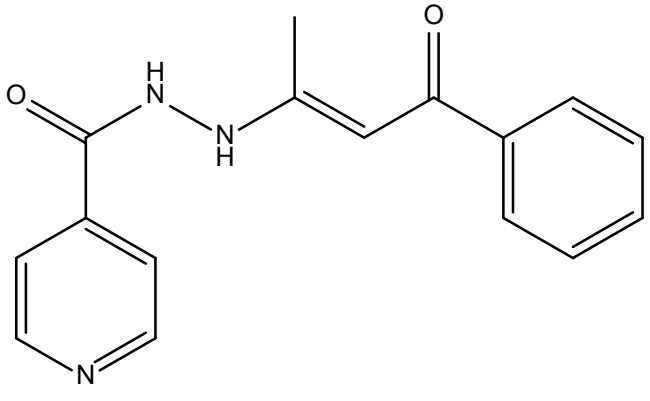


Figure 25: Compound 24

20 (Figure 22); (MIC = 0.56 μM) when evaluated against INH (MIC = 2.04 μM). It was non-toxic against Vero cell-lines (IC50 > 17.68 μM) and had a SI of > 30. It also reduced bacterial load almost equally with INH in mice at a 25 mg/kg dose. Similar benzylidene-INH derivatives comprising the e-withdrawing group showed inferior *in vitro* anti-TB activity in comparison to INH against

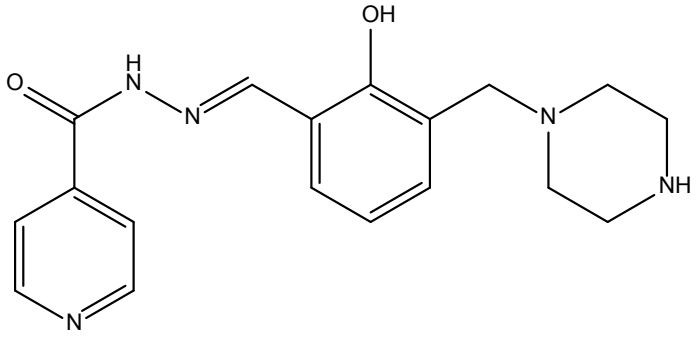
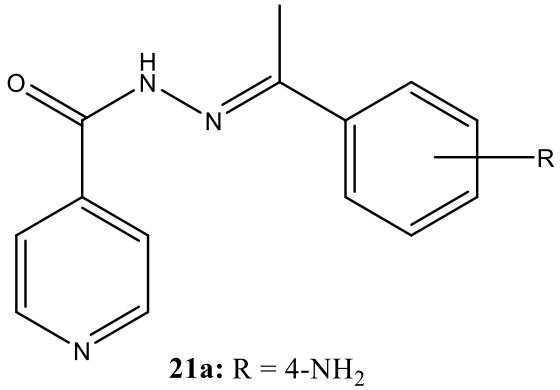


Figure 22: Compound 20

An investigation of benzylidene-INH derivatives as anti-MTB H37Rv (Malhotra, Sharma, Monga, Deep, Sahu & Samad, 2011) offered Compound **21a** (Figure 23); (MIC = 0.23 μM) which was non-toxic until 100 μg/mL to the host cell and because more potent and effective than INH (MIC = 1.45 μM). Another Compound, **21b** (Figure 23); (MIC = 2 nM) reported by Judge *et al*. (Judge, Narasimhan, Ahuja, Sriram, Yogeeswari, Clercq, Pannecouque & Balzarini, 2012) also almost had a similar profile as **21a**. The SAR revealed that an e-withdrawing group like -Br or -NO_2 on the benzene ring potentiates the anti-TB activity. Investigation of other types of benzylidene-INH derivatives resulted in mild to moderately active anti- MTB H37Rv Compounds (Lourenço, Ferreira, de Souza, Peralta, Vasconcelos & Henriques, 2007).

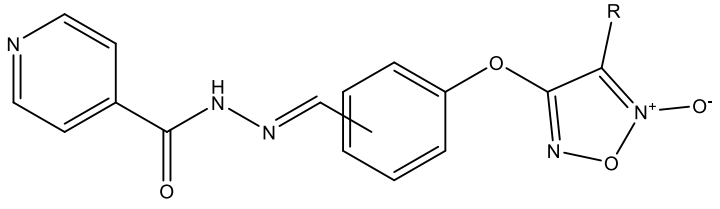


21a: R = 4-NH_2
21b: R = 4-Br

Figure 23: Compounds 21a and 21b

of bacteria (Voskuil, Schnappinger, Visconti, Harrell, Dolganov, Sherman, & Schoolnik, 2003; Chan, Chan, & Schluger, 2001). This led to the development of furoxan-INH hybrids as anti-MTB H37Rv agents (Fernandes, de Souza, Marino, Chegaev, Guglielmo, Lazzarato, Fruttero, Chung, Pavan, & Dos Santos, 2016), wherein the hybrids **18a**, **18b** and **18c** (Figure 20) comprising phenyl sulfonyl moiety displayed promising anti-TB potential against INH, rifampin and ethambutol resistant-TB isolates with a MIC value range of 7-50 μM. It was postulated that these Compounds displayed promising activity against MD-MTB because of their ability to generate nitric oxide. This strategy has provided a new direction for anti-TB drug research.

An *in vitro* study on INH-hydrazones (Sriram, Yogeeswari, & Madhu, 2005) revealed an almost four times potent anti-MTB H37Rv Compound



18a: INH fragment at -ortho & R = -SO_2Ph
18b: INH fragment at -meta & R = -SO_2Ph
18c: INH fragment at -para & R = -SO_2Ph

Figure 20: Compounds 18a, 18b and 18c

Several INH hydrazine-hydrazides have been screened as anti-MTB H37Rv (Naveen, Parumasivam, Jumaat, Ibrahim, Asmawi & Sadikun, 2014) that have provided a promising Compound **19** (Figure 21) (MIC = 0.28 μM) when compared with INH (MIC = 0.28 μM). It was observed that most of the promising Compounds comprised of an e-donating group like -OEt on the phenyl ring.

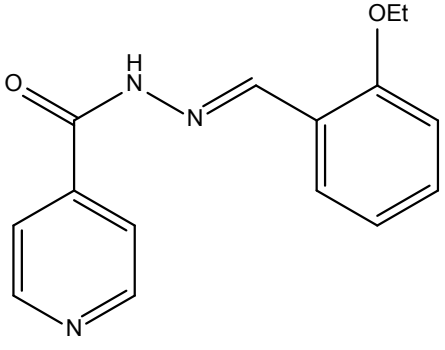


Figure 21: Compound 19

identified as one of the targets to develop an anti-TB agent (Patel, Keum, & Park, 2014; Lele, Raju, Ray, Rajan, & Degani, 2014). Many triazine-INH derivatives were evaluated *in vitro* as anti-MTB H37Rv agents (Sunduru, Gupta, Chaturvedi, Dwivedi, Sinha, & Chauhan, 2010). However, all of the developed Compounds were inferior to INH.

Compound **16** (MIC = 1.56 μg/mL); (Figure 18) was the most potent and non-toxic in mouse bone marrow-derived macrophages (MBMDMQs) and Vero cells.

Furoxan derivatives can generate nitric oxide, which is also generated by macrophages during an MTB infection as it can disrupt the DNA

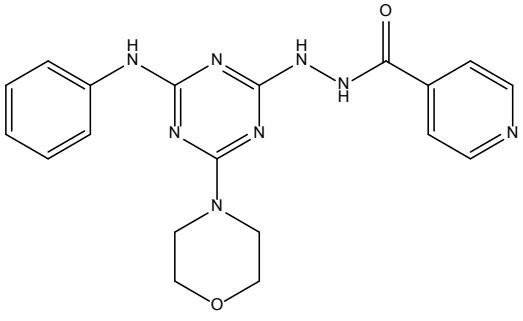
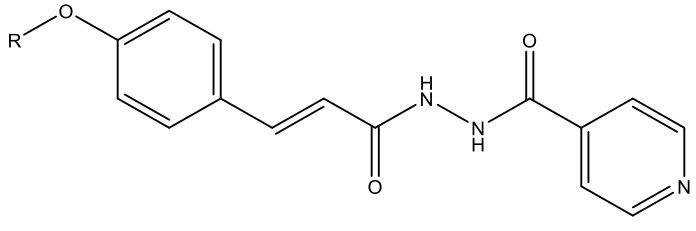


Figure 18: Compound 16

Similarly, another report (Shabadi, Shelar & Shelar, 1999) mentioned triazine-INH hybrids as moderately active anti-MTB H37Rv agents.

3.9. Other INH Hybrids

A combination of *trans*-cinnamic acid along with INH, RIF, and EMB produces a synergistic effect for DR-TB (Rastogi, Goh, Horgen & Barrow, 1998), and cinnamic acids also possess anti-TB activity (Yoya, Bedos-Belval, Constant, Duran, Daffé & Baltas, 2009). Accordingly, cinnamic acid-INH derivatives were developed (De**,** Koumba**,** Constant**,** Bedos-Belval**,** Duran**,** Saffon**,** Daffé & Baltas**,** 2011), wherein the derivative **17a** (Figure 19); (MIC = 0.3 μM) exhibited double potency as anti-MTB H37Rv than INH (MIC = 0.6 μM), and low toxicity (IC_{50} = 168 μM) for THP-1 cells. Another hybrid **17b** (Figure 19); (MIC = 2.3 μM) also inhibited mycolic acid synthesis like INH. These results have provided the possibility to develop mycolic acid inhibitors as anti-TB agents.



17a: R = -Me; **17b:** R = -i-pentenyl

Figure 19: Compounds 17a and 17b

16) exhibited a MIC value of 1.7 µM with respect to INH (MIC = 0.7 µM) and low *in vitro* toxicity in Vero cells (IC50 >1000 µM) as well as a high SI of >160. It was also observed that an e-attracting group in the phenyl ring potentiates anti-TB profile while an e-donating group failed to increase the anti-TB profile of the reported INH-derivatives. This report has suggested further optimisation of the Compound **14**.

3.8. Triazine-INH Hhybrids

Triazine ring targets dihydrofolate reductase,

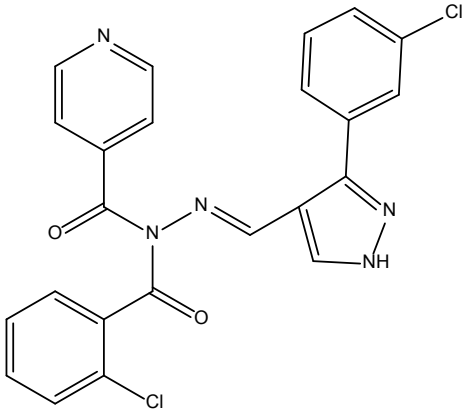
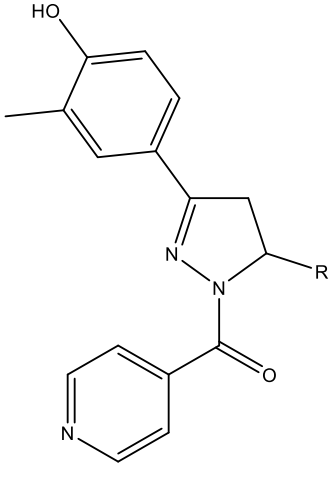


Figure 16: Compound 14

A new series of pyrazoline-INH hybrids with excellent activity against isoniazid-resistant MTB-strains and MTB-H37Rv with MIC values starting from 0.23 μM to 0.58 μM in comparison to INH (MIC=0.73 for MTB H37Rv; MIC=11.37 μM for INH-resistant MTB strains) has been developed (Shaharyar, Siddiqui, Ali, Sriram & Yogeeswari, 2006). Compounds **15a-b** (Figure 17) bearing the halogen groups exhibited better anti-TB activity than INH. Two Compounds, **15a** and **15c** (MIC ≤ 0.27 μM); (Figure 17) were about 2.5-times superior than INH for MTB H37Rv, wherein the Compound **15a** (MIC = 0.26 μM), and **15b** (MIC = 0.23 μM) displayed almost 50-times superiority than INH for INH-resistant MTB. The **15a-c** were also non-toxic (IC50) even at 62.5 μg/mL when evaluated in mammalian Vero cell-line. It was suspended that further modification of these derivatives might provide a promising lead Compound as an anti-TB agent.



15a: R = 2-ClPh; **15b:** R = 2,6-di-ClPh; **15c:** R = 4-FPh

Figure 17: Compounds 15a-c

derivatives investigated as anti-MTB H37Rv by *in vitro* method (Aragade, Palkar, Ronad, & Satyanarayana, 2013) provided Compound **12** (Figure 14; MIC= 0.625 µg/mL), that inhibited the growth of MTB H37Rv by 80%. It has been suggested that the anti-MTB H37Rv activity increases with the presence of an e-withdrawing group (-Cl, -F and -NO_2) in the substituted phenyl ring.

Another anti-MTB H37Rv series of INH-pyrazole derivatives has been reported (Nayak, Ramprasad, , & Dalimba, 2015) wherein Compound **14** (Figure

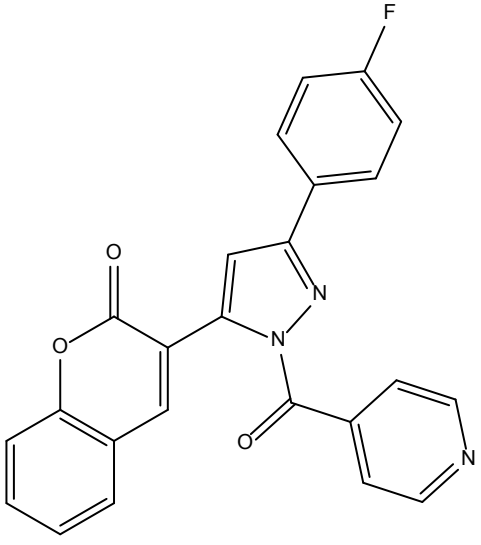
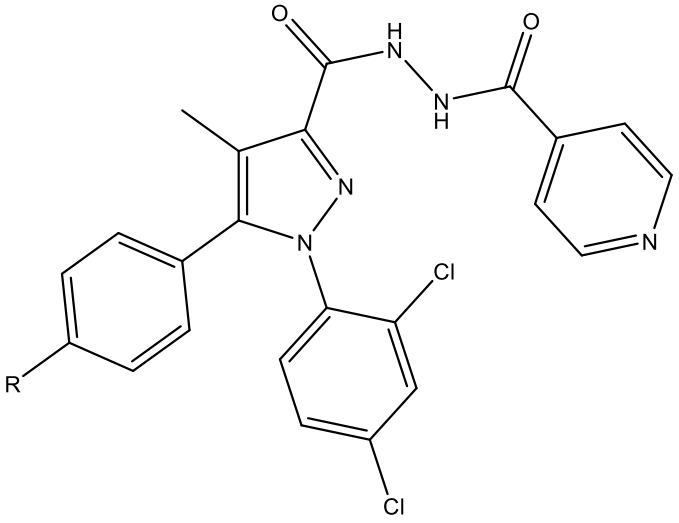


Figure 14: Compound 12

Gajbhiye *et al.* published anti-TB pyrazole-INH derivatives (Gajbhiye, More, Patil, Ummanni, Kotapalli, Yogeeswari, Sriram & Masand, 2015), wherein Compounds **13a** and **13b** (Figure 15) displayed superior anti-MTB H37Rv activity than pyrazinamide; however, it was inferior to INH.



13a: R = Cl; **13b: R** = H

Figure 15: Compounds 13a and 13b

3.6. Triazole-INH Hybrids

The mechanism of bacterial cell wall disruption is similar for triazoles and INH (Mir, Shafi, Zaman, Kalia, Rajput, Mulakayala, Mulakayala, Khan, & Alam, 2014). Therefore, some INH-triazole conjugates/hybrids have been synthesised (Kumar, Beena, Khare, Kidwai, Tyagi, Singh, & Rawat, 2014) as anti-TB agents. These Compounds displayed better anti-MTB H37Rv activity having MIC values of 0.195-1.56 µM and none of them was toxic for THP-1 cell-line until 50 µM strenght. Among this series, Compound **10** (Figure 12) with a MIC value of 0.195 exhibited higher potency than INH (MIC=0.39 µM). The *in vivo* studies of **10** in the murine model also revealed that it had the potential to decrease the bacillary load in the spleen and lungs after 10-weeks' treatment. This Compound was considered to be a promising lead Compound in this study.

3.7. Pyrazole and Pyrazoline-INH Hybrids

Pyrazole and coumarin derived INH

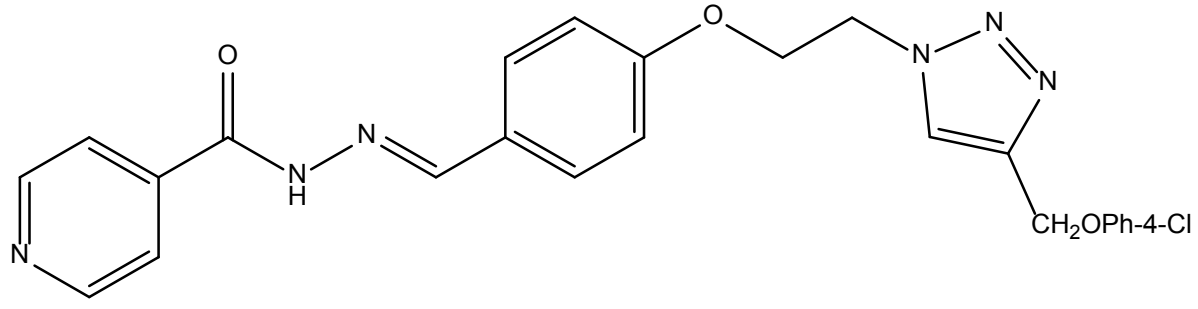
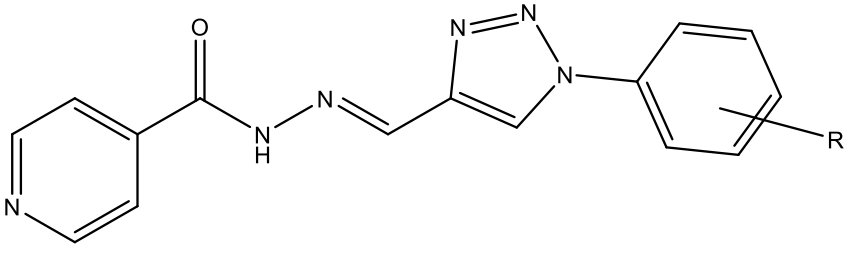


Figure 12: Compound 10

Another INH-triazole hybrid's *in vitro* anti-MTB H37Rv (ATCC 27294) has been published (Boechat, Ferreira, Ferreira, de Lourdes, Ferreira, de C da Silva, Bastos, Dos, Lourenço, Pinto, Krettli, Aguiar, Teixeira, da Silva, Martins, Bezerra, Camilo, da Silva & Costa, 2011). The Compounds of this study displayed appreciable activity. Four potent Compounds, namely, **11a-d** (Figure 13) (MIC values=0.62 μM) displayed minimum toxicity against the liver cells (MDL_{50} >1000 μM) and the kidney cells (MDL_{50} >1000 μM). These Compounds also revealed excellent selectivity index SI of >1612 for liver and kidney cell lines. It was also observed that an e-attracting group at C-4 of the triazole ring provides increased activity against MTB H37Rv. Further, the modification has been recommended of these four Compounds to identify more potent and non-toxic anti-TB agents.



11a: R = 4-Cl; **11b:** R = 3-Cl; **11c:** R = 4-NO_2; **11d:** R = 2-OMe

Figure 13: Compounds 11a-d

Based on the similarity of quinoline and quinoxaline rings, new quinoxaline-INH hybrids were also developed. However, these hybrids were less active than INH (Torres, Moreno, Ancizu, Barea, Galiano, Aldana, Monge, & Pérez-Silanes, 2011).

INH-quinolone hybrids as anti- H37Rv MTB template have recently been developed (Beteck, Seldon, Jordaanc, Warner, Hoppe, Laming, Legoabe, & Khanye, 2019) wherein Compound **8** (MIC = 0.8 μM) (Figure 10) displayed drug-likeliness property, better water solubility and metabolic stability in spite of its lower activity with respect to INH (MIC = 0.2 μM). This Compound showed no toxicity against HeLa cell lines at 20 μM concentration.

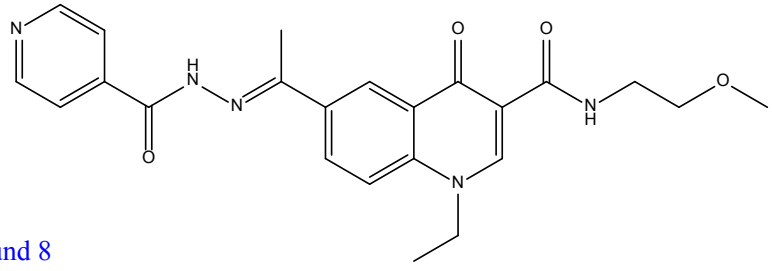


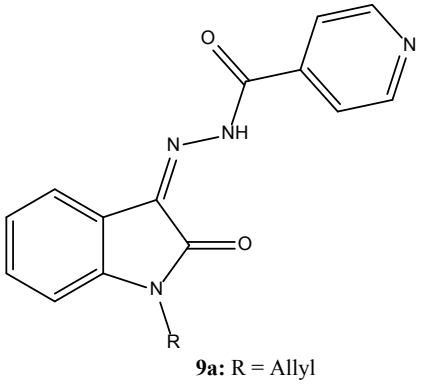
Figure 10: Compound 8

3.4. Thiazolidinone-INH Hybrids

Many chemotherapeutic agents possess thiazolidinone or thiazolidinedione ring systems (Jaju, Palkar, Maddi, Ronad, Mamledesai, Satyanarayana & Ghatole, 2009). However, the INH-hybrids of these systems provide moderately active anti-TB hybrids in comparison to INH (Pattan, Kedar, Pattan, Dengale, Sanap, Gharate, Shinde & Kadam, 2012).

3.5. Isatin-INH Hybrids

Isatin derivatives possess anti-TB activity by its lipophilic character (Feng, Liu, Wang, Chai, Hao, Meng & Guo, 2010; Feng, Liu, Zhang, Chai, Wang, Zhang, Lv, Guan, Guo & Xiao, 2011; Feng, Liu, Wang, Chai, Li & Guo, 2012; Xu, Zhang, Gao, Fan, Zhao, Lv & Feng, 2017). Accordingly, Aboul-Fadl *et al.* (Aboul-Fadl, Mohammed & Hassan, 2003; Aboul-Fadl, Abdel-Aziz, Abdel-Hamid, Elsaman, Thanassi & Pucci, 2011) have reported remarkable lipophilic isatin-INH derivatives, **9a** and **9b** (Figure 11) that were more potent (MIC = 2.7 and 3.5 μg/mL) than INH (non-active) towards human-resistant MTB strains. The bioavailability of these Compounds was also superior to INH. Other reports also mention lesser active isatin-INH derivatives as anti-TB agents concerning INH (Hussein, Aboul-Fadl, & Hussein, 2005; Sriram, Aubry, Yogeeswari, & Fisher, 2006).



9a: R = Allyl
9b: R = Bn.

Figure 11: Compounds 9a and 9b

Balabon, Huss, Cunningham, Lopez-Roman, Joossens, Augustyns, Ballell, Bates, & Van der Veken, 2016). However, the most promising Compound of this series, **5** (Figure 7) (MIC=0.62 μM), was only moderately active as compared to INH.

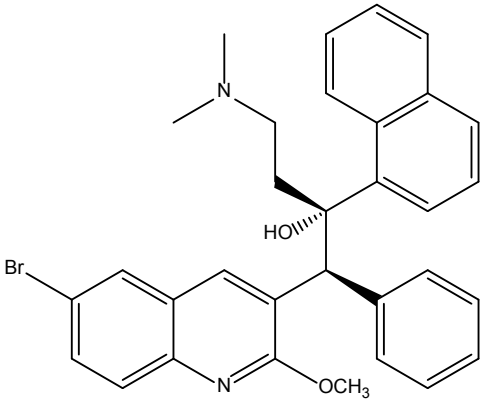


Figure 6: TMC 207

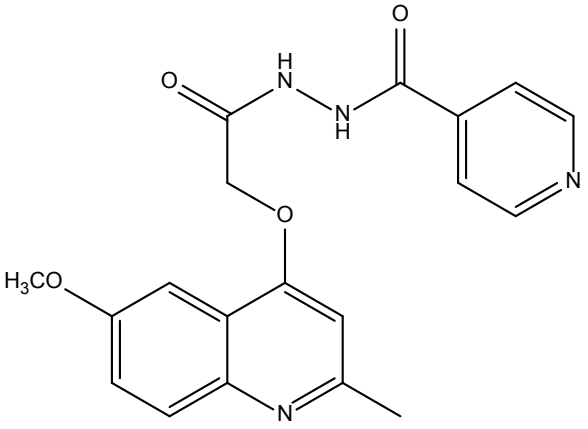


Figure 7: Compound 5

Quinoline ring is also present in fluoroquinolone, and these fluoroquinolones are used as anti-TB drugs (Xu, Zhang, Gao, Fan, Zhao, Lv & Feng, 2017). The INH incorporated fluoroquinolone derivatives have resulted in potent anti- MTB H37Rv Compounds, wherein Compound **6** (MIC = 1.26 μM) (Figure 8), and **7** (MIC = 1.30 μM) (Figure 9) had about double potency than INH (MIC = 2.04 μM) with low toxicity (IC50 > 100 μM). These results have increased the interest to prepare quinoline-INH derivative as anti-TB agents (Sriram, Yogeeswari & Madhu, 2005).

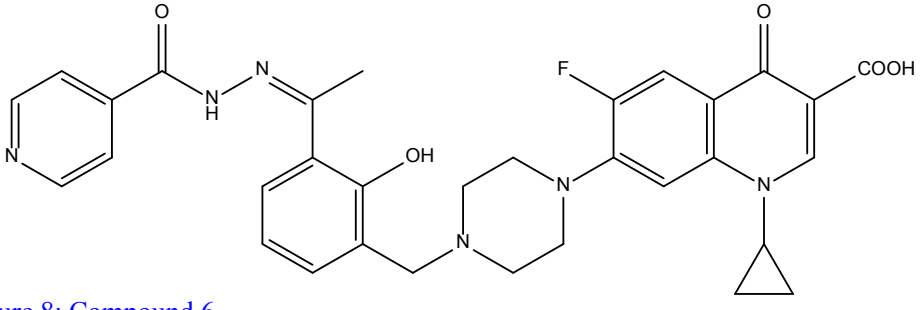


Figure 8: Compound 6

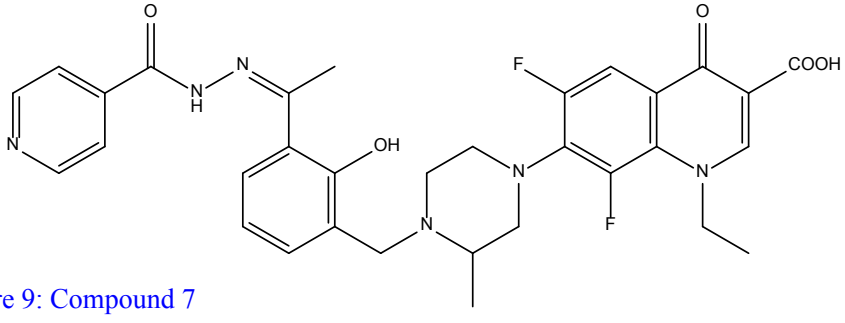


Figure 9: Compound 7

novel anti-tubercular therapies.

3. RECENTLY DEVELOPED ISONIAZID DERIVATIVES

3.1. Pyrrole-INH Hybrids

A significant number of pyrroles possess *in vitro* anti-TB activity (Hearn, Chen, Terrot, Webster, & Cynamon, 2010). Accordingly, pyrrole-INH hybrid **1** (Figure 3; MIC = 3.2 μg/mL for MTB Erdman strain) was developed having a bactericidal effect as per its study in mice infected with TB (Hearn, Chen, Terrot, Webster, & Cynamon, 2010) and was also non-toxic at therapeutic doses.

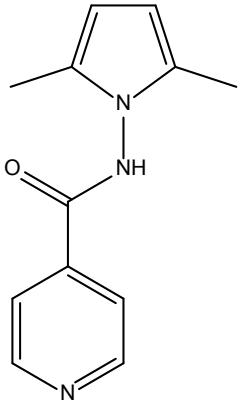


Figure 3: Pyrrole-INH hybrid 1

3.2. Azetidinone-INH Hybrids

Many antibacterial agents like carbapenem, penicillin and cephalosporin contain 2-azetidinone rings as an essential part of their structure. As per the literature, the azetidinone ring also possesses anti-TB activity (Joshi, More, Parkale, Aminabhavi, & Gadad, 2015). This observation led to the development of azetidinone-INH hybrids as anti-MTB H37Rv agents having MIC values of 3.125-12.5 μg/mL (Joshi, More, Parkale, Aminabhavi, & Gadad, 2015). The SAR revealed that Compound 2 (Figure 4) (MIC = 3.125 μg/mL) displayed enhanced anti-MTB H37Rv because of an e-donating group, -OCH_3.

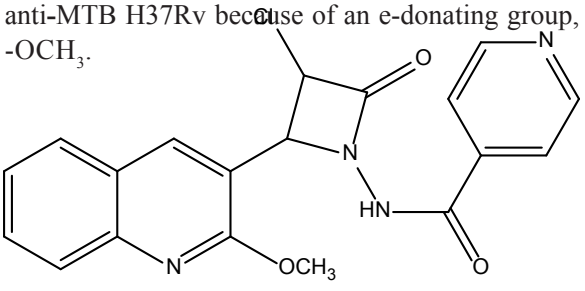
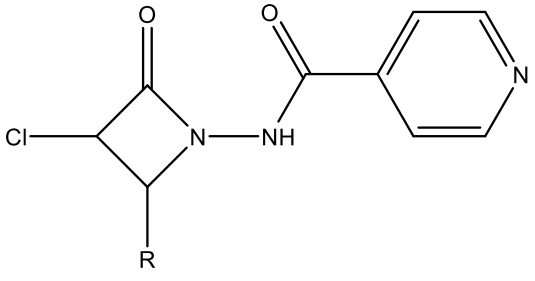


Figure 4: Compound 2

Another anti- MTB H37Rv activity of azetidinone-INH hybrids by the MABA method (Jaju, Palkar, Maddi, Ronad, Mamledesai, Satyanarayana, & Ghatole, 2009) provided Compounds **3** & **4** (MIC = 0.62 and 0.31 μg/mL, respectively) as moderately active hybrids (Figure 5). It was observed that azetidinone-INH hybrids with an unsubstituted phenyl ring resulted in non-active anti-TB agents.



3: R = 2-OHPh
4: R = 4-OH-3-OMe-Ph

Figure 5: Compounds 3 and 4

3.3. Quinoline and Quinoxaline-INH Hybrids

Quinoline is an identified pharmacophore in a recently discovered anti-TB Compound, **TMC 207** (Figure 6) (Hu, Zhang, Zhao, Gao, Feng, Lv, Xu , & Wu, 2017). Therefore, a series of quinoline-INH derivatives was prepared (Pitta, Rogacki,

challenges to the scientific community to produce novel anti-TB agents. Further, negligible newer anti-TB medicines have been discovered since 1960 (Vilchèze & Jacobs, 2019). This creates a necessity to establish newer anti-TB agents to fight with the challenges of MDR and TDR TBs. Accordingly, new hybrids of isoniazid and its derivatives have been prepared with the expectation of possessing potency against DR-TB, lesser toxicity/drug interactions with current drugs, reduced duration of therapy and having a new mechanism of action to avoid recurrence of MDR (Lu, You, & Chen, 2010; Ginsberg, 2010; Nuermberger, Spigelman, & Yew, 2010). These derivatives have been prepared mainly with two objectives .i.e. blocking the possibility of acetylation of hydrazine moiety that converts to hepatotoxic species in the fast acetylators and incorporates the lipophilic group in the isoniazid framework to disrupt the lipophilic components of the cell wall of the mycobacterium (Hu, Zhang, Zhao, Gao, Feng, Lv, Xu, & Wu, 2017). This strategy has been successful in identifying a promising INH-pyrrole hybrid called LL-3858 (Figure 2) which underwent a phase II clinical trial as an anti-TB agent (Martins, Santos, Ventura, Elvas-Leitão, Santos, Vitorino, Reis, Miranda, Correia, Aires-de-Sousa, Kovalishyn, Latino, Ramos, &Viveiros, 2014; Hu, Zhang, Zhao, Gao, Feng, Lv, Xu, &Wu, 2017). Similarly, many different isoniazid hybrids/ derivatives have been reported in the literature. This review provides a summary of recently reported ones used as anti-TB agents. The article will provide updates on the recent developments concerning the novel and more lipophilic isoniazid derivative to researchers working on it that may help them to explore the new possibility of developing better

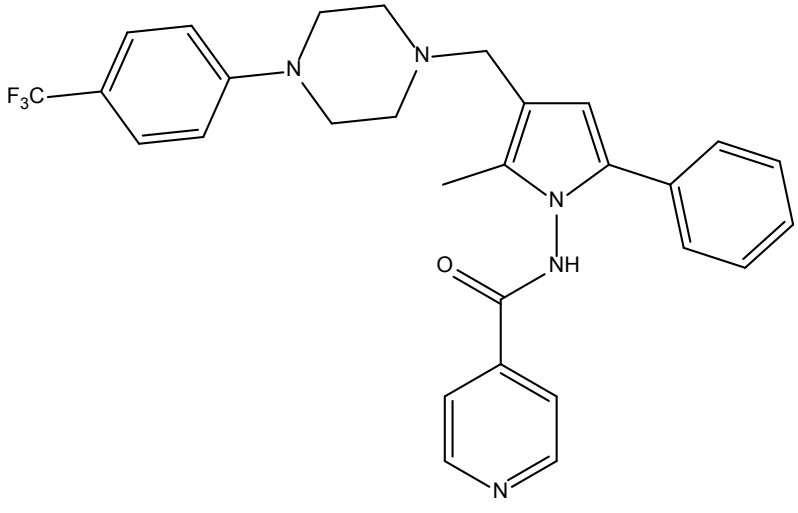


Figure 2: LL-3858

2. DATA COLLECTION

The references related to isoniazid were collected by using different keywords of isoniazid, such as *isoniazid, INH, isonicotinic hydrazide, 4-pyridinecarboxylic acid hydrazide, registry number* 54-85-3 in the general search of PubMed and Google Scholar. The references related to isoniazid derivatives were selected to write this article.

1. INTRODUCTION

Tuberculosis (TB) is a global pandemic (Miotto, Zhang, Cirillo, & Yam, 2018). This infectious disease mainly affects the lungs and can be transmitted through one person to another by sneezing, coughing, direct contact with patients and breathing in a bacteria polluted environment (Cox, Cox, Pai, Stillo, Citro, & Brigden, 2019). If untreated, the disease also affects different parts of the body such as bones, liver, heart, brain and kidney (Cox, Cox, Pai, Stillo, Citro, & Brigden, 2019). TB is an infectious disease of concern because its symptoms appear after many weeks or months, or in some cases after years of the infection. During this period another person may also become infected (Vasava, Nair, Rathwa, Patel, & Patel, 2018). TB is also a leading cause of deaths among AIDS patients (Dianatinasab, Joulaei, Ghorbani, Zarei, Rezaeian, Fararouei, & Greenwald, 2018). TB is the ninth root cause of death at the global level; about 10.4 million people suffered from the disease in the year 2016 and it ranks eleventh among the root causes of death in Saudi Arabia (Imran, Bawadekji, & Ali, 2018). TB is treated either with first-line treatment drugs like isoniazid or with second-line treatment drugs for about six to nine months (Gegia, Winters, Benedetti, van Soolingen, & Menzies, 2017). However, the recently emerged drug-resistant tuberculosis (DR-TB) cases have amplified the challenges to eradicating tuberculosis at the international level (Vilchèze & Jacobs, 2019).

Isoniazid (INH) was first reported in 1912, whereas its anti-TB property was disclosed in 1951. INH is also called isonicotinic acid hydrazide or isonicotinyl hydrazine. It has the molecular formula of $C_6H_7N_3O$ with a molecular weight of 137.14 and a melting point of 171°C to 173°C. The structure of isoniazid is provided in Fig. 1 (Fernandes, Salgado, & Santos, 2017).

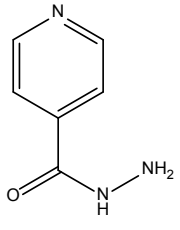


Figure 1: Isoniazid

Isoniazid (INH) is an extensively studied anti-TB medicine. According to the Orange Book data of the USFDA (https://www.accessdata.fda.gov/Scripts/cder/ob/search_product.cfm), accessed on March 5, 2019, INH is approved as tablets having strengths of 50 mg, 100 mg, 120 mg and 300 mg: as a capsule having strengths of 150 mg and 300 mg , as a syrup having a concentration of 50 mg/ml and as an injection of 100 mg/ml strength (Nagel, Streicher, Klopper, Warren, &Van Helden, 2017). The drug exerts its effect by inhibiting the production of mycolic acid, an essential component of the highly lipophilic cell-wall of the mycobacteria (Unissa, Subbian, Hanna, & Selvakumar, 2016). Recently, it has been discovered that isoniazid is a prodrug that after metabolic activation provides reactive species and acylates the enzyme system specific for the mycobacteria (Scior & Garcés-Eisele, 2006; Timmins & Deretic, 2006). These findings indicate that the isonicotinic acid part is essential to the anti-TB potential of isoniazid. The drug metabolism of isoniazid has been studied extensively (Metushi, Uetrecht, & Phillips, 2016). INH is metabolized by the N-Acetyltransferase (NAT) enzyme to yield N-acetylisoniazid. In some patients, this process is slow (slow acetylators) and in others, the process is fast (fast acetylators) depending on their genetic variations (Hassan, Guo, Yousef, Luyong, & Zhenzhou, 2015). The N-acetylisoniazid on hydrolysis gives acetylhydrazine, which after N-oxidation, provides reactive acetyl species. These species covalently bind with the tissues of the liver and lead to hepatotoxicity (Hassan, Guo, Yousef, Luyong, & Zhenzhou, 2015). Accordingly, the fast acetylators of the isoniazid are on a higher risk of hepatotoxicity as compared to its slow acetylators (Wang, Pradhan, Zhong, & Ma, 2016). This finding indicates that the hepatotoxicity of isoniazid is attributed to the acetylation of the hydrazine group of isoniazid.

During the isoniazid therapy, an emergence of the drug resistance was observed that was overcome partly by using the combination of first-line agents (Vilchèze & Jacobs, 2019). However, recently emerged MDR and TDR TBs have posed

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بحث مرجعي

المشتقات الحديثة للآيزونيازيد كعامل مضاد للسل: دراسة مرجعية

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(قدم للنشر في 1440/07/29 هـ؛ وقبل للنشر في 1441/01/05 هـ)

ملخص: يعتبر السل من الأمراض الوبائية على مستوى العالم. ومركب الآيزونيازيد هو من الأدوية المضادة لهذا المرض والمدروسة على نطاق واسع. ومع ذلك فإنه قد ظهر حديثاً السل المقاوم للأدوية وكذلك السل ذو المقاومة المطلقة تجاه الأدوية، وقد طرح ذلك تحدياتٍ كبيرةٍ قبل عملية التكاتف العلمي لإنتاج مركبات أكثر فعالية وذات نشاط قوي تجاه هذين النوعين من السل المقاوم وذي المقاومة المطلقة أو التامة تجاه الأدوية المتعددة، بحيث تكون هذه الأدوية ذات سمية وتداخل أقل مع غيرها من الأدوية، إضافة إلى تخفيض مدة العلاج، وامتلاكها بالوقت ذاته لآلية عمل جديدة لتجنب عودة ظاهرة المقاومة تجاه الأدوية. وتبعاً لذلك واستناداً إلى الطابع الشاذ للدهون الموجودة في الجدار الخلوي لبكتريا السل، فقد تم تحضير مشتقات جديدة من الآيزونيازيد المحبة للدهون، مثل هجين من البيرولات والبيرازول والبيرازولين والتريازين والآزيتيدينون والكينولين والكينولون والثيازوليدينون والايساتين وحمض السينامك والفوركسان وكذلك قواعد شيف *(Schiff)* لبعض الألدهيدات والكيتونات. وقد اسفرت هذه الإستراتيجية عن تطوير بعض الأنواع الهجينة من الآيزونيازيد الواعدة كعوامل مضادة للسل، وعلى سبيل المثال المركب *(LL3858)* الذي تطور إلى المرحلة المتقدمة من الطور الثاني من التجارب السريرية. وتقدم هذه الدراسة ملخصاً عن مركبات الآيزونيازيد الجديدة الهجينة أو المشتقة كعوامل مضادة للسل. ومن المتوقع أنه في المستقبل القريب، ستتم الموافقة على أدوية أكثر أمناً وفعالية، بما في ذلك مركبات الآيزونيازيد الهجينة أو المشتقة وذلك من قبل السلطات التنظيمية للعقاقير على الصعيد العالمي.

كلمات مفتاحية: الآيزونيازيد، الآيزونيازيد الهجينة/المشتقة، السل، المقاومة المتعددة للأدوية، المقاومة المطلقة للأدوية.

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Review Article

Recent Isoniazid Derivatives as Anti-Tubercular Agents: Review Article

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Abstract: Tuberculosis (TB) remains a global pandemic. Isoniazid (INH) is an extensively studied anti-TB medicine. However, the recently emerged MDR and TDR TBs have posed challenges to the scientific community to produce more effective anti-TB agents possessing potent effects on the two strains of TB, lesser toxicity and drug interactions with current drugs, reduce the duration of therapy, and have a new mechanism of action to avoid recurrence of MDR. Accordingly, based on the abnormal lipophilic character of the cell wall of M. tuberculosis, novel lipophilic isoniazid derivatives have been prepared as hybrids of pyrrole, pyrazole, pyrazoline, triazole, triazine, azetidinone, quinoline, quinolone, thiazolidinone, isatin, cinnamic acid, furoxan, and Schiff bases of certain aldehydes and ketone. This strategy has resulted in the development of some promising INH hybrids as anti-TB agents, for example, LL-3858 that progressed to the advanced stage of phase II clinical trial. This review provides a summary of the novel INH-hybrids/derivatives as anti-TB agents. It is expected that safer and effective drugs, including these INH-hybrids/derivatives, may be approved in the future by drug regulatory authorities globally.

Keywords: Isoniazid, INH-hybrids/derivatives, Tuberculosis, MDR-TB, TDR-TB.

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activity in apoptosis mediated by TF–β1 integrin. The cells were grown and counted to optimize TF transfection. Two samples of 105 HDBECs/well were transfected with 1 µg/ml of plasmid to express wild-TF. The plasmid was isolated from E. coli and plasmid purification was carried out. In addition, two samples of 105 HDBECs/well were transfected to express mutant-TFAla253, and t-GFP was used in two samples as a control. After TF expression, the samples were activated with PAR2-AP (20 µM) for 120 minutes. Two samples of 105 HDBECs/well were used as controls in the study. One HDBEC sample was not transfected which was used as a negative control. The other control sample was activated only with PAR2-AP as a positive control. Three samples of 105 HDBECs/well samples were treated with the β1 inhibitor for 20 minutes while three were not treated with the β1 integrin inhibitor. The purpose was to examine the effect of the β1 integrin on the Src pathway mediated by TF. In addition, the influence of the mutant-TF or wild TF on inducing apoptosis in endothelial cells was assessed. The SDS-PAGE technique was used to detect the Src level in the cell lysate after the β1 integrin inhibition. The results confirmed that the β1 integrin affects the Src pathway in TF signaling for inducing apoptosis. Furthermore, substitution of Ser253 with alanine in the TF sequence showed an effect of TF signaling compared with wild-TF. Samples treated with the β1 integrin inhibitor exhibited a low level of Src activity during TF signaling, mainly, the sample transfected to express mutant-TFAla253 had the lowest Src activity ratio. In contrast, the samples that were not treated with the β1 integrin inhibitor had a higher Src ratio. Thus, the results showed a significant difference between cells treated with the β1 integrin inhibitor and cells that were not so treated.

The results revealed the apoptosis pathway was induced by the TF–β1 integrin complex. This investigation explained the mechanism of the TF–β1 integrin apoptosis pathway. As TF signaling has mainly been known for cell proliferation and metastasis, previous studies on TF–β1 integrin inducing apoptosis are limited. The present findings may assist in the explanation of certain diseases and metastasis, as well as improving medication for tumours induced by TF and angiogenesis. Thus, observation of Src activity in TF–β1 integrin–induced apoptosis facilitates the understanding of the apoptosis mechanism.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

TF: Tissue factor, MAPK: Mitogen-activated protein kinase, Src: Non-receptor protein tyrosine kinase that plays roles in cell signaling, MPs: Microparticles, PARs: Protein-activated receptors, Fl-TF: Full length, as-TF: alternative spliced, BAX: Bcl-2-associated X protein, ECM: Extracellular matrix, VEGF: Vascular endothelial growth factor, FAK: Focal adhesion kinase, TGF-β: Transforming growth factor-β, MMPs: Metalloproteases, JNK: Jun NH2-terminal kinases, CDK: Cyclin-dependent kinase, SFKs: Src family kinases, SHs: Src Homology, the β1-integrin: Beta-1-integrin, P53: protein 53, P38: protein 38.

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the β1 integrin inhibition, but mutant-TFAla253 had a lower Src activity. Thus, the mutation of TF in Ser253 substitution with alanine affected the TF signaling pathway. This contrasted with wild-TF, which was not affected by the β1 integrin inhibition to the same extent as mutant-TFAla253. This suggested that the TF-mediated apoptosis pathway is altered when TF Ser253 was replaced with alanine. This was evidence of a relationship between TF mutation and apoptosis.

For instance, mutant-TF Ala253 caused an accumulation of TF in the cells then induced apoptosis through the β1 integrin inhibition. In contrast, mutant-TFAsp253 did not induce apoptosis as Ala253. Therefore, mutant-TFAla253 had a strong influence in TF signaling for inducing apoptosis.

The release of TF-bearing MPs also had a role in inducing apoptosis. These were factors that induced apoptosis in the endothelial cells mediated by TF–the β1 integrin. The activation of TF signaling in the cell triggered PAR2, thereby inducing apoptosis or cell proliferation. PAR2 activation was correlated with TF expression in inducing cell signaling for proliferation and apoptosis. Indeed, cell proliferation increased with PAR2 activation. Thus, TF regulated its proliferation function in the presence of PAR2 (Kocaturk & Versteeg, 2013). TF was not expressed normally in endothelial cells, like tumour cells. This was evidence that TF overexpression is associated PAR2 activation.

In addition, expression of TF in endothelial cell plasmid transfection was required to express TF in endothelial cells. Moreover, PAR2 cleavage triggered the TF signaling pathway for cell proliferation, migration and survival. Thus, PAR2-AP activated the signaling pathway in the cell (Guo, Zhou, Wu, Zhou, Wen, & Zhang, 2011). Treating HDBECs with PAR2-AP (20 μM) for 120 minutes triggered cell signaling. Src activity increased after activation with PAR2-AP. Therefore, Src activation was induced by TF activated PAR2. The level of Src activity was elevated during PAR2 activation. Whereas, un-transfected and non-activated PAR2 samples had a low Src activity compared to PAR2 activated control. Furthermore, Src activity ratio remained high, due to cell activation with PAR2, induced by TF signaling. However, Src activity was affected by the β1 integrin. In this study, the activity of Src phosphorylation activity was affected by the β1 integrin inhibitor, as was shown above. Therefore, the β1 integrin played an important role in TF signaling. As well as TF residue site, it affected the signaling pathway. This was shown in HDBECs sample which was transfected with mutant-TFAla253 as it had a low Src phosphorlyatiin activity ratio. Overall, mutant-TFAla25 altered signaling pathway in the cell.

5. CONCLUSION

TF has coagulant and non-coagulant functions in the cell. It interacts with the β1 integrin to maintain harmostasis. Overexpression of TF- β1 integrin is considered an abnormal condition leading to thrombosis in cancer and metastasis. It has been reported in recent studies that TF is an overexpression. Thus, malignancy is always associated with TF overexpression in cancer cells, as TF increases the risk of metastasis, as well as angiogenesis. In contrast, TF has been reported to induce apoptosis in endothelial cells through the TF–β1 integrin complex, as reported in this study. This mechanism involves an accumulation of TF in the endothelial cells and knockdown of the β1 integrin; which induces apoptosis as the cells lose their attachment. The apoptosis process activates P38 MAPK, P53 and caspases. The cell signaling involves the Src pathway; therefore, the β1 integrin inhibition affects Src activity. Moreover, the β1 integrin inhibition reduces Src activity in the cells. Thus, it can be confirmed that Src is linked to the β1 integrin in the TF pathway for the apoptosis mechanism. In addition, different types of TF play a role in inducing apoptosis in the cell. As recent studies have suggested, the mutation of TF in Ser253 to Ala253 substitution causes the accumulation of TF inside the cells. Therefore, it will activate the apoptosis signaling pathway.

As shown in this study, the signaling pathway is characterized by a low Src activity. Here, HDBECs were employed in the experiment to observe the Src

(van den Berg, van Den Hengel, Myers, Ayachi, Jordanova, Ruf, Spek, Reitsma, Bogdanov, & Versteeg, 2009). According to previous studies, prolonged exposure to TF in endothelial cells will cause apoptosis. Therefore, accumulation of TF in the cell has been reported to cause cell apoptosis through the cellular signal pathway (ElKeeb et al., 2015). The signaling pathway for apoptosis involves TF–β1 integrin complex formation. This will activate the P38 MAPK pathway, P53, as well as caspases-3 cleavage. Moreover, TF mediates signaling pathway for apoptosis in endothelial cells, and is characterized low phosphorylation Src (Figure 1). Indeed, inhibition of the β1 integrin is characterized by low Src activity. The results showed a significant low Src phosphorylation ratio in the absence of the β1 integrin. The results confirmed that the β1 integrin modulates Src phosphorylation in the cells. Thus, the β1 inhibition affects TF signaling for inducing apoptosis (Arderiu, Espinosa, Peña, Crespo, Aledo, Bogdanov, & Badimon, 2017). On the other hand, a high expression of Src is correlated with the β1 integrin in cancer progression. Therefore, targeting the β1 integrin will induce cell apoptosis. The β1 integrin can induce apoptosis in endothelial cells, as the cells lose their attachment; this is because the β1 integrin is considered the main factor controlling cell attachment.

The β1 integrin mediates cell adhesion, and facilities cell migration and survival. Thus, the β1 integrin expression in the cell is correlated with apoptosis resistance. Therefore, the β1 integrin inhibition stimulates the apoptosis process in the cell (Renner, Janouskova, Noulet, Koenig, Guerin, Bär, Nuesch, Rechenmacher, Neubauer , & Kessler, 2016). Indeed, the β1 integrin inhibition is involved in the activation of the proapoptotic pathway and P38 MAPK. Furthermore, the β1 integrin inhibition activates tumour suppression via P53, and activates caspases-3 cleavages which reduces cell proliferation. The apoptosis induced by the TF–β1 integrin complex is characterized by a low Src phosphorylation activity (Figure 1). Therefore, Src inhibition accelerates cell death and Src inhibitors are involved in blocking the FAK pathway (Takadera, Fujibayashi, Koriyama, & Kato, 2012).

As previous studies have shown, Src inhibitors can induce apoptosis in the cells (Wang, Zhan, Shao, Jiang, & Wang, 2015). Thus, down regulation of Src activity will alter the signaling pathway induced by TF (Mandal, Pendurthi, & Rao, 2007). This can be stimulated when TF is activated in the cell with the β1 integrin inhibition. Moreover, the β1 integrin inhibition will affect Src activity in the cell. Thus, the results confirmed that adding the β1 integrin antibody reduced Src activity as apoptosis is characterized by low Src activity while Src activity was increased during cell proliferation (Indovina, Casini, Forte, Garofano, Cesari, Iannuzzi, Del Porro, Pentimalli, Napoliello, & Boffo, 2017). Thus, Src upregulation is considered evidence for cell proliferation and resisting apoptosis.

Therefore, targeting Src can induce apoptosis in cancer. Previous studies have shown that metastasis is accompanied with high expression of Src phosphorylation. Another finding is that the β1 integrin upward regulation promotes cell proliferation and targeting it by an antibody will induce apoptosis in endothelial cells mediated by TF. Thus, the integration between the TF and the β1 integrin is essential for cell proliferation in endothelial cells. For example, endothelial cells depend on the interaction of TF with the β1 integrin to stimulate cell proliferation (van den Berg et al., 2009). Therefore, any disturbance in the TF–β1 integrin will reduce cell growth in vivo.

Thus, in this study, HDBECs were affected by the β1 integrin inhibition, as the signaling pathway was altered. Then, Src activity was downward regulated. Indeed, the β1 integrin inhibition induced TF signaling for apoptosis. Thus, the β1 integrin inhibition was responsible for reducing Src activity. The β1 integrin affects Src activity during apoptosis induction in endothelial cells. Moreover, Src activity was affected by TF–β1 integrin–induced signaling due to the disassociation of TF and the β1 integrin. TF signaling was disrupted because of the TF structure change. Therefore, TF lose its function in the cell, causing an accumulation in the cell. For example, wild-TF had a moderate Src activity during

apoptosis in endothelial cells through the TF- β1 integrin signaling mechanism. Indeed, Targeting the TF- β1 integrin signaling will induce apoptosis. Furthermore, mutation in TF residue will alter TF signaling and can induce apoptosis. For example, Ser253 substitution with Ala in a TF amino acid sequence will increase the ability of the cell to induce apoptosis (Collier & Ettelaie, 2011). In addition, the β1 integrin inhibition has an influence on endothelial cells to induce apoptosis. The interaction between TF and the β1 integrin affects cell survival (Aberg, Eriksson, & Siegbahn, 2015). As overexpression of TF–β1 integrin signaling induces cell proliferation. Thus, TF–β1 integrin disruption will reduce cell proliferation (Kocaturk & Versteeg, 2013). It can be done in vitro by adding the β1 integrin inhibitor to the cell. Therefore, cell signaling will be altered and apoptosis can be induced. During induced apoptosis in endothelial cells by the β1 integrin inhibition, the Src phosphorylation was altered (Figure 1). Therefore, the β1 integrin affects Src activity during apoptosis mediated by TF- β1 integrin signaling. Therefore, there was a strong correlation between the β1 integrin and Src during apoptosis. Previous studies have shown that Src activity inhibition, will reduce cell proliferation (Jin, Nam, Park, Bang, Bang, & Oh, 2017). In this study, the Src phosphorylation ratio decreased during apoptosis in the endothelial cells. Indeed, the β1 integrin inhibition reduced Src activity in the cell. Thus, the β1 integrin affects Src in TF signaling pathway. These mechanisms occurr during endothelial cell apoptosis mediated by TF. Thus, TF can induce apoptosis in endothelial cells via activation to the P38 MAPK and P53 apoptosis pathway. Apoptosis was induced in HDBECs due to external stimuli, which was induced by the β1 integrin inhibition on the cell. Therefore, the β1 integrin inhibitions will recruit P38 MAPK and P53 to activate apoptosis pathway (Bottone, Santin, Aredia, Bernocchi, Pellicciari, & Scovassi, 2013). Indeed, apoptosis in HDBECs was induced by the TF–β1 integrin complex mechanism. It was elicited by adding the β1 integrin inhibitor to the cells. Then, Src activity was measured by detecting the Src antibody using western blotting. Thus, Src activity was correlated with the β1 integrin expression as in the previous study. The present research demonstrated that Src activity depends on the β1 integrin expression in the cell, as the β1 integrin overexpression and hyperactivity are associated with cell proliferation. Thus, interaction between TF and the β1 integrin has a crucial role for cancer progression. Thus, targeting the β1 integrin will decrease cell proliferation. Therefore, the cells will undergo apoptosis with the β1 integrin, as the attachment is lost (Ishikawa, Ushida, Mori, & Shibanuma, 2015). Then, there will be an impact on cell proliferation and migration as a result of cell s disassociation. This suggests that the mechanism of inducing apoptosis is mediated by the β1 integrin inhibition. Furthermore, endothelial cells undergo apoptosis for physiological and pathological conditions to maintain haemostasis and remodeling (Santos, Sato, Moro, Bazzoli, & Rizzo, 2008). For example, vascular injury inflammation and chronic cardiovascular diseases induce apoptosis in endothelial cells. Then, the apoptotic cells will induce P53 as a result of cell damage due to external stimuli and P38 MAPK activation causing cell arrest. The process of P38 MAPK activation. In addition, the Src activity ratio is an indicator of apoptosis in endothelial cells.

Indeed, Src signaling is affected by the β1 integrin in TF mediated apoptosis in endothelial cells. Thus, the expression of the β1 integrin maintains cell survival and migration and Src also mediates the β1 integrin adhesion. Therefore, the β1 integrin is a target for Src, to prevent cell proliferation (Qin et al., 2011). The β1 integrin has an important role in cell regulation for proliferation, migration and differentiation as well as survival. This arises from cell cycle regulation mediated by the β1 integrin signaling (Moreno-Layseca & Streuli, 2014). TF can induce apoptosis in endothelial cells through TF signaling in the cell. However, TF's induction of apoptosis has been subject to debate. TF signaling is involved in cell proliferation and migration as well as survival. Overexpression of TF is seen in pathological conditions, such as cancer or angiogenesis. Thus, blocking it pathway or modifying the TF structure will induce apoptosis

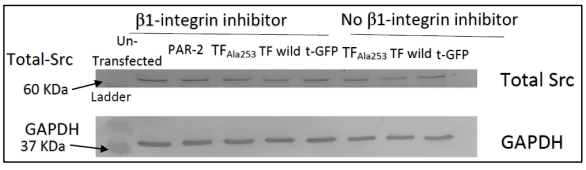

Figure 4: Effect of β1-integrin inhibitor on total-Src phosphorylation.

HDBECs (105/well) were seeded in a 12-well plate, followed by transfection to express mutant-TF and wild-TF, as well as plasmid control t-GFP. Samples were activated by PAR2-AP (20 μM) for 120 minutes. Three samples were treated with β1-integrin inhibitor for 20 minutes, and three samples were not treated with β1-integrin inhibitor (negative control). Samples were lysed in Laemmli buffer, and then the proteins were separated in 12% SDS-PAGE. Protein samples were transferred to nitrocellulose membranes and cut into two halves for total Src and GAPDH (control). The membrane was probed for total-Src using rabbit anti-human total Src antibody. The secondary membrane was probed for GAPDH using a goat anti-human GAPDH antibody, followed by secondary antibodies. Finally, bands formed in the two membranes. The bands were analysed by ImageJ, and the Src ratios were calculated.

3.3 Effect of Mutant-TF on Src Phosphorylation

The inhibition of the β1 integrin exhibited different Src ratios for both wild-TF and mutant-TFAla253 in the cells. Thus, two HDBEC samples were transfected to express mutant-TFAla253 to observe the effect of TF mutation for Src signaling. To examine the effect of the β1 integrin on Src activity induced by mutant-TF Ala253, the amino acid sequence of TF Ser253 was substituted with alanine amino acid. Then, the two samples were activated with PAR2-AP (20 μM) for 20 minutes. One sample was treated with 10 μg/ml of the β1 integrin inhibitor for 120 minutes. The second was not treated with it that was used as a negative control. After adding phospho-Src and total Src antibodies to these samples, Src was detected for both phospho-Src and total Src in the TF-mutant samples. The ratio of Src activity was low in the mutant-TFAla253 samples, for samples with and without the β1 integrin inhibitor, compared with wild-TF samples. The Src ratio was the lowest in the β1 integrin inhibitor mutant-TFAla253 among the samples (Figure 1). In contrast, wild-TF transfected HDBEC samples had a higher Src ratio and the Src activity ratio was not reduced like it was in mutant-TFAla253. The results suggested that mutation of TF amino acid Ser253 to Ala253 substitution had the effect of reducing Src activity in the cell signaling. In addition, mutant-TFAla253 sample was affected by the β1 integrin inhibition; thus, the Src ratio decreased.

4. DISCUSSION

A high expression of TF is associated with, cell proliferation and migration. Thus, a high level of TF expression in endothelial cells leads to tumour angiogenesis and/or metastasis as well as cell survival (Han, Guo, Li, & Zhu, 2014). This process is initiated by TF interaction with the β1 integrin, and the activation of P38 MAPK. The mechanism of cell proliferation, migration and survival includes Src activation in TF–β1 integrin pathway. TF-FVlla stimulates the Src expression in the cells (Versteeg, Hoedemaeker, Diks, Stam, Spaargaren, Henegouwen, Deventer, & Peppelenbosch, 2000). Src mediates TF–β1 integrin function for cell adhesion. Thus, the Src activation is correlated with the β1 integrin expression (Qin, Chen, Wu, Feng, He, Wang, Liao, & Xu, 2011). Both Src and the β1 integrin are overexpressed in cancer. Therefore, cancer cells express high levels of the β1 integrin. Thus, cells proliferation and survival are stimulated by the TF- β1 integrin integration to activate the Src signaling pathway. In contrast, TF can induce

3.2 Effect of the β1 Integrin Inhibition on Src Phosphorylation

To examine the effect of the β1 integrin on the Src pathway for apoptosis in endothelial cells, three HDBEC samples (105 cells/well) were treated with the β1 integrin inhibitor to determine the β1 integrin inhibition of Src activity. Each cell sample was transfected with a different TF. The first HDBEC sample was transfected to express wild-TF. The second HDBECs were transfected to express mutant-TFAla253, as well as a plasmid control t-GFP. The samples were activated by PAR2-AP (20 µM) for 120 minutes, followed by 10 µg/ml of the β1 integrin inhibitor for 20 minutes. Then, the samples were run on SDS-PAGE and transferred to nitrocellulose membranes to examine the total Src and phospho-Src. In addition, three HDBEC samples (105 cells/well) were not treated with the β1 integrin inhibitor to observe the total Src and phospho-Src. These samples were used as the negative control for the experiment. After adding the total Src and phospho-Src antibodies to the β1 integrin inhibitor and non–β1 integrin inhibitor samples, different bands formed in the membranes (Figures 3&4).

The Src activity ratio with the β1 integrin inhibitor was obtained by calculating the concentrations of phospho-Src and total Src using the ImageJ software programme.

Src activity was obtained after dividing phospho-Src by total Src for all the samples (Figure 1). The data showed a significant difference in Src phosphorylation with the β1 integrin inhibitor. Indeed, the samples treated with the β1 integrin inhibitor had a low Src activity ratio, whereas samples that were not treated with the β1 integrin inhibitor had a higher Src activity ratio (Figure 2). Therefore, blocking the β1 integrin in the cell affected the Src phosphorylation of the TF signaling pathway in the cell. Inhibition of the β1 integrin knockdown phosphorylation of the Src activity ratio. Especially, the sample transfected with mutant-TFAla253 had the lowest Src activity compared with the wild-TF sample. In contrast, the Src activity ratio was higher in samples that were not treated with the β1 integrin inhibitor. Therefore, the ratio of Src activity phosphorylation in HDBEC samples was higher in the absence of the β1 integrin (Figure 2). However, the total-Src was similar for all samples after detection of the total Src antibodies (Figure 4). Inhibition of the β1 integrin had no influence on any total Src samples. Thus, after obtaining the calculation of phospho-Src and total Src, the ratios varied among the samples. The data suggest a correlation between a reduced Src ratio and the β1 integrin inhibition.

Figure 3: Effect of β1-integrin inhibitor on phospho-Src activity.

HDBECs (105/well) were seeded in a 12-well plate, followed by transfection to express mutant-TF and wild-TF, as well the plasmid control t-GFP. The samples were activated by PAR2-AP (20 µM) for 120 minutes. Three samples were treated with β1-integrin inhibitor for 20 minutes, and three samples were not treated with β1-integrin inhibitor (negative control). Samples were lysed in Laemmli buffer, and then the proteins were separated in 12% SDS-PAGE. Protein samples were transferred to nitrocellulose membranes and cut into two halves for phospho-Src and GAPDH (control). The membrane was probed for total Src using the rabbit anti-human total Src antibody. The secondary membrane was probed for GAPDH using a goat anti-human GAPDH antibody, followed by secondary antibodies. Finally, bands formed in the two membranes. Bands images were recorded and analysed by ImageJ. The Src activity ratio was obtained by dividing phospho-Src by total-Src to observe the effect of β1-integrin on Src signalling induced by TF. There were different Src concentrations, relating to the TF types. Mutant-TFAla253 had the lowest Src activity compared with wild-TF. Especially, β1-integrin inhibition reduced the Src ratio.

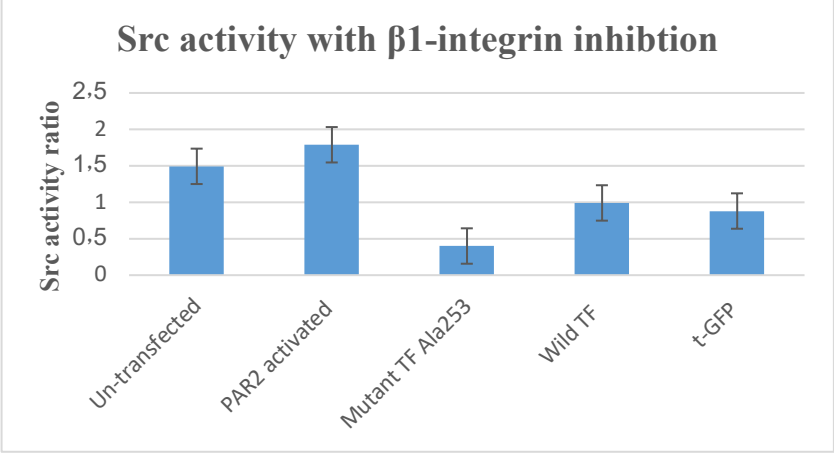

Figure 1: Src activity ratio for samples with β1-integrin inhibitor.

HDBECs (105) were transfected with wild-TF and mutant-TFAla253, followed by PAR2-AP for 20 minutes (20 µM). The untransfected, unactivated sample and PAR2-AP activated, untransfected sample were used as controls. The three samples were treated with β1-integrin inhibitor for 90 minutes. Then, the samples were transferred to western blotting membranes and probed with phospho-Src and total Src antibodies. The ratio of Src activity was determined by calculating the ratio of phospho-Src and total-Src. The β1-Integrin inhibition showed a low Src ratio compared with samples without it. Mutant-TFAla253 had the lowest Src ratio compared with wild-TF.

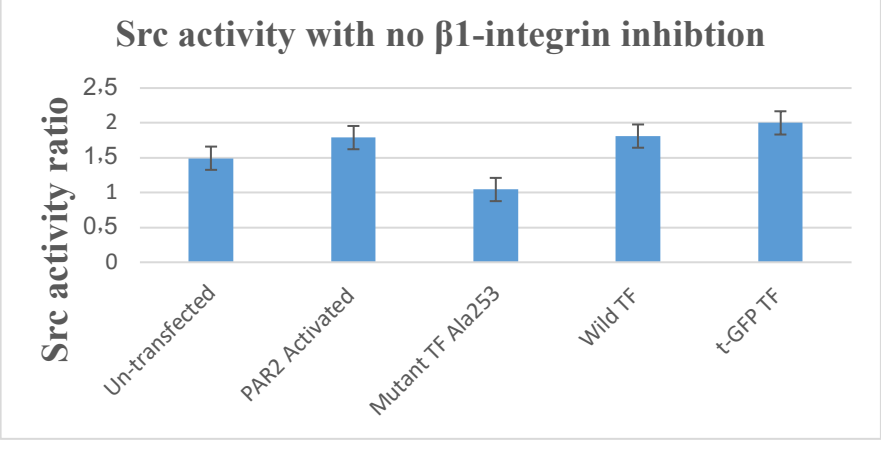

Figure 2: Src activity ratio for samples without β1-integrin inhibitor.

HDBECs (105) were transfected with wild-TF and mutant-TFAla253, followed by PAR2-AP for 20 minutes (20 µM). The untransfected, unactivated sample and PAR2-AP activated, untransfected sample were used as controls. The three samples were treated with β1-integrin inhibitor for 90 minutes. Then, the samples were transferred to western blotting membranes and probed with phospho-Src and total Src antibodies. The ratio of Src activity was determined by calculating the ratio of phospho-Src and total-Src. The Src activity in HDBECs was higher without the β1-integrin inhibitor.

Then, the cell samples were activated with 20 μM of PAR2 agonist peptide for 120 minutes. Moreover, 10 μg/ml of the β1-integrin inhibitor (AIIB2) was added to samples 1–7 for 90 minutes. Then, the samples were lysed with 100 μl of Laemmli buffer.

When the samples were ready for SDS-PAGE and the gel electrophoresis was prepared, the samples were loaded as described above. The samples were run on two different gels, one for phospho-Src and the other for total Src. Bands were formed for both gels after 1 hour. The samples were then transferred to two different nitrocellulose membranes and run over night at 4°C. Following this, the two membranes were cut into halves, one for phospho-Src and GAPDH and the other for total-Src and GAPDH according to their molecular weights. The two GAPDH membranes were probed with primary goat anti-human GAPDH antibodies. In addition, the phospho-Src and total-Src membranes were probed with specific primary rabbit anti-human antibodies for each membrane overnight. Then, the membranes were washed three times with TBST. Then, the samples were treated with secondary mouse anti-rabbit alkaline phosphatase–conjugated antibody for both phospho-Src and total Src and mouse anti-goat alkaline phosphatase– conjugated antibody for GAPDH. Then, the two membranes were washed three times with TBST, followed by distil H_2O wash. Alkaline phosphatase was used for all membranes to observe the formed bands. Finally, the band intensity was measured using ImageJ software.

3. RESULTS

3.1 Influence of TF activation on Src activity

The activity of Src phosphorylation in TF after the β1-integrin inhibition was determined using the Src antibody for both phospho-Src and total Src. First, the cell samples were activated by PAR2-AP (20 μM) for 120 minutes to observe the Src signaling activity mediated by TF–β1-integrin. To accomplish this, 105 HDBECs were seeded into a 12-well plate. The first cell sample was untransfected and PAR2-AP inactivated (negative control). The second cell sample was PAR2-AP activated without transfection (positive control). The cell activation by PAR2-AP increased the activity of Src in the activated samples, whereas the negative control sample had lower Src activity compared with PAR2-AP positive control. However, the Src activity increased in endothelial cells samples with PAR2 activation (Figures 1&2). Consequently, the Src activity ratio in TF signaling increased with PAR2-AP activation compared with the inactivated PAR-2 HDBEC control sample. Therefore, the activation of HDBECs with PAR2-AP activated cell signaling and the increased phosphorylation for Src were compared with the untransfected and unactivated samples.

The activated samples had the highest Src activity ratios among the samples. Therefore, there was a significant value for PAR2-AP-activated cell signaling of Src in HDBECs. PAR2-AP activation induced the signaling pathway in endothelial cells. Thus, PAR-2 activated cell signaling in HDBECs and triggered the proliferation or apoptosis pathway induced by the TF–β1 integrin. Src activity was influenced by cell activation as well as the β1 integrin blocking.

The samples were treated with a β1-integrin inhibitor to observe Src signaling during the β1-integrin inhibition for wild-TF, mutant-TFAla253 and plasmid control t-GFP. The ratio of Src phosphorylation with the β1-integrin inhibitor was obtained by measuring the intensity of western blotting bands by dividing phospho-Src by total Src (Figures 3 & 4). The level of Src phosphorylation was different in each sample according to the β1-integrin inhibition treatment. In addition, GAPDH was used as a control for all samples. All samples had similar values for GAPDH; thus, the samples were not affected by the β1 integrin inhibition for GAPDH. The GAPDH concentration was not significantly changed, unlike that of phospho-Src and total Src (Figure 3 & 4). Therefore, the activation of HDBECs with PAR2-AP affected Src signaling as PAR2-AP activated Src in the cell control sample.

overnight. The next day, the media were changed and the cells were washed with PBS in preparation for the transfection process. For the transfection process, the Trans IT 2020 Transfection Reagent was warmed to room temperature prior to use. Then, 100 µl of Opti-MEM I reduced-serum medium was placed in a sterile tube. Following this, 1 µg of the plasmid DNA was added to the tube and mixed gently and 2 µl of the TransIT-2020 reagent was added to dilute the DNA plasmid mixture. Then, the mixture was incubated at room temperature for 20 minutes. Next, 1 µl of plasmid was added to each well, with 900 µl of 5% MV media added to each well and incubated at 37°C under 5% CO2 for 4 hours. After the cells had been transfected, the medium was changed and the cells were washed with PBS and incubated for 48 hours. Finally, the transfected cells were visualised under the microscope and the transfection efficiency was determined by flow cytometer (Becton Dickinson FACSCalibur flow cytometer) to measure the t-GFP expression in each well. After transfection, samples followed by activation by PAR2.

2.4 SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

A 12% agarose gel concentration was used to prepare the gel for western blotting. The gel was prepared by mixing 3.3 ml of distilled water, 2.6 ml of separating buffer (1.5 M Tris HCl, pH 8.8, 0.4% SDS), 4 ml of acrylamide and 100 µl of 10% ammonium persulphate in a beaker. Then, 10 µl of TEMED (N, N, N', N'-tetramethylethylenediamine) was added to the mixture and the solution was poured immediately into the gel plate of the caster. A drop of butanol was poured into the edge to avoid air bubbles inside the gel. Then, the gel was left for 1 hour. Next, the stacking gel was prepared by mixing 0.65 ml of acrylamide, 1.3 ml of stacking buffer (0.5 M Tris-HCl, pH 6.8, 0.4% SDS), 3 ml of distilled water and 100 µl of 10% ammonium persulphate. Following this, 10 µl of TEMED was added to the mixture and poured into the gel plate. The combs were inserted in the gel caster and left over night. After the gel became solid, it was transferred from the gel caster into an electrophoresis tank. The electrophoresis buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycine, 0.035% SDS) was poured into the electrophoresis tank. Then, 10 µl of protein ladder marker (10–260 kDa) was loaded into the first well, while 10 µl each of eight protein samples were loaded into the next wells. The electrophoresis tank was connected to the power and water tape was placed on the bottom for cooling the generated heat. The gel was run at 100V for 1 hour until the blue band migrated to the end of the gel.

The gel was transferred to a nitrocellulose membrane (filter paper, membrane and gel) and run at 4°C and 16 mA overnight. Then, the membrane was blocked with Tris-buffered saline Tween-20 (TBST) solution (20 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.05% Tween-20) and incubated on a shaker at room temperature for 1 hour. After blocking, the membrane was incubated with 15 ml of primary antibody (mouse anti-human) for GAPDH (Goat anti-human) as a control over night. The membrane was washed three times with TBST, followed by a secondary antibody (goat anti-rabbit) incubation for 1 hour on a shaker at room temperature. Finally, the membrane was washed with distilled water and treated with alkaline phosphatase substrate. The GAPDH bands appeared in the 37 kDa line after 10 minutes and the bands were recorded as described previously (Alabdulmonem, Alhomaidan, Rasheed, Madar, Alasmael, Alkhatib, & Al Ssadh, 2018).

2.5 Src detection by western blotting

Eight samples were prepared for both phospho-Src and total Src to observe the effect of the β1-integrin inhibition on Src. The first sample was untransfected and unactivated, as it was used as a negative control. The second sample was activated only as a positive control. The other seven HDBEC samples were transfected with pCMV-A6-tGFP. In addition, the mutant-TFAla253 samples were already prepared and transfected to HDBECs to express mutant-TFAla253, using specific siRNA.

medium) media under sterile conditions; these conditions were maintained for the media and all reagents to avoid any contamination. A vial of 0.7 ml of HDBECs was seeded in a T25 (25 cm²) flask; the cells were incubated at 37°C with 5% CO2 overnight. The next day, the medium was changed and washed with Phosphate-buffered saline (PBS), to detach the cells from the flask, followed by trypsin neutralization treatment. The cells were examined under a microscope to check cell movement. The medium was changed daily for 3 days to maintain the cells. Then, cell counts were performed using a haemocytometer to calculate the number of cells required for transfection. The cell count was 5.8 × 105, which is considered abundant. Finally, after optimizing the cell count, the medium was removed and the cells were washed with PBS and trypsin. The excess cells were transferred into cell vials, followed by gradual cryopreservation.

2.2 Plasmid Isolation from *Escherichia Coli*

The plasmid was obtained from Escherichia coli to express TF in endothelial cells, as TF is not expressed normally like cancer lines. The PCMV6A-TF-tGPF plasmid was used in the experiment and an LB medium was prepared for bacterial growth. First, 1 ml of *E. coli* containing the plasmid was subculture in a 100 ml LB broth in a flask and incubated in a shaking incubator at 37°C overnight under sterile conditions. Then, the media flask was transferred into two 50-ml tubes and centrifuged in an ultra-cold centrifuge (4°C) at 2500 rpm for 15 minutes. The bacterial pellet was formed in the bottom and the supernatant was discarded. The pellet was resuspended with 3 ml of lysis buffer and 4 ml of neutralisation buffer (provided in the Promega kit) in 20-ml tubes and inverted gently 4–6 times. After gentle mixing, 20 ml of the lysates were centrifuged at 3500 rpm for 20 minutes. The clear supernatant was transferred into two 15-ml tubes and centrifuged again at 3500 rpm for 15 minutes to ensure that all the bacterial cell debris had been removed. Then, 10 ml of DNA purification resin was added to the Midprep column and the supernatant lysate was transferred to the column. The lysate was cleared into the Midprep column using a vacuum. Then, the column was washed with 20 ml of washing buffer using the vacuum. Finally, the Midprep bottom was cut and transferred into a 1.5-ml Eppendorf tube. The DNA plasmid was eluted with 300 µl of nuclease-free water and centrifuged for 3 minutes at 13,400 rpm in a microcentrifuge. At this point, the eluted plasmid was ready for plasmid participation.

As a next step, 150 µl of 5 M sodium acetate (pH 5.2) and 600 µl of absolute ethanol (100%) were added to 150 µl of the plasmid in a 1.5-ml Eppendorf tube. Then, the plasmid samples were incubated in the freezer at –20°C for 30 minutes. After the incubation, the sample tubes were centrifuged at a maximum speed for 10 minutes in a microcentrifuge. Then, the samples were washed with 70% ice-cold ethanol and centrifuged again at the maximum speed for 10 minutes in the microcentrifuge. The ethanol was removed and the DNA was resuspended in 150 µl of nuclease-free water. Once the DNA plasmid was ready for transfection, optimization was carried out to calculate the required plasmid for transfection. The plasmid concentration was measured in a quartz cuvette at A260 and A280 using a spectrophotometer.

DNA agarose gel was employed to ensure plasmid quality. The agarose gel was prepared by adding 0.3 g of agarose to 50 ml of TBE. The mixture was heated in a microwave for 2 minutes. After the gel cooled, it was poured into the gel tray and left until it became solid. Then, 10 µl of plasmid DNA was mixed with 1 µl of SYBR Green I dye and 3 µl of loading buffer. In addition, the DNA ladder was prepared by adding 10 µl of DNA marker and 1 µl of SYBR Green I dye. The samples were run on electrophoresis at 100V for 1 hour. Finally, the gel was transferred to a UV transilluminator and the DNA plasmid bands were visualized.

2.3 Transfection Using TransIT®-2020

HDBECs (105 per well) were seeded in 12-well plates containing 2 ml of 5% MV media and 2% of FCS and incubated at 37°C under 5% CO2

1. INTRODUCTION

The association of tissue factor (TF) with the inhibition of cell apoptosis in tumours has long been established (Versteeg, 2004 & Fang, 2008). Moreover, TF expression in cells controls the balance between cell proliferation and apoptosis. Thus, it has been suggested that metastasis and angiogenesis are regulated by TF expression in the cell (Mackman, 2004). The process involved in the overexpression of TF in cancer mediates the intracellular signaling pathway for proliferation (Pradier & Ettelaie, 2008). Indeed, apoptosis in endothelial cells can be induced by the TF–β1 integrin complex. TF can interact with cell β1 integrin, leading to various intracellular pathways, such as cell proliferation and migration (Collier & Ettelaie, 2011). In contrast, interaction of the TF–β1 integrin complex can induce apoptosis via the cell signaling pathway (Kocaturk & Versteeg, 2013). TF has a non-coagulation role in malignancies, which suggests a relationship between TF and cancer. Indeed, TF activates signaling pathways in the cell, leading to cell proliferation, cell migration and gene expression (Collier & Ettelaie, 2011). Thus, TF overexpression is predominant in many cancers and its upregulation correlates with metastasis (Shaker, Harrison, Clarke, Landberg, Bundred, Versteeg & Kirwan, 2017). TF overexpression is also characterized by apoptosis resistance. Therefore, the accumulation of TF in cancer cells is common and cancer progression is influenced by TF expression (Kocaturk & Versteeg, 2013). The activation of TF is stimulated by triggering via the cleavage of G-protein receptors, termed protein-activated receptors (PARs). There are four PAR types (Soh, Dores, Chen, & Trejo, 2010). PAR-1 and PAR2 are the most common activation factors for TF signaling in the cell (Schaffner, Versteeg, Schillert, Yokota, Petersen, Mueller, & Ruf, 2010). Thus, the activation of TF by PAR2 increases tumour growth (Kocaturk & Versteeg, 2013). Moreover, Kocaturk, & Versteeg (2013) discovered that TF depends on PAR2 activation via integrin ligands to induce angiogenesis. There is a strong correlation between TF types for inducing apoptosis. Mutants in the TF residue will cause a defect in coagulation and cell proliferation (Ke, Yuan, & Morrissey, 2014). This is because mutation in a TF amino acid will alter its activity. In addition, modification of the TF structure leads to changes in its signaling pathway. For example, Ser253 substitution in TF with alanine 253 (Ala253) has been shown to reduce the release of TF in cells (ElKeeb, Collier, Maraveyas, & Ettelaie, 2015). Thus, TF will accumulate in cells and induce apoptosis. In contrast, aspartate 253 (Asp253) substitution exhibits the increased release of TF in the cell, causing cell proliferation. TF and the β1 integrin interact together in endothelial cells to form the TF–β1 integrin complex in tumour growth. The inhibition of TF also has affects on the β1 integrin via the disruption of the TF–β1 integrin interaction. Thus, TF is a key regulator of the β1 integrin in tumours to promote cell proliferation and metastasis. The regulation of the TF–β1 interaction is controlled by the TF ligand and PAR2 activity (Ruf & Versteeg, 2010).

Practically, endothelial cells are transfected with plasmid to express wild-TF and mutant-TFAla253, followed by cell activation with PAR2. After the activation, the cells are treated with a β1 inhibitor for western blotting to observe the effect of the β1 integrin inhibition on Src activity, using specific Src antibodies. Thus, the Src activity is used to indicate the apoptotic effect induced by the TF–β1 integrin pathway.

Given the gaps in knowledge outlined above, the aim of the present study was to investigate the effect of the β1 integrin on Src activity during the apoptosis mechanism induced by the TF–β1 integrin in endothelial cells.

2. MATERIAL AND METHODS

2.1 Cell Culture

Human dermal blood endothelial cells (HDBEC) were used throughout this study as a model for studying endothelial cells. The cells were warmed in an incubator at 37°C (Scientific Laboratory Supplies Ltd, Hessle, UK) prior to use. The cells were cultured in 5% MV2 (Endothelial cell growth

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دور العامل النسيجي البروتيني بيتا أنتجرين على نشاط بروتين الكاينيز في الخلايا السرطانية خلال آلية موت الخلايا المبرمج في الخلايا البطانية

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ملخص: يزداد العامل النسيجي البروتيني في الأنسجة والخلايا وخاصة بعد تحفيزها. ويزداد تعبيرها وبالاخص مع ارتباطها في الحالات المرضية المزمنة بما فيها تجلط الدم والأوعية الدموية وأمراض القلب. وفي هذا الصدد هدفت هذه الدراسة إلى البحث في آلية موت الخلايا المبرمج في الخلايا البطانية من خلال العامل النسيجي البروتيني (بروتين بيتا أنتجرين 1) من خلال استخدام خلايا الدم البطانية البشرية، وتم تقسيمها إلى ثلاث مجموعات. ففي المجموعة الأولى تم نقل البلازميد للتعبير عن النوع البري للعامل النسيجي البروتيني، بينما في المجموعة الثانية تم نقل البلازميد للتعبير عن النوع المتحور للعامل النسيجي، وفي المجموعة الثالثة تم نقل البروتين الفلوري الأخضر لها. وبعد ذلك تم استخدام مهبط للعامل النسيجي البروتيني في المجموعات الثلاث كلها للفحص على تأثير المهبط على الخلايا المستخدمة. وقد أظهرت نتائج هذه الدراسة تأثيرا معنويا على العامل النسيجي البروتيني وخاصة في عملية الفسفرة. وأضحت الدراسة أن عملية الفسفرة لبروتين الكاينيز خلال موت الخلايا المبرمج يقل أثناء تثبيط العامل النسيجي البروتين (بروتين بيتا أنتجرين 1).

كلمات مفتاحية: العامل النسيجي، بروتين بيتا أنتجرين ، سيرين الفسفره، موت الخلايا.

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The Role of Tissue Factor- β1- Integrin Complex Formation on Src Activity during the Apoptosis Mechanism in Endothelial Cells.

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Abstract: Tissue factor (TF) is expressed by cells and tissues, specifically endothelial cells following activation. In addition, an overexpression of TF in the endothelium contributes to a variety of chronic pathological conditions including thrombosis in cancer, metastasis, angiogenesis and cardiovascular disease. The aim of this study was to investigate the mechanisms of apoptosis in endothelial cells through the TF–β1 integrin complex formation. Throughout the study, human dermal blood endothelial cells (HDBECs) were transfected with plasmid to express wild-TF or alternatively, transfected to express mutant-TF. Other cells incubated with green fluorescent protein (t-GFP) were used as a plasmid control. Inhibition of the β1-integrin was enhanced by treatment with 10 µg/ml of the β1-integrin inhibitor in three samples to test the effect of the β1-integrin inhibitor on wild-TF, mutant-TFAla253 and t-GFP. Moreover, samples were run on sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) followed by western blotting for Src antibody detection, using phospho-Src and total Src. The ratio of Src activity for the samples was obtained by dividing phospho-Src by total Src. The results revealed a significant effect of the β1 integrin inhibition on Src phosphorylation. The samples that were treated with the β1 integrin inhibitor showed lower Src activity ratios. Specifically, the cell sample that was transfected to express mutant-TFAla253 had the lowest Src activity among the HDBEC samples treated with the β1-integrin inhibitor. In contrast, the Src activity ratios were higher in cell samples that were not treated with the β1-integrin inhibitor. Therefore, both the β1-integrin inhibitor and the mutant-TFAla253 were major factors inducing apoptosis. The results suggested that the β1-integrin affects TF mediated apoptosis. The results also suggested that Src is reduced during apoptosis through the β1-integrin inhibition.

Keywords: Tissue factor, β1 integrin, Serine-phosphorylation, p38-MAP kinase, p53 activation, Apoptosis.

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sues with acidic and fatty foods, new formulations of PCZ are being developed. This is evident from the filing of patent applications associated with the pharmaceutical compositions of PCZ (Heimbecher & David, 2012; Ramana, Naidu, Chary, Kumar, & Hendrik, 2017). The injection of PCZ is compatible with many other drugs via IV route. This gives the PCZ an edge over other azole antifungal agents. This property of the PCZ has also opened doors to develop new IV dosage forms that can be prepared with/without the use of a solubilizing agent (Heimbecher & David, 2012). PCZ has an advantage over ICZ and FCZ for the treatment of Aspergillus infection because it is active against both enzymes, CYP51A and CYB51B of *Aspergillus* spp. (Moore, Healy & Kraft, 2015). This property of PCZ is being utilized to develop a newer PCZ derivative that can be effective against CYP51A and CYB51B, of *Aspergillus* spp. (Zhiguo, 2017). The patented inventions related to the polymorph of PCZ and its particle size give positive evidence that the nano-medicine of PCZ can be more beneficial therapeutically (Imran, Nayeem, & Bawadekji, 2018; Raghavendra, Chandre, Shanmughasamy, & Manikandan, 2017). Further, there are current clinical studies underway, which may provide more clinical uses of PCZ against IFIs in immunocompromised patients (Maertens, Cornely, Ullmann, Heinz, Krishna, Patino, Caceres, Kartsonis, Waskin, & Robertson, 2014; *ClinicalTrial.gov*). Furthermore, the pharmaceutical composition of PCZ and an anticancer agent for the treatment of cancer has also been developed, which may provide a better therapeutic regime for cancer patients. However, it will be interesting to see how many new formulations of PCZ and nano-medicine of PCZ will be available in future.

10. CONCLUSION

PCZ is a better choice for the treatment of IFIs as compared to FCZ / ICZ. It is a lipophilic and water-insoluble azole antifungal drug. These two parameters affect the absorption of PCZ and impact its therapeutic effects. Therefore, researchers have developed different dosage forms of the PCZ and have also studied the impact of its particle size on solubility and bioavailability. The new findings concerning the nano-particles of PCZ are providing an insight that nano-particles of PCZ may have better water solubility, better absorption, better bioavailability and ultimately better therapeutic effects. These nano-particles of PCZ may also have a positive impact on its spectrum of activity and potency against many fungal strains. Therefore, it is recommended to develop the nano-medicine of PCZ with the expectation that the nano-medicine will provide better therapeutic effects of PCZ.

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Table 3: Current clinical Phase I to Phase III studies of Posaconazole (PCZ) referenced in *ClinicalTrial.gov* database.

S. No.	Title of the Study	Condition / Disease	Phase	Estimated Completion Date
1	Intravenous PCZ in the Chinese population at high risk for IFIs	Fungal infections	I	Completed
2	Genetic variation and variability in PCZ pharmacokinetics in children	Fungal infections	I	December 2018
3	Safety & efficacy of PCZ vs VCZ for invasive aspergillosis	Fungal infections	III	September 16, 2019
4	Study of ABT-199 in combination with azacitidine/decitabine in patients with acute myelogenous leukaemia	Acute myelogenous leukaemia	II	March 27, 2020
5	PCZ vs ICZ in preventing IFD post-HSCT	IFD Post-HSCT	III	April 1, 2020
6	PCZ prophylaxis during ATG treatment for hMDS/AA patients	Aplastic anaemia/ Myelodysplastic syndromes / Fungal infections	II	June 30, 2020
7	Universal prophylaxis vs pre-emptive therapy using PCZ post-lung transplant (UPPRITE)	Fungal infections	II	September 30, 2020
8	Peripheral blood stem cells transplantation from the family donors	Transplant-related hematologic malignancy leukaemia/ Acute myelodysplastic syndrome / Graft vs host disease fungal infections	II	September 15, 2022

13, 2019, a lot of clinical trials on PCZ are under development for various diseases. Table 3 provides information about some conditions or diseases for which the clinical trials on PCZ are completed or under progress. This table includes information about the phase I to Phase III clinical trials.

9. DISCUSSION

In the last two decades, an escalation in the statistical cases of IFIs, especially in immunocompromised patients, has been reported. The azole class of antifungals, including PCZ, is in clinical practice for the IFIs (Moore, Healy, & Kraft, 2015). PCZ is a Class II drug, which means that it has high permeability and lipophilicity, but low water solubility. To address the issues of water solubility, phosphate ester prodrug of PCZ was developed. However, this prodrug could not become a clinically useful medicine (Leung, Poulakos, & Machin, 2015; Lee, Eckert, Gala, Schwartz, Renton, Pergamen, Whttington, Schumacher, Heimark, & Shipkova, 2001; Kim, Kumari, Lin, & Nomeir, 2002). Therefore, researchers are focussing on the nano-particles of the PCZ to improve its water solubility (Imran, Nayeem, & Bawadekji, 2018; Moore, Healy, & Kraft, 2015; Krishna, Moton, Ma, Medlock, & McLeod, 2009). Similarly, to avoid absorption related is-

beneficial effects. Imran, Nayeem, & Bawadekji (2018) have recently reported a nano-particle of PCZ wherein the nano-particles had a particle size of about 325 nm and about 25 times more water solubility than regular PCZ powder, which has an average particle size of 111.2 µm. The reported nano-particles are reported to be stable and can be used to prepare different dosage forms (Moore, Healy, & Kraft, 2015; Krishna, Moton, Ma, Medlock, & McLeod, 2009).

7. PATENT LITERATURE

The patent literature search was conducted through the World Intellectual Property Organization (WIPO) website for information about the latest research on PCZ. The recent and significant Patent Cooperation Treaty (PCT) publications by the World Intellectual Property Organization (WIPO) are discussed below.

The PCT publication number WO 2007/143390 (Scott & Gary, 2007) assigned to Elan Pharma International Ltd., indicates PCZ as having an average particle size of 2000 nm or less with improved water solubility and improved bioavailability. This PCT publication also shows the parenteral dosage forms of nano-sized particles of PCZ. The PCT publication number WO 2011/158248 (Milind, Vivek, Reddy, Ganesh, Jitendra, & Ahmed, 2011) assigned to Glenmark provides crystalline polymorphic Form V of PCZ. The PCT publication number WO 2011/003992 (Josef, Arthur, Andreas, Ulrich, & Christoph, 2011) assigned to Sandoz provides a crystalline form N-S of PCZ and crystalline form IV of PCZ. The PCT publication number WO 2012/005973 (Heimbecher & David, 2012) assigned to Merck provides an intravenous dosage form containing PCZ and cyclodextrin Compounds. The PCT publication number WO 2015/092595 (Domenico, Claudio, & Alberto, 2015) assigned to Avanthera provides a crystalline form A of PCZ, and pharmaceutical compositions comprising crystalline form A of PCZ. The PCT publication number WO 2016/061863 (Zunliang & Xilin, 2016) assigned to Jiangsu Hansyn Pharmaceutical Co. Ltd., provides crystalline polymorph I of the PCZ and the process of its preparation. The PCT publication number WO 2017/147893 (Zhiguo, 2017) assigned to Zhejiang Ausun Pharmaceutical, indicates PCZ-oxazolidine derivatives and their pharmaceutical compositions. The PCT publication number WO 2017/133632 (Jun, Penggao, Li, Hongwei, Anxiao, Yongkai, & Chaodong, 2017) assigned to Wuhan LL Science and Technology Development Co., Ltd., shows PCZ derivatives with potent antifungal activity, safety and better water solubility, which do not require a solubilising agent to make dosage forms. The PCT publication number WO 2017/051342 (Raghavendra, Chandre, Shanmughasamy & Manikandan, 2017) assigned to Biocon Limited, provides the amorphous form of the PCZ, a crystalline B-1 form of the PCZ, crystalline B-2 form of the PCZ and crystalline B-3 form of the PCZ. However, this publication does not provide any comparative information about the disclosed polymorphs. The PCT publication number WO 2017/025292 (Ramana, Naidu, Chary, Kumar, & Hendrik, 2017) assigned to Alfred E. Tiefenbacher, contains tablet and capsule dosage forms containing the PCZ, an enteric coated material and an antioxidant. The PCT publication number WO 2018/191541 (Bhagwandin, 2018) assigned to Scynexis Inc., shows pharmaceutical compositions comprising the PCZ and an anticancer agent for the treatment of cancer, for example, adrenal cortical carcinoma, neuroblastoma, cervical cancer, colon cancer, colorectal cancer or small-cell lung cancer.

8. CURRENT CLINICAL STUDIES OF THE PCZ

The United States National Library of Medicine maintains a database at ClinicalTrial.gov. It provides information about the funded clinical trials under progress throughout the world. According to this website, accessed on March

Table 2: Comparison of the spectrum of activity of Posaconazole (PCZ) Voriconazole (VCZ), and Itraconazole (ICZ)

Organisms	PCZ		VCZ		ICZ	
	MIC (mcg/mL)					
	90%	90	50%	90	50	90%
A. flavus	0.25	0.5	0.5	1.0	0.5	1.0
A. famigatus	0.125	0.5	0.25	0.5	0.5	1.0
A. niger	0.25	0.5	0.5	2.0	1.0	2.0
A. terreus	0.25	0.25	0.25	0.5	0.5	0.5
All *Zygomycetes*	0.5	4.0	16.0	128.0	1.0	32.0
Rhizopus spp.	1.0	8.0	16.0	128.0	4.0	32.0
Mucor spp.	1.0	16.0	64.0	128.0	2.0	32.0
Absidia spp.	0.125	0.25	16.0	128.0	0.125	0.5
Cunninghamella spp.	0.031–1	0.031–1	8–128	8–128	0.125–2	0.125–2
Apophysomyces	0.031–4	0.031–4	16–128	16–128	0.031–8	0.031–8
Saksenaea spp.	0.016–2	0.016–2	0.5–4	0.5–4	0.016–0.125	0.5–4
Rhizomucor spp.	0.016–0.25	0.016–0.25	2–16	2–16	0.016–0.25	0.016–0.25
Cokeromyces spp.	0.25–4	0.25–4	16–64	16–64	0.25–8	0.25–8
All *Fusarium* spp.	16	32	16	32	16	32
F. solani	32	32	16	32	ND	ND
F. oxysporum	2.0	4.0	4.0	32	ND	ND
F. moniliforme	1.0	1.0	1.0	1.0	ND	ND
Other *Fusarium* spp.	16	16	4.0	16.0	ND	ND
All *Candida* spp.	0.063	1.0	0.031	0.5	0.125	1.0
C. krusei	0.5	1.0	0.25	0.5	1.0	1.0
C. lusitaniae	0.063	0.25	0.031	0.063	0.25	2.0
C. guillermondii	0.25	1.0	0.063	8.0	0.5	4.0
C. dubliniensis	0.031	0.125	0.016	0.125	0.063	0.5
Other Candida	0.25	2.0	0.063	0.25	0.5	1.0
Cryptococcus spp.	0.125	0.5	0.063	0.125	0.125	0.5
Scedosporium prolificans	16.0	32	ND	ND	64	64
Scedosporium apiospermum	0.25	1.0	ND	ND	1.0	32
Coccidioides spp.	0.125	0.25	ND	ND	0.125	0.25
Blastomyces	0.063	0.125	ND	ND	0.031	2.0
Histoplasma	0.019	0.25	ND	ND	0.019	0.063
Pseudallescheria	0.25	1.0	ND	ND	0.5	1.0
All *Aspergillus* spp.	0.125	0.5	0.25	0.5	0.5	2.0
C. albicans	0.031	0.063	0.008	0.063	0.063	0.25
C. glabrata	1.0	2.0	0.25	2.0	1.0	4.0
C. parapsilosis	0.063	0.25	0.031	0.125	0.5	0.5
C. tropicalis	0.063	0.125	0.063	0.5	0.5	0.5

MIC 50% = Minimum inhibitory concentrations (MIC) at which the growth of 50% isolates were inhibited; MIC 90% = Minimum inhibitory concentrations (MIC) at which the growth of 90% isolates were inhibited; b = When $n < 10$, MIC ranges are presented. ND = not determined.

PCZ suspension is pH dependent (Neofytos, Avdic, & Magiorakos, 2010; Cojutti, Candoni, Simeone, Franceschi, Fanin, & Peaa, 2013; Kraft, Chang, van Iersel, Waskin, Krishna, & Kersemaekers, 2014; Jung, Tverdek, & Kontoyiannis, 2014).

5. SPECTRUM OF ACTIVITY OF PCZ

PCZ has been reported to possess an extensive spectrum of activity for many pathogens comprising Zygomycetes, Rhizopus spp., Absidia spp., Cunninghamella spp., Apophysomyces, Saksenaea spp., Rhizomucor spp., Cokeromyces spp., Fusarium spp., Histoplasma and Pseudallescheria. Pharmacodynamics studies on PCZ have been reported alone or in combination with other drugs against the standard drugs, for example, Cryptococcus neoformans, Candida glabrata, Aspergillus and Candida spp., Aspergillus fumigatus, Blastomycesdermatitidis, Trichosporon spp., Scedosporium spp., Coccidioidesimmitis, Mucor spp., Pseudallescheriaboydil and Dermatophytes (Sabatelli, Patel, Mann, Mendrick, Norris, Hare, Loebenberg, Black, & McNicholas, 2006; Barchiesi, Arzeni, Camiletti, Simonetti, Cellini, Offidani, & Scalise, 2001; Barchiesi, Caggiano, Maracci, Arzeni, Scalise, & Montagna, 2003; Barchiesi, Schimizzi, Caselli, Giannini, Camiletti, Fileni, Giacometti, Di Francesco, & Scalise, 2001; Barchiesi, Schimizzi, Najvar, Bocanegra, Caselli, Cesare, Giannini, Francesco, Giacometti, Carle, Scalise, & Graybill, 2001; Pfaller, Messer, Boyken, Hollis, Rice, Tendolkar, & Diekema, 2004; Oliveira, Fothergill, Kirkpatrick, Coco, Patterson, & Redding, 2005; Cacciapuoti, Loebenberg, Corcoran, Menzel, Moss, Norris, Michalski, Raynor, Halpern, Mendrick, Arnold, Antonacci, Parmegiani, Yarosh-Tomaine, Miller & Hare, 2000; Sugar and Liu, 1996; Paphitou, Ostrosky-Zeichner, Paetznick, Rodriguez, Chen, & Rex 2002; Carrillo and Guarro, 2001; Gonzalez, Tijerina, Najvar, Bocanegra, Rinaldi, Loebenberg, & Graybill, 2002; Gonzalez, Tijerina, Najvar, Bocanegra, Rinaldi, Loebenberg, & Graybill, 2003; Sun, Najvar, Bocanegra, Loebenberg, & Graybill, 2002; Manavathu, Alangaden, & Chandrasekar, 2003; Heeres, Meerpoel, & Lewi, 2010). A comparison of the spectrum of activity of PCZ, ICZ and Voriconazole is provided in Table 2.

6. DOSAGE FORMS AND COMPOSITIONS OF THE PCZ

Currently, three dosage forms of PCZ are approved by the USFDA, as listed in Table 1. The oral suspension was the first dosage form that was approved in 2006 by the USFDA. However, for optimum absorption, the PCZ suspension has to be administered with a meal or fatty nutritional additive. It is also reported that the absorption of the oral suspension is boosted when co-administered beside acidic beverages. However, the absorption of the PCZ decreases when co-administered beside antacids, proton pump inhibitors (PPIs) and H_2-receptor antagonists (Courtney, Radwanski, Lim, & Laughlin, 2004; Cojutti, Candoni, Simeone, Franceschi, Fanin, & Peaa, 2013; Leung, and Poulakos, 2008). Therefore, a dose adjustment is sometimes required while treating a patient with the oral suspension of PCZ. To subdue the problems linked with the oral suspension of PCZ, an oral delayed release tablets were developed. The oral delayed-release tablets were approved by the USFDA in 2013. These tablets have no significant clinically relevant pharmacokinetic interaction with food, acidic beverages or antacids. The oral delayed release tablet can be given with antacids, PPIs and H_2-receptor antagonists (Leung, and Poulakos, 2008). In 2014, USFDA approved the intravenous dosage form of the PCZ. This aqueous IV dosage form (comprising sulfobutyl ether beta-cyclodextrin as solubilizer) is indicated for patients 18 years or older (Li, Theuretzbacher, Clancy, Nguyen, & Derendorf, 2010).

PCZ is a poorly water-soluble drug, which affects its bioavailability. The water solubility of a drug depends on its particle size (Ezzet, Wexler, Courtney, Krishna, Lim, & Laughlin, 2005). The literature contains many reports wherein a reduction in the particle size has produced

Svetaz, Vicente, & Zacchino, 2014; Milind, Vivek, Reddy, Ganesh, Jitendra, & Ahmed, 2011; Odds, 2001).

The Noxafil injection is physically compatible with many diluents, for example, dextrose (5%) and NaCl (0.9%), dextrose (5%) and NaCl (0.45%), dextrose (5%) and 20 mEq KCl and dextrose (5%) in water (Leung, Poulakos, & Machin, 2015). The Noxafil injection is incompatible with sodium bicarbonate (4.2%), dextrose (5%) with Lactated Ringer's solution. It is also adaptable with many drugs, for example, lorazepam, famotidine, filgrastim, levofloxacin, meropenem, amikacin sulphate, norepinephrine bitartrate, caspofungin, ciprofloxacin, potassium chloride, daptomycin, dobutamine hydrochloride, hydromorphone hydrochloride, gentamicin sulphate, morphine sulphate, micafungin, morphine sulphate and vancomycin hydrochloride (Moore, Healy, & Kraft, 2015).

4. PHARMACOLOGY OF PCZ

PCZ inhibits the CYP-450 reliant enzyme lanosterol 14α-demethylase that is required for the synthesis of ergosterol from lanosterol (Moore, Healy, & Kraft, 2015). Ergosterol is a crucial part of the fungal cell wall. PCZ has many advantages over other azole antifungal agents, for example, it has an extensive spectrum of antifungal activity, particularly for Aspergillus and other common nosocomial infections that are resistant to other antifungals (Jang, Colangelo, & Gobburu, 2010). It has been reported that Aspergillus has two distinct 14α-demethylase, namely, CYP51A and CYB51B. FCZ and ICZ inhibit only CYB51B and not CYB51A. The remaining CYB51A can convert lanosterol to ergosterol. PCZ, on the other hand, is active against both CYP51A and CYB51B, which gives PCZ an edge over ICZ and FCZ (Lipp, 2010). Therefore, it is also approved for oropharyngeal candidiasis encompassing oropharyngeal candidiasis that is unmanageable to ICZ and/or FCZ. PCZ is a highly protein bound drug, because of its lipophilicity, with a half-life of around 25 hours to 35 hours. It is primarily metabolized by Phase II glucuronic acid conjugation and has very little interaction with the oxidative CYP450 metabolizing enzymes. There are no reports of any active metabolite of PCZ (Lipp, 2010). PCZ is eliminated unchanged in the faeces of healthy people. The safety and tolerability of PCZ are good. The primary adverse reactions of PCZ are hypersensitivity, arrhythmias and QT prolongation and hepatotoxicity. Other mild side effects have also been reported (Li, Theuretzbacher, Clancy, Nguyen, & Derendorf, 2010).

PCZ inhibits CYP3A4 enzyme, and it may boost the plasma concentration of the drugs that are metabolized by the CYP3A4 enzyme. Accordingly, a dose adjustment of the drugs that are precursors of the CYP3A4 enzyme (for example, vincristine, verapamil, diltiazem, nifedipine, nicardipine, felodipine, midazolam, simvastatin, ergotamine, dihydroergotamine, ritonavir, atazanavir, sirolimus, tacrolimus, and cyclosporine), is required when PCZ is coadministered with them. (Vaes, Hites, Cotton, Bourguignon, Csergö, Rasson, Ameye, Bron, Jacobs, & Aoun, 2012; Krishna, Ma, Prasad, Moton, Martinho, & O'Mara, 2012; Krishna, Persons, Kantesaria, & Mant, 2007; Krishna, Vickery, Ma, Yu, Noren, Power, Beresford, & Medlock, 2011; Courtney, Sansone, Statkevich, Martinho, & Laughlin, 2003; Courtney, Wexler, Statkevich-Lim, Batra, & Laughlin, 2002; Courtney, Statkevich, Laughlin Pai, Lim, Clement, & Batra, 2001; Heinz, Grau, Ulrich, Helle-Beyersdorf, Zirkel, Schirmer, Lenker, Einsele, & Klinker, 2012; Poulakos, Henneman, & Leung, 2014 **;** Skiest, Vazquez, Anstead, Graybill, Reynes, Ward, Hare, Boparai, & Isaacs, 2007). Similarly, the UDP-glucuronidase inducer drugs, for example, phenytoin, rifabutin, fosamprenavir and efavirenz, will increase the metabolism of the PCZ and reduce its plasma concentration (Courtney, Radwanski, Lim, & Laughlin, 2004; Cojutti, Candoni, Simeone, Franceschi, Fanin & Peaa, 2013; Wexler, Courtney, Richards, Banfield, Lim, & Laughlin, 2004; Leung and Poulakos, 2008).The dose monitoring of a PCZ suspension is also required when it is co-administered with proton pump inhibitors because the absorption of a

the PCZ is depicted in Figure 1. Chemically PCZ is nomenclated as 4-[4-[4-[4-[[(3R,5R)-5-(2,4-difluorophenyl) tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)-3-furanyl] methoxy] phenyl]-1-piperazinyl] phenyl]-2-[(1S,2S)-1-ethyl-2-hydroxy propyl]-2,4-dihydro-3H-1,2,4-triazol-3-one with Chemical Abstract Service Number 171228-49-2; molecular formula of $C_{37}H_{42}F_2N_8O_4$; MW of 700.8 and melting point of 170°C to 172°C (Leung, Poulakos & Machin, 2015).

PCZ has four chiral carbons, which give rise to 16 isomers. However, only the RRSS isomer is used as the Active Pharmaceutical Ingredient (Ravi, Srinivasa, Sreenu, Venkata, Dilip, & Raja, 2016). PCZ is a white solid with high lipophilicity. The USFDA lists PCZ as a Class II drug, which means that it has a high permeability but a low solubility (Moore, Healy, & Kraft, 2015). To address the solubility issue of PCZ, the phosphate prodrug of SCH 56592 (PCZ) has been developed (SCH-59884) (Lee, Eckert, Gala, Schwartz, Renton, Pergamen, Whttington, Schumacher, Heimark, & Shipkova, 2001; Kim, Kumari, Lin, & Nomeir, 2002). The chemical structure of SCH-59884 is depicted in Figure 2.

PCZ is a structural analogue of ICZ (Figure 3), which is also a USFDA approved triazole antifungal agent. However, PCZ contains a tetrahydrofuran ring in place of a dioxolan ring, which also accounts for its better properties (Castelli, Butassi, Monteiro,

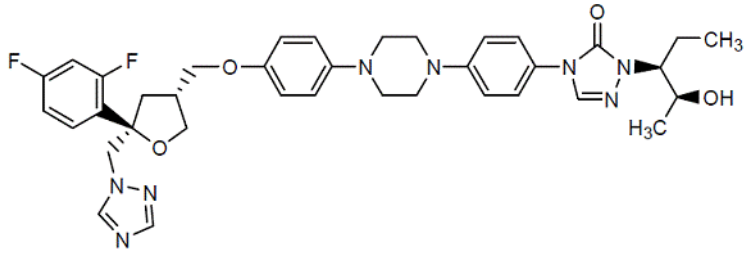

Figure 1: Chemical framework of PCZ (Saksena, Girijavallabhan, Lovey, Pike, Wang, Liu, Ganguly, & Bennett, 1997).

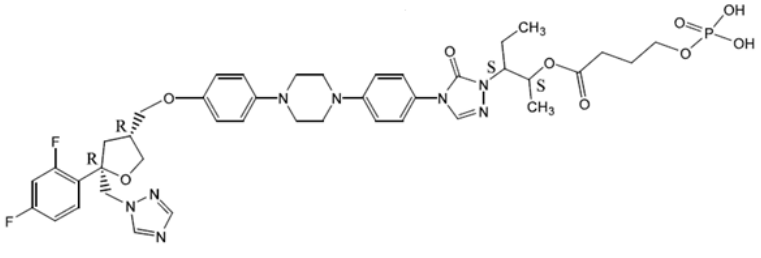

Figure 2: Chemical structure of SCH-59884 (Lee, Eckert, Gala, Schwartz, Renton, Pergamen, Whttington, Schumacher, Heimark, & Shipkova, 2001).

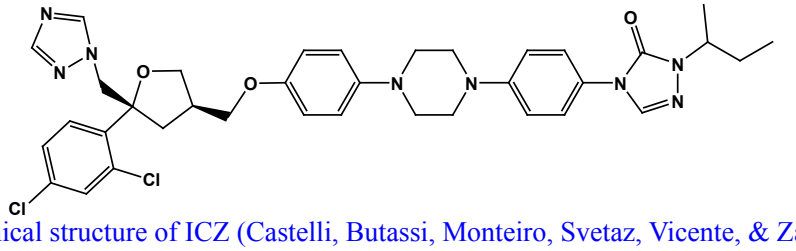

Figure 3: Chemical structure of ICZ (Castelli, Butassi, Monteiro, Svetaz, Vicente, & Zacchino, 2014).

1. INTRODUCTION

Fungal infections are caused by fungi. There are four main types of fungal infections. These are: *superficial fungal skin infection, superficial fungal mucosal infection, allergic fungal infection and invasive fungal infection* (Pfaller & Diekema, 2004). The term *invasive fungal infections* (IFIs) generally means systemic fungal infection, sterile body sites fungal infections or invasion of fungal infection into the organ tissue. The number of cases of IFIs keeps on increasing for the last two decades. IFIs are generally observed in many types of patients; for example, transplant recipients, AIDS patients, immune-compromised patients and patients on immune-suppressant therapy (Keating, 2005). Increasing resistance of IFIs towards certain clinically used antifungal agents has caused concern among health care sector personnel (Leung, Poulakos, & Machin, 2015).
Azole is a chemical class of antifungal agents. It is further classified into two sub-classes, namely *imidazole antifungal* and *triazole antifungal*. Examples of imidazole antifungal agents include Econazole, Ketoconazole and Miconazole. Examples of triazole antifungal agents include Fluconazole (FCZ), Isavuconazole, Voriconazole, Itraconazole (ICZ) and Posaconazole (Peyton, Gallagher, & Hashemzadeh, 2015; Aldorkee, 2017). These azole antifungals disrupt the construction and other functions of the cell wall of the fungus by inhibiting the enzyme lanosterol 14α-demethylase (Munayyer, Mann, Chau, Yarosh-Tomaine, Greene, Hare, Heimark, Palermo, Loebenberg, & McNicholas, 2004). The azole antifungals are used against many types of fungal infections, encompassing invasive fungal infections. Posaconazole, an anti-fungal agent, has been approved by the USFDA for the prophylaxis of IFIs. This review article briefly discusses the chemistry, pharmacology, reported antimicrobial spectrum, recent clinical trials, and other vital updates on Posaconazole.

2. POSACONAZOLE (PCZ)

PCZ, marketed as Noxafil, is a second generation 1,2,4-triazole antifungal agent (New Drug Application Number 22-003). It was first approved by the USFDA on September 15, 2006, as an oral suspension to Schering Corporation (New Drug Application Number 22-027). The oral tablets were approved on November 25, 2013, to Merck Sharp and Dohme Corporation (New Drug Application Number 205053). The intravenous solution of PCZ was approved by the USFDA on March 13, 2014 (New Drug Application Number 205596). All the three dosage forms have been approved for the prophylaxis of the invasive *Candida* and *Aspergillus* infections and also for oropharyngeal candidiasis, comprising oropharyngeal candidiasis that is unmanageable to ICZ and/or FCZ (Moore, Healy, & Kraft, 2015). The details of the USFDA approved dosage forms of PCZ are given in Table 1.

3. CHEMISTRY OF PCZ

PCZ was first patented in the United States as Patent Number 5661151 (Saksena, Girijavallabhan, Lovey, Pike, Wang, Liu, Ganguly, & Bennett, 1997). During the development phase, it was coded as SCH-56592 (Nomeir, Pramanik, Heimark, Bennett, Veals, Bartner, Hilbert, Saksena, McNamara, Girijavallabhan, Ganguly, Lovey, Pike, Wang, Liu, Kumari, Korfmacher, Lin, Cacciapuoti, Loebenberg, Hare, Miller, & Pickett, 2008; Nomeir, Kumari, Hilbert, Gupta, Loebenberg, Cacciapuoti, Hare, Miller, Lin, & Cayen, 2000; Oakley, Moore, & Denning, 1997). The chemical framework of

Table 1: USFDA approved dosage forms of the PCZ.

S. No.	Brand Name	Active Ingredient	Concentration	Approved Dosage Form (Route)	Status
1	Noxafil	Posaconazole	40 mg/ml	Suspension (Oral)	Prescription
2	Noxafil	Posaconazole	100 mg	Delayed Release Tablet (Oral)	Prescription
3	Noxafil	Posaconazole	300 mg / 16.7 mL (18 mg / mL)	Solution (IV)	Prescription

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بحث مرجعي

عقار البوساكونازول: مقالة مرجعية

محمد عمران[1*]، نيرة نعيم[1]، عبدالحكيم بوادقجي[2]

(قدم للنشر في 1440/04/16 هـ؛ وقبل للنشر في 1440/07/07 هـ)

ملخص: تمت الموافقة من قبل هيئة الغذاء والدواء الأمريكية لاعتماد عقار البوساكونازول (نوكسافيل) الذي يمثل عامل مضاد للفطريات من الجيل الثاني (1،2،4-تريازول) للوقاية بشكل عام من العدوى الناتجة من (*Candida sp.*) و(*Aspergillus* sp.) وكذلك العدوى الفموية الناتجة من (*Candida sp.*) المقاومة لعقار إيتراكونازول أو فلوكونازول. حيث يتم تسويق هذا الدواء المحب للدهون كمعلق أو كحبوب -تطلق المادة الفعالة بشكل متدرج أو كحقن، وقد تم نشر العديد من النتائج الصيدلانية الجديدة فيما يتعلق بهذا الدواء الأولي الجديد من حيث أشكال الجرعة والجزيئات النانوية والتعدد الشكلي والتجارب السريرية للبوساكونازول. يناقش هذا البحث المرجعية باختصار الأشكال المختلفة للجرعة المسوقة، وكيمياء التأثير، والتأثير الصيدلاني، ونطاق النشاط، والتركيبات الجديدة، وبراءات الاختراعات الهامة السابقة، والتجارب السريرية قيد التنفيذ المتعلقة بالبوساكونازول. وقد وجدنا أنه حتى تاريخ هذه الورقة العلمية المرجعية، لا يوجد ما هو منشور يتعلق بالجوانب الصيدلانية للبوساكونازول. وستكون هذه الدراسة مفيدة للصيادلة والأطباء والمهنيين شبه الطبيين، ليكون لديهم فكرة تتعلق بعقار البوساكونازول.

كلمات مفتاحية: بوساكونازول، المركبات الحلقية من الآزولات، مضاد فطري، علم العقاقير، براءات الاختراع.

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Review Article

Posaconazole: A Pharmaceutical Review

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(Received 25/12/2018; accepted 14/03/2019)

Abstract: Posaconazole (Noxafil), a second generation 1,2,4-triazole antifungal antibiotic, has been approved by the USFDA for the prophylaxis of invasive *Candida* spp. and *Aspergillus* spp. infections and for oropharyngeal candidiasis, comprising oropharyngeal candidiasis that is unmanageable to Itraconazole and/or Fluconazole. This lipophilic drug is marketed as a suspension, a sustained release tablet, and as an injection. Many new pharmaceutical findings have been published regarding its prodrug, dosage forms, nano-particles, polymorphs and clinical trials. This review article briefly discusses the various marketed dosage forms, chemistry, pharmacology, spectrum of activity, new compositions, important patent literature and ongoing clinical trials related to Posaconazole. Till the date of this manuscript, there is no published review article that provides information about the pharmaceutical aspects of Posaconazole. This article will be useful for pharmacists, medical and paramedical professionals seeking to know better about the pharmaceutical aspects of Posaconazole.

Keywords: Posaconazole, Azole, Antifungal, Chemistry, Pharmacology, Patents.

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Mamourian, M. Shirvana, K.M., & Pop, I. (2016). Sensitivity analysis for MHD effects and inclination angles on natural convection heat transfer and entropy generation of Al_2O_3-water nanofluid in square cavity by response surface methodology. *International Communication in Heat and Mass Transfer*, *79*, 46–57. doi: 10.1016/j.icheatmasstransfer.2016.10.001.

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- Entropy production is minimized for α = 90° and maximized for a magnetic field inclination α = 60°. Thus, with the aim of minimizing the entropy generation, the latter inclination must be avoided.

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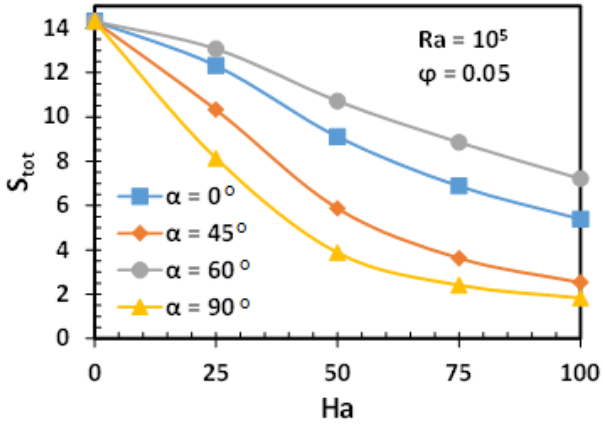

Figure 11: Total generated entropy versus Hartmann number for Ra=10^5, φ=5% and various inclination.

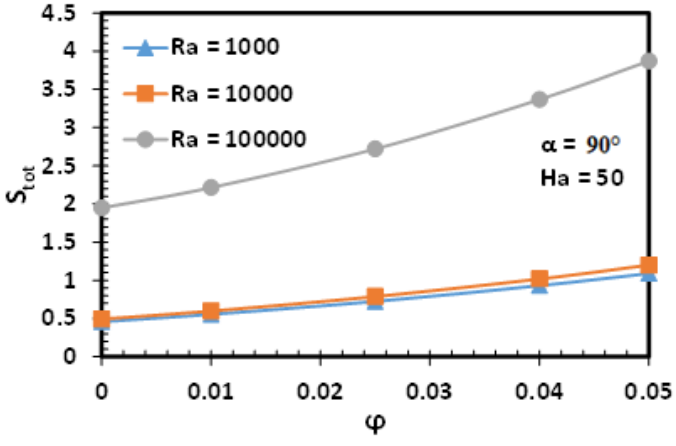

Figure12: Total generated entropy versus nanoparticles volume fraction for Ha=50, α = 90° and various Rayleigh numbers

5. CONCLUSIONS

In this investigation, a 3D numerical study was carried out to study the behaviour of the different kinds of entropy generations caused by magnetohydrodynamic free convection over an open sided trapezoidal domain filled with a suspension of carbon nanotubes in water. The cavity has a hot wall in the left side and a cold entrance from the open boundary in the right side. This computational analysis was achieved for the above mentioned parameters and the main conclusions are:

- Adding nanoparticles to the pure fluid leads to an increase in the total created entropy with or without a magnetic field application for different Hartmann number values.
- Regardless of the values of the magnetic field inclination angles and Hartmann number, the total created entropy is found to be increased by increasing Rayleigh number.
- Viscous dissipations are dominated by thermal irreversibilities which control the total entropy creation.
- Applying an external magnetic force causes convective heat transfer suppression and a reduction in the total entropy production rate.

Figure 11 illustrates the generated entropy variation along with Hartmann number at various inclination angle of the magnetic force, Ra=10^5 and φ= 0.05. As shown from the figure, the rate of produced entropy is reduced due to an increase in the Hartmann number regardless of the magnetic field inclination. Certainly, the application of a uniform magnetic field minimizes the irreversibility production by slowing down the nanofluid motion. It should also be mentioned that, as expected, the maximum of entropy generation occurs with an inclination α=60°; whereas, its minimum occurs with α=90°. Figure 12 displays the effects of the Rayleigh number as well as nanoparticles concentration on the created entropy for an inclination angle α=90° and Ha=50. For a particular value of the Hartman number and inclination angle, the entropy production increases with the increase of nanoparticle volume fraction irrespective of the Rayleigh number. Nevertheless, the effect of nanoparticle concentration is significant at Ra=10^5 but it is almost imperceptible at low Raleigh number values.

Furthermore, entropy generation shows a growing trend with the an increase in Ra values for water and CNT-water nanofluid.

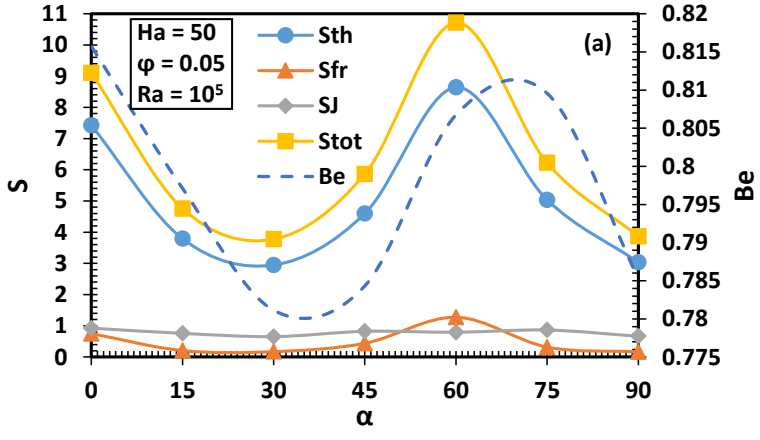

Figure 9: Generated entropies and Bejan number versus inclination angle for Ha=50, Ra=10^5 and φ=5%.

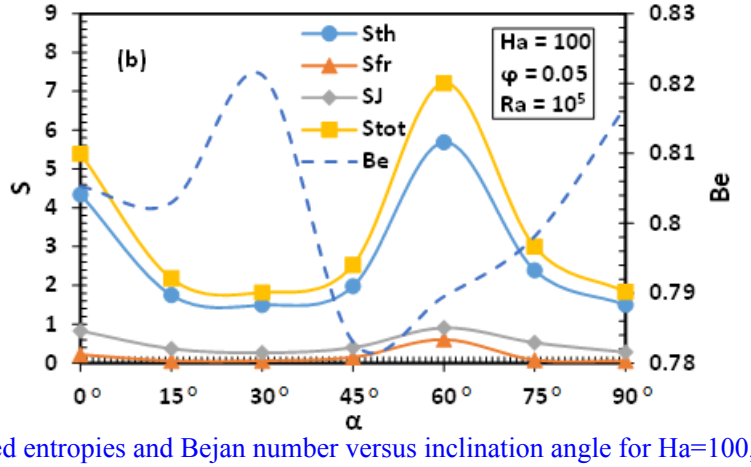

Figure 10: Generated entropies and Bejan number versus inclination angle for Ha=100, Ra=10^5 and φ=5%.

Furthermore, with the aim of minimizing the entropy generation, the application of a magnetic field having an inclination of α = 30° or α = 90° may be a good solution. The Bejan number is found to be ranging from 0.78 to 0.82 and ensures that heat transfer induced irreversibilities predominated fluid friction irreversibilities.

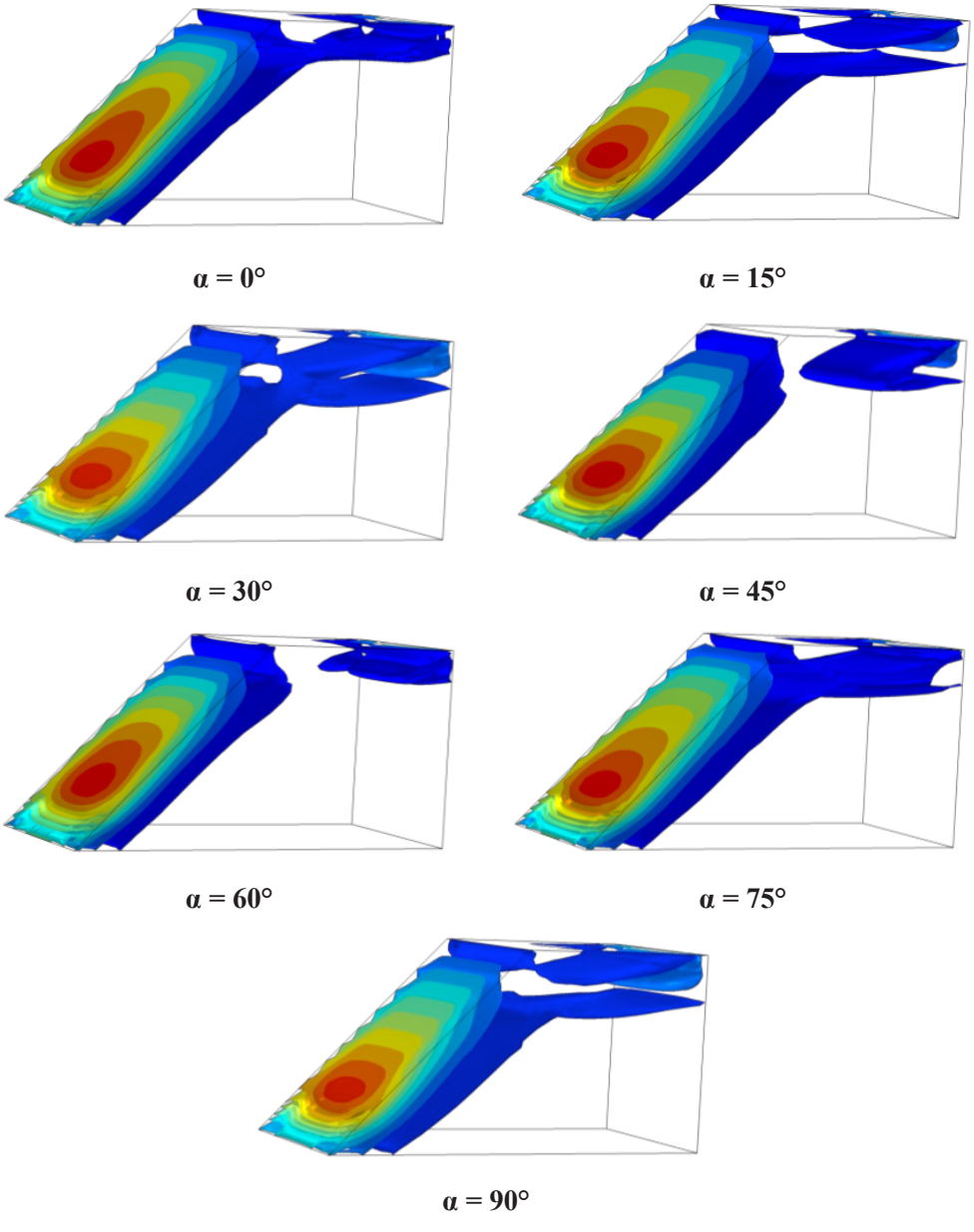

Figure 8: Iso-surfaces of total generated entropy for Ha=25, Ra=10^5, φ=5% and various inclinations.

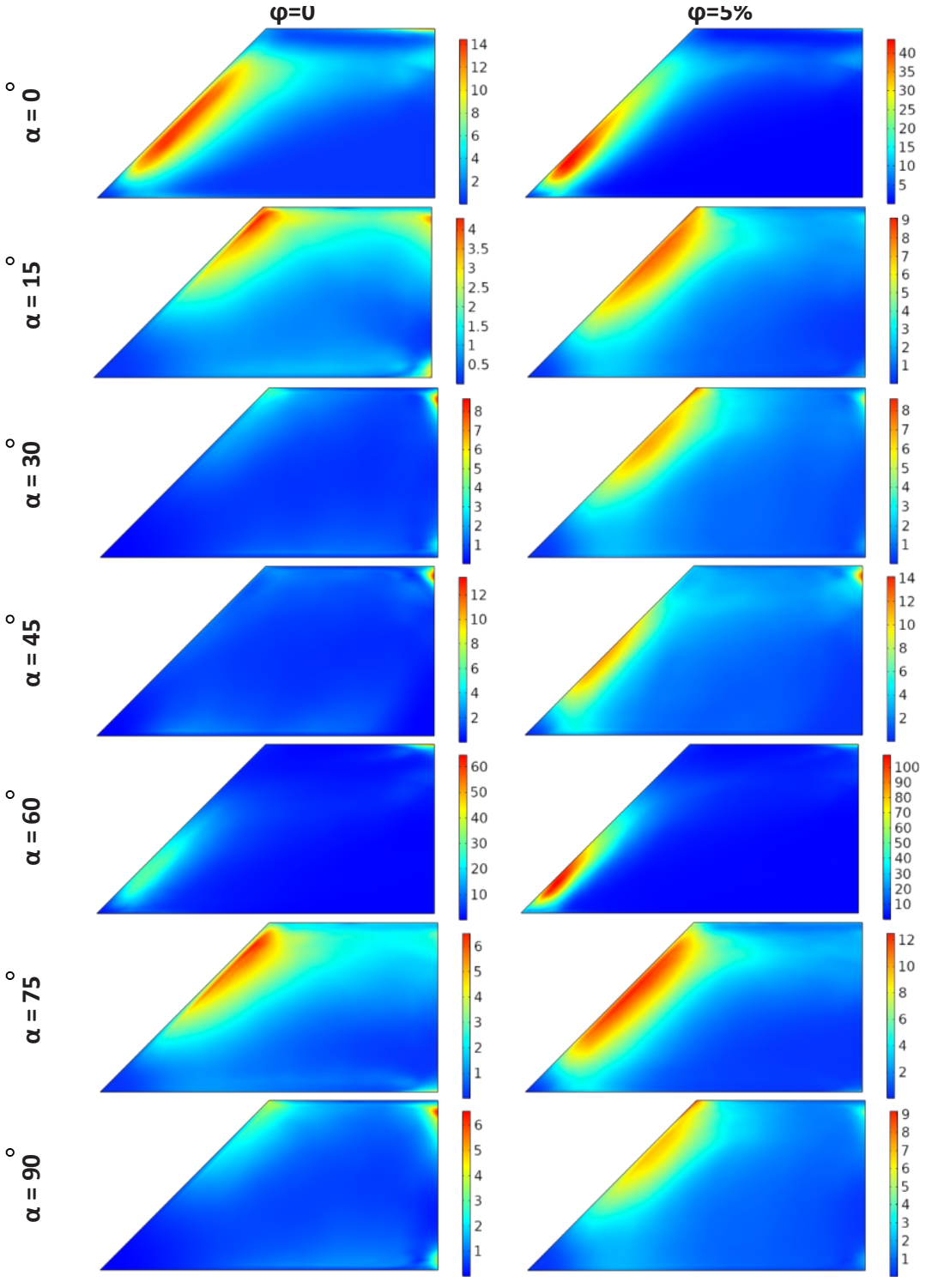

Figure 7: Patterns of total entropy contours at the central XY-plan for Ha=100, Ra=10^5 and various inclination.

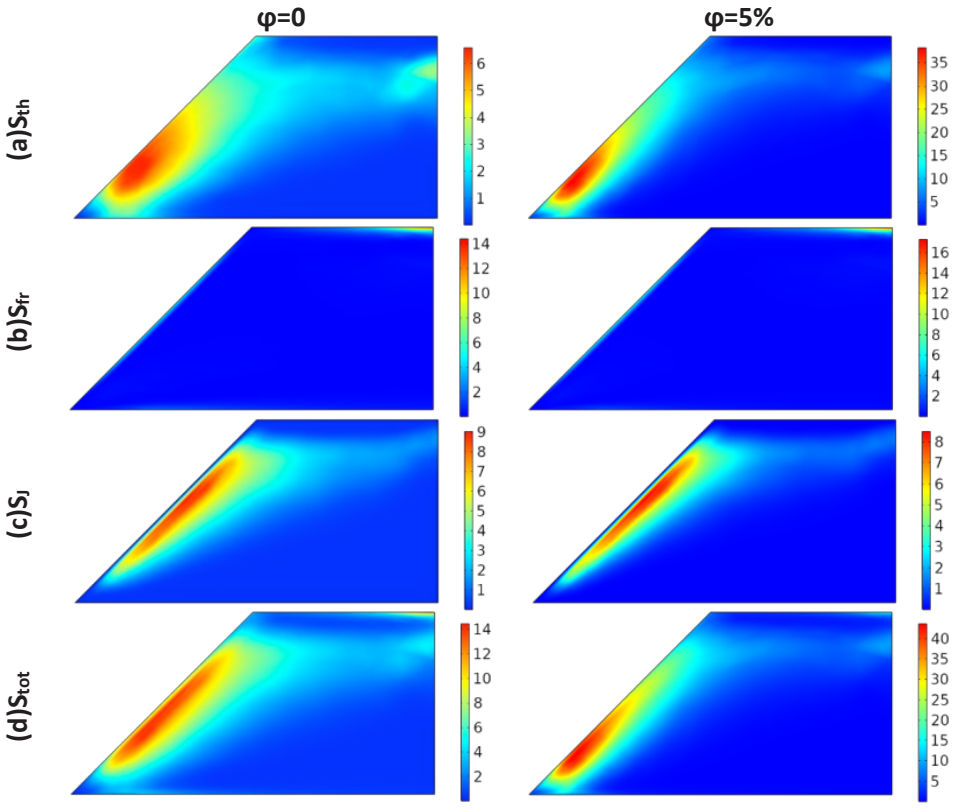

Figure 6: Patterns of entropy contours at the central XY-plan plan for Ha=100, α=0° and Ra=10^5, (a)Thermal; (b)Viscous; (c); Joule effect (d) Total and (e) Bejan

analyze the 3D aspect of the produced entropy, its iso-surfaces are drawn for different magnetic field inclinations (Ha=25) for φ=0.05 and Ra=10^5 (figure 8). As it can be observed, the highest value of local irreversibility production is considerably greater near the central region of the hot wall irrespective of the magnetic force inclination. The fluid flow over the cavity is composed of the motion of cold nanofluid entering from the lower part of the open boundary throughout the bottom side pursued by an upward stream over the inclined hot side which comes back again through the top wall to the open boundary yielding place to the cold nanofluid for further cooling of the enclosure. Due to larger velocity gradients induced by the cooling of the heated side, the total entropy generated is concentrated utmost in the central portions of the left heated side followed by the upper part of the cavity.

The variation of the generated entropy and the Bejan number with the magnetic field inclination while Ra=10^5 and φ= 0.05 are shown in figures 9 and 10 for Ha=50 and Ha=100 respectively. It should be mentioned that in both cases, the maximum values of the total entropy generated occur with an inclination angle α = 60° followed by α = 0°. Moreover, the entropy generation is mostly controlled and predominated by heat transfer irreversibilities with weak contributions of Joule effects and fluid friction.

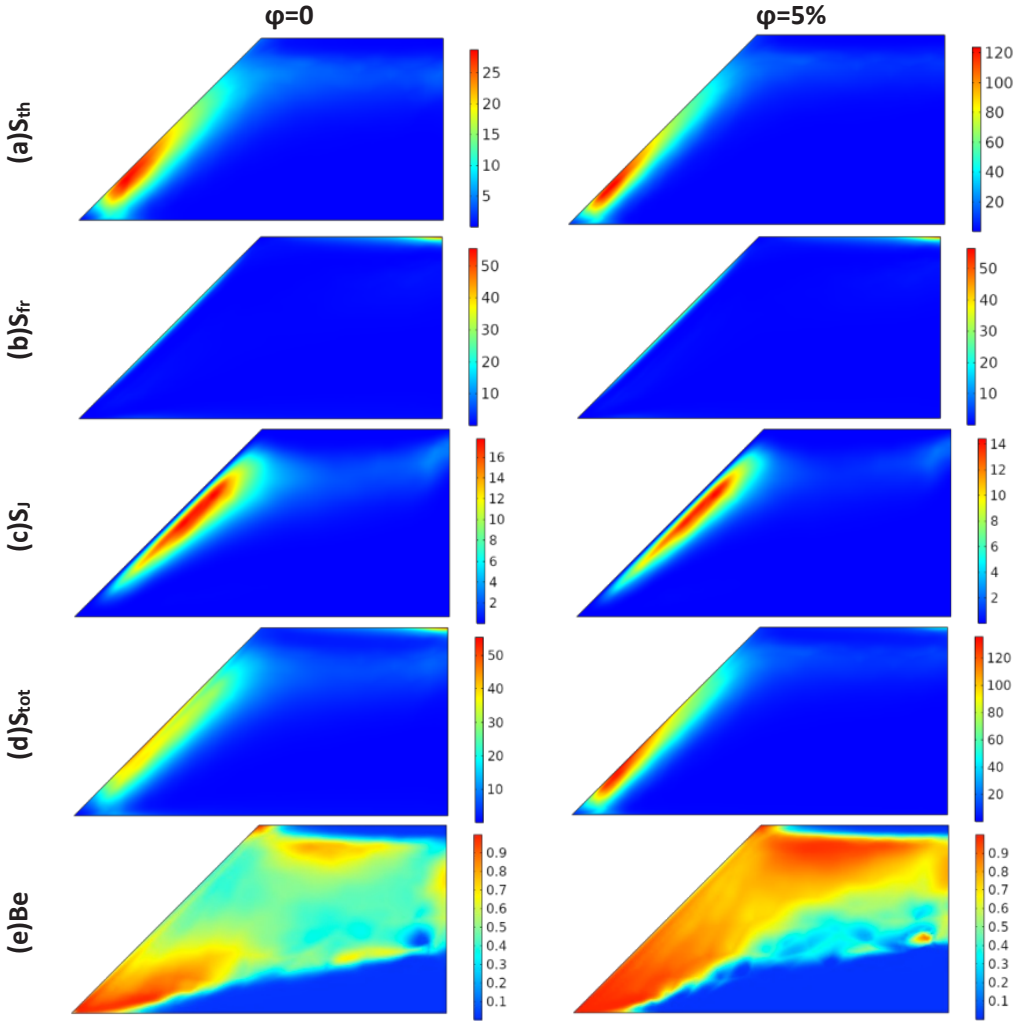

Figure 5: Patterns of entropy contours and Bejan number at the central XY-plan for Ha=50, α=0° and Ra=10^5, (a)Thermal; (b)Viscous; (c); Joule effect (d) Total and (e) Bejan

For a better description of the magnetic force influence on the produced entropy, the patterns of total entropy contours at the midplane z= 0.5 for different magnetic force inclinations at Ra = 10^5 (Ha = 100) are drawn in figure 7 for pure water (φ = 0) and CNT-water nanofluid (φ = 0.05). Obviously, the increase in nanoparticles' concentration for Ha=100 is accompanied by an increase in total produced entropy regardless of the angle of inclination. However, the highest value of generated entropy takes place close to the lower part of the inclined heated side for an inclination angle α = 60°; whereas, its minimum occurs for inclinations α = 15°, α = 30°and α = 90°. Therefore, the enhancement of natural convection heat transfer rate inside the cavity can be achieved by avoiding an inclination angle α = 60° for which the generated irreversibilities are maximized. As a means to

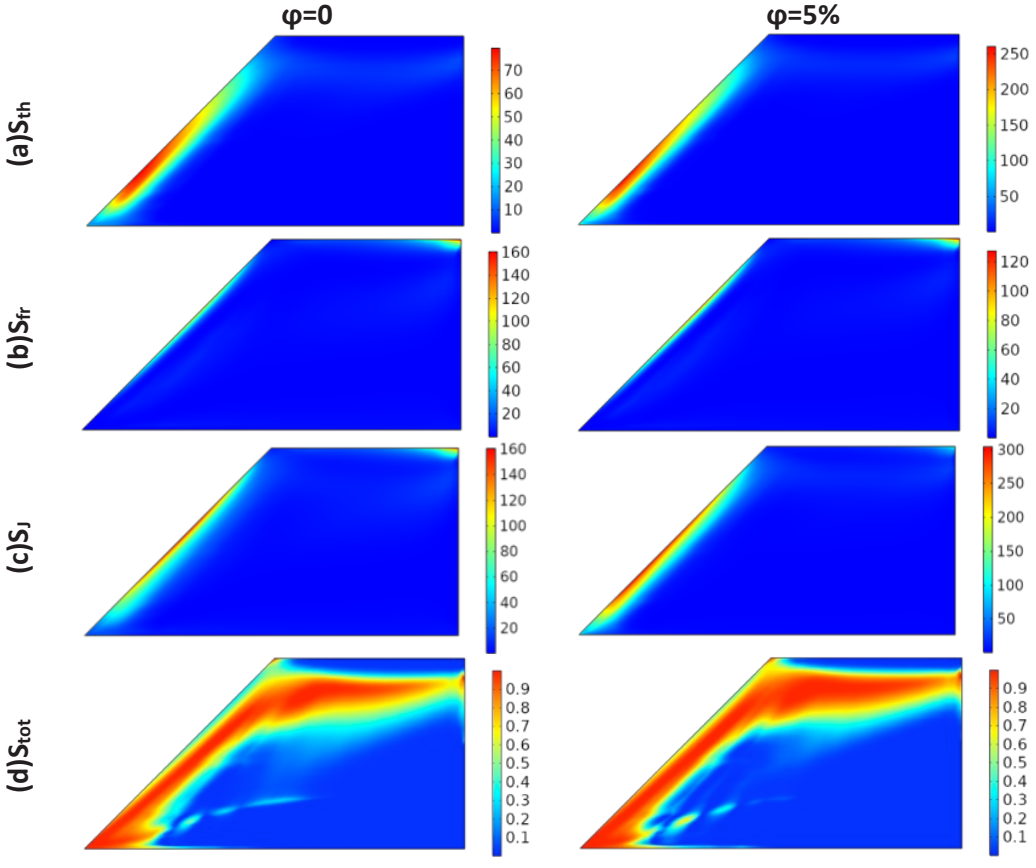

Figure 4: Patterns of entropy contours and Bejan number at the central XY-plan for Ha=0 and Ra = 10^5, (a) Thermal; (b)Viscous (c) total and (d) Bejan number

As discussed above, the distribution of locally generated entropy is mainly located close to the active inclined wall and slightly in the upper part of the cavity leading to the discharging area where the rising heated nanofluid leaves giving way to better cooling of the heated side by the entering of cold nanofluid in the down part charging area. However, when we observe the scale of entropy magnitude in figure 6 and compare it with that of figure 5, we notice that the increase of the Hartman number (from 50 to 100) leads to a decrease in the total generated entropy. This finding is reasonable since the imposed magnetic force reduces the produced entropy via natural convection by slowing down the nanofluid motion which implies that the external magnetic field is opposing the boundary layer phenomenon met for the great Rayleigh number. Moreover, it can be noticed that both the produced entropy due to the Joule effects and fluid friction remain s almost constant with the existence of the magnetic force. Furthermore, it is to be mentioned that in spite of the increase in fluid viscosity as a consequence of augmenting nanoparticles concentration, the contribution of viscous dissipation irreversibilities in the rate of the total produced entropy remains very weak. Indeed, the total entropy generation is mostly controlled by thermal irreversibility leading to a Bejan number approaching unity in all cases of the present study.

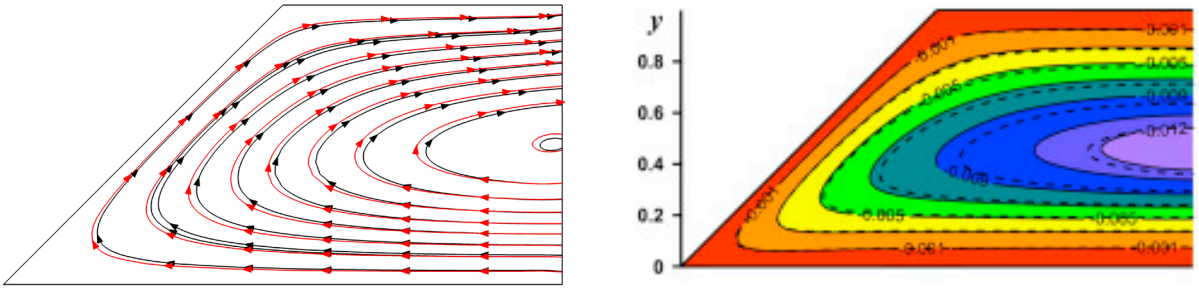

Figure 3: Comparison with the 2D results of Miroshnichenko et al. (2016) for Ra= 104, Ha = 50 and α = 0: φ = 0.04 (red), φ = 0 (black).

4. GRID INDEPENDENCE STUDY

The grid independence study was performed for φ=0.05 and Ha = 50 (α=0). The total entropy generation was chosen as a sensitive parameter. As presented in Table 2, four grids were tested (E1→E4). The increase in the total entropy generation between grids E3 and E4 was only 0.243%. The spatial mesh E3 (209534) was retained to combine result accuracy and time economy.

Table 2: Grid sensitivity analysis for Pr=6.2, Ra=105, φ=0.05, Ha=100 and LB=0.5

	Elements	S_{tot}	Increase (%)
E1	34871	8.8305	-
E2	104495	9.0121	2.056509
E3	209534	9.10232	1.001099
E4	590377	9.1245	0.243674

5. RESULTS AND DISCUSSION

For natural convection, thermal gradients and viscous effect irreversibilities are the main sources of entropy production. To show the pure effect of increased nanoparticles concentration on the entropy generation, its contours are presented at the z-central plan for φ=0 and φ=5% without applying the magnetic field as shown in figure (4). As it can be seen, increased nanoparticle concentration in water is accompanied by an increase in entropy generation.

Furthermore, as a result of higher temperature gradients, the produced entropy is mainly located close to the hot inclined surface and the upper part where the heated nanofluid is leaving giving way to the cold nanofluid to further cool the cavity. As the Bejan number is near unity in these regions, irreversibility is mainly provoked by temperature gradients. To introduce the combined effects of applying magnetic forces and nanoparticles addition on entropy generation, figure 5 shows the patterns of entropy contours and the Bejan number for a zero angle of magnetic dip (Ha=50) and $Ra=10^5$. A noteworthy fact is that, in the presence of a magnetic force, the increase in nanoparticle volume fraction has an unimportant influence on the produced entropy induced by fluid friction as well as the Joule effect; whereas, the thermal entropy generation increases considerably and the total generated entropy is clearly predominated by thermal irreversibility. Consequently, the Bejan number draws near the unity along a wider area of the enclosure owing to the enhancement of heat transfer rate as a result of increasing the nanoparticle volume fraction.

2.3. Numerical Methode

To solve the governing equations, the FEM based on Galerkin weighted residual method is used. Due to the existence of non-overlapping domains, the approximation of variables within these domains is done using the Lagrange finite elements.

To handle the problem of boundary layers, non-uniform elements are used with finer elements at the wall regions. The steps of the solution procedures are:

(1) the governing equations are transformed to integral forms using Galerkin method of weighted residuals;

(2) the pressure and second order derivative terms are integrated using Gauss theorem;

(3) the pressure terms are substituted by the penalty function:

$$P = -\gamma\left(\frac{\partial U_x}{\partial x} + \frac{\partial U_y}{\partial y} = \frac{\partial U_z}{\partial y}\right)$$

where γ is fixed to 10^8;

(4) the nonlinear governing equations are descritised using the FEM to obtain the algebraic equations;

(5) finally, these equations are solved via the Newton-Raphson scheme.

More details can be found in the works of Basak et al. (2007), Fu and Shieh (1988) and Reddy (1993).

3. VALIDATION OF THE CODE

To make the code validation, a results' comparison with the work of Alrashed et al. (2017b) is performed. In the latter work, the authors analyzed the influence of the magnetic field inclination on the CNT-water nanofluid flow inside a 3D closed cavity. As it can be seen from figure 2, the concordance of results is acceptable. Figure 3 presents a second comparision with a similar 2D configuration studied by Miroshnichenko et al. (2016) for $Ra=10^4$, Ha=50, and $\alpha=0$. The red streamlines correspond to $\varphi=0.04$ (red) and the black are for $\varphi=0$. It is to be mentioned that they used the Cu-O as a nanoparticle. For that, a simulation with the thermophysical properties used by this author is performed. The comparision of streamlines shows a good similarity but the results are not identical due to the 3D behavior in our case. It should also be metioned that the flow structure presented in fig. 3a is at the central plan (z=0.5)

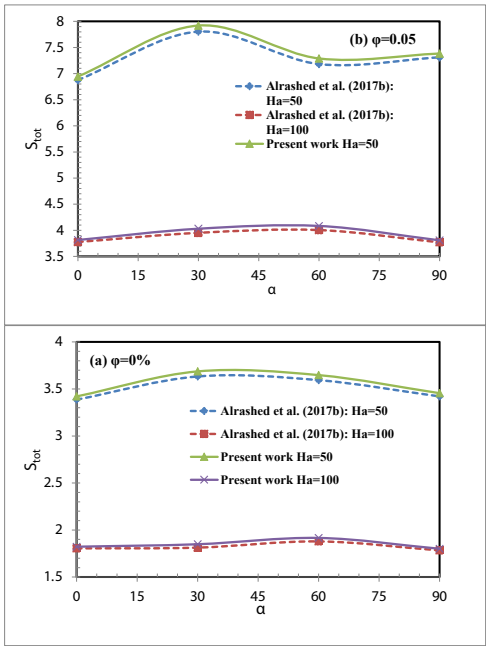

Figure 2: Comparison with 3D results of Alrashed et al. (2017b) for cubic cavity. (a) Pure water and (b) CNT-water nanofluid.

$$\sigma_{nf} = \sigma_f \left[1 + \frac{3.\varphi.\left(\frac{\sigma_s}{\sigma_f} - 1\right)}{\left(\frac{\sigma_s}{\sigma_f} + 2\right) - \varphi.\left(\frac{\sigma_s}{\sigma_f} - 1\right)} \right]. \tag{14}$$

The thermophysical properties of water and CNT nanoparticles are presented in Table 1.

Table 1: Thermophysical properties of water and CNT nanoparticles

Physical properties	Water	CNT
$C_p[kJ.kg^{-1}.K^{-1}]$	4.179	0.425
r $[kg.m^{-3}]$	997.1	2600
$k\,[W.m^{-1}.K^{-1}]$	0.613	6600
b $[1/K]$	21.10^{-5}	$1.6.10^{-6}$
$\sigma\,[\Omega^{-1}.m^{-1}]$	0.05	$4.8.10^{-7}$

2.2. Boundary Conditions

The boundary conditions relative to the studied configuration are:

$\theta = 1$ on inclined wall, (15)

$\frac{\partial \theta}{\partial n} = 0$ on adiabatic walls, (16)

$\theta_{in} = \theta c$ for $n.U < 0$ and $\left.\frac{\partial \theta}{\partial n}\right|_{out} = 0$ for $n.U \geq 0$ at the open side. (17)

$U_x = U_y = U_z = 0$ on all walls, (18)

$\frac{\partial U_x}{\partial x} = \frac{\partial U_y}{\partial x} = \frac{\partial U_z}{\partial x} = 0$ At the open right side. (19)

$\frac{\partial \phi}{\partial n} = 0$ at all walls, (20)

$\phi = 0$ at open boundary, (21)

$\vec{J}.\vec{n} = 0$ on all walls. (22)

The local dimensionless entropy generation (S_{loc}) is expressed by:

$$S_{loc} = \overbrace{\frac{k_{nf}}{k_f}.\left[\left(\frac{\partial\theta}{\partial x}\right)^2+\left(\frac{\partial\theta}{\partial y}\right)^2+\left(\frac{\partial\theta}{\partial z}\right)^2\right]}^{S_{l-th}:\ Thermal\ \text{Entropy generation}} + \underbrace{\gamma\frac{\mu_{nf}}{\mu_f}.\left\{\begin{array}{l}2.\left[\left(\frac{\partial U_x}{\partial x}\right)^2+\left(\frac{\partial U_y}{\partial y}\right)^2+\left(\frac{\partial U_z}{\partial z}\right)^2\right]+\\ \left[\left(\frac{\partial U_y}{\partial x}+\frac{\partial U_x}{\partial y}\right)^2+\left(\frac{\partial U_z}{\partial y}+\frac{\partial U_y}{\partial z}\right)^2+\left(\frac{\partial U_x}{\partial z}+\frac{\partial U_z}{\partial x}\right)^2\right]\end{array}\right\}}_{S_{l-fr}:\ Viscous\ \text{Entropy generation}} \quad (7)$$

$$+\overbrace{\gamma\frac{\mu_f}{\mu_{nf}}\frac{\sigma_{nf}}{\sigma_f}Ha^2.\left(J_x^2+J_y^2+J_z^2\right)}^{S_{l-j}:\ Joule\ \text{effect Entropy generation}}$$

Where $\gamma=\left(\frac{\alpha}{L.\Delta T}\right)^2.T_0$

The total generated entropy (S_{tot}) and Bejan number are written respectively as:

$$S_{tot}=\int_V S_{loc}dV=\int_V\left(S_{l-th}+S_{l-fr}+S_{l-j}\right)dV=S_{th}+S_{fr}+S_J \quad (8)$$

$$Be=\frac{S_{th}}{S_{th}+S_{fr}+S_J} \quad (9)$$

The nanofluid's thermophysical properties are determined according to the following expressions (Al-Rashed et al. 2017b):

- Effective density:

$$\rho_{nf}=\phi\rho_s+(1-\phi)\rho_f. \quad (10)$$

- Heat capacitance:

$$(\rho C_p)_{nf}=(1-\varphi)(\rho C_p)_f+\varphi(\rho C_p)_s. \quad (11)$$

- Effective thermal conductivity:

$$\frac{\lambda_{nf}}{\lambda_f}=\frac{1-\phi+2\phi\frac{\lambda_s}{\lambda_s-\lambda_f}\ln\left(\frac{\lambda_s+\lambda_f}{2\lambda_f}\right)}{1-\phi+2\phi\frac{\lambda_f}{\lambda_s-\lambda_f}\ln\left(\frac{\lambda_s+\lambda_f}{2\lambda_f}\right)}. \quad (12)$$

- Effective dynamic viscosity:

$$\mu_{nf}=\frac{\mu_f}{(1-\varphi)^{2.5}}. \quad (13)$$

$$t=\frac{t'}{L'^2/\alpha};\ (x,y,z)=\frac{(x,y,z)'}{L'};\quad (U_x,U_y,V_z)=\frac{(U_x,U_y,U_z)'}{\alpha_f/L'};\ P=\frac{P'}{\rho_f(\alpha_f/L')};\ \Phi=\frac{\Phi'}{L'.\upsilon_0.B_0};$$

$$\vec{B}=\frac{\vec{B}'}{B_0};\ \vec{J}=\frac{\vec{J}'}{\sigma.\upsilon_0.B_0}\ \text{and}\ \theta=(\theta'-\theta_c')/(\theta_h'-\theta_c').$$

$$\nabla.\vec{U}=0 \tag{1}$$

$$\frac{\partial\vec{V}}{\partial t}+(\vec{U}.\vec{\nabla})\vec{U}=-\vec{\nabla}P+\frac{\sigma_{nf}\rho_f}{\sigma_n\rho_{nf}}Ha^2\,\mathrm{Pr}(\vec{J}\times\vec{B})+\mathrm{Pr}\left(\frac{\nu_{nf}}{\nu_f}\right)\Delta\vec{U}+\left(\frac{\beta_{nf}}{\beta_f}\right)Ra\,\mathrm{Pr}\,\vec{T}_g \tag{2}$$

$$\frac{\partial\theta}{\partial t}+\vec{U}.\nabla T=\frac{\alpha_{nf}}{\alpha_f}\nabla^2\theta \tag{3}$$

$$\vec{j}=-\vec{\nabla}\Phi+\vec{U}\times\vec{e}_B \tag{4}$$

$$\vec{\nabla}^2\Phi=\vec{\nabla}.(\vec{U}\times\vec{B}) \tag{5}$$

with

$$\mathrm{Pr}=\frac{\nu_f}{\alpha_f},\ Ra=\frac{g.\beta_f.\Delta T.L^3}{\nu_f.\alpha_f}\ \text{and}\ Ha=B_0L\sqrt{\frac{\sigma}{\rho_f\nu_f}}. \tag{6}$$

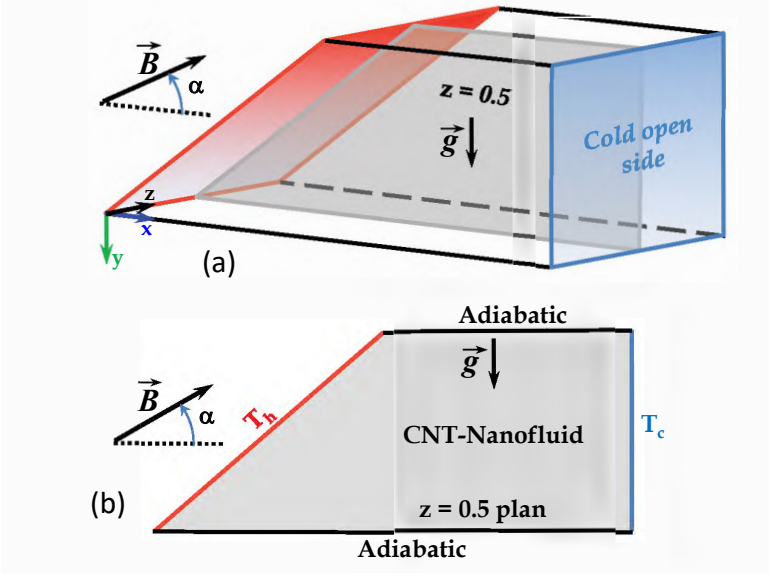

Figure 1: a) Studied configuration with coordinates; b) central XY-plane.

Ismael, & Chamkha, 2017; Al-Mudhaf & Chamkha, 2004; Sathiyamoorthy & Chamkha, 2010; Sarris, Kakarantzas, Grecos, & Vlachos, 2005; Sheikholeslami et al., 2016 and Mehryan, Kashkooli, Soltani, & Raahemifar, 2016). In recent years, a great deal of research has been undertaken on the MHD control of generated entropy during nanofluids convection within two-dimensional confined spaces for the optimization of available energy use. Chamkha, Ismael, Kasaeipoor, & Armaghani, (2016) considered the MHD convection in a C-shaped enclosure containing CuO/water nanofluid. They concluded that Lorentz forces behave like an attenuator of both the natural convection effects and irreversibility production and that this attenuation can be optimized via the addition of nanoparticles to the special case of the magnetic field's magnitude (Ha=30).

The horizontal magnetic field's effect on non-Newtonian-nanofluid convection was investigated by Kefayati (2016). The author concluded that the Hartman number was very effective on all types of entropy generations. Moreover, entropy generations were found to be reduced with the increase in the Hartman number. The mutual effects of the presence of the magnetic force and internal heat generation on the MHD convection of the nanofluid were carried out by Selimefendigil and Oztop (2016). For low Ra values, irreversibility production was slightly affected by the Hartman number; this effect became more important for higher Ra values.

Selimefendigil and Oztop, (2015) studied numerically the magnetohydrodynamic natural convection in 2D trapezoidal shaped cavities containing nanofluids. They concluded that the entropy generation rate diminishes considerably due to the externally applied magnetic field.

Mamourian, Shirvana, & Pop, (2016) studied numerically the entropy production in an inclined cavity that contained oxide aluminum/water-nanofluid with a focus on the MHD effects. They concluded that the combination of the Hartman number and inclination can minimize the irreversibility production.

The magnetic influence on entropy production due to Cu-water nanofluid natural convection in a wavy cavity was studied by Cho (2016). Obtained results show that the irreversibility production rate is more affected by the magnetic forces of high Ra values. It should be noted that an excellent review paper on entropy production due to free convective flows in confined and semi-confined spaces (with examples) is Biswal and Basak (2017).

However, the magnetic control of entropy production due to 3D convection in an open cavity filled with nanofluid has attracted only limited attention to date despite the interest that it represents in the minimization of irreversibility production. Among these studies, Kolsi et al. (2010) investigated numerically the effect of a horizontal magnetic field on entropy production of mercury contained inside a 3D enclosure. It has been observed that due to the generated Lorentz forces entropy is minimized and behaves similarly to a 2D configuration. The present work aims to study the magnetic control of the entropy generated due to 3D natural convection in a trapezoidal shaped cavity with a fully opened right side and containing a Carbone Nanotubes-water nanofluid.

2. PROBLEM FORMULATION

As presented in Fig.1, the physical domain consists of a 3D trapezoidal shape enclosure with an opened right side and heated inclined wall while the other sides are assumed to be insulated.

The magnetohydrodynamic convection is induced by an inclined external uniform magnetic field. The gravitational force is imposed in the positive y-direction while buoyancy induced fluid motion is considered unsteady, laminar and incompressible flow. Properties of the CNT-water/nanofluid are considered to be constant and a Boussinesq approximation is considered. More details on the mathematical formulation can be found in the work of Al-Sayegh (2018)

The parameters used to write the dimensionless governing equations (1-5) are:

NOMENCLATURE

$\vec{B}$ Magnetic field ($=\vec{B}'/B_0$)
Be Bejan number
C_p Specific heat at constant pressure (J/kg. K)
$\vec{E}$ Dimensionless electric field
$\vec{e}_B$ Direction of magnetic field
g Gravitational acceleration (m/s^2)
$\vec{J}$ Dimensionless density of electrical current
λ Thermal conductivity (W/m.K)
n unit vector normal to the wall.
Nu Local Nusselt number
Pr Prandtl number
Ra Rayleigh number
S'_{tot} Generated entropy (kJ/kg.K)
t Dimensionless time ($t'.a/l^2$)
$\vec{U}$ Dimensionless velocity vector ($\vec{U}'.l/a$)
x, y, z Dimensionless coordinates (x'/l , y'/l , z'/l)

Greek symbols

α Thermal diffusivity (m^2 /s)
β Thermal expansion coefficient (1/K)
ρ Density (kg/m^3)
μ Dynamic viscosity (kg/m.s)
ν Kinematic viscosity (m^2/s)
θ Dimensionless temperature $[(\theta'-\theta'_c)/(\theta'_h-\theta'_c)]$
θ'_c Cold temperature (K)
θ'_h Hot temperature (K)
φ Nanoparticles volume fraction
γ Penalty parameter
σ Electrical conductivity

Subscripts

av	Average
f	Fluid
fr	Friction
j	Joule effect
nf	Nanofluid
s	solid (nanoparticle)
x, y, z	Cartesian coordinates

Superscript

'	Dimensional variable

1. INTRODUCTION

Entropy production caused by natural convection is very relevant in many applications dealing with the competent management of irreversibility production. Furthermore, thermal energy system conception needs more and more strenuous efforts to allow the achievement of high level heat generation while reducing the available surface area for heat removal. Therefore, by dint of their highly enhanced properties and increased heat transfer coefficient, the use of liquid nanofluids coolant can be an alternative to accomplish this challenge. Nevertheless, heat transfer processes are usually followed by thermodynamic irreversibility or entropy generation. Thus, in thermal system design, substantial considerations that lead to the minimization of entropy generation should be taken into account in order to reduce irreversibilities and benefit from the available energy.

Several studies have investigated the natural convection in confined spaces (Karatas & Derbentli, 2017; Hong, 2008; Aich, Kolsi, Borjini, Al-Rashed, ben-Aissia, Oztop, & Abu-Hamdeh, 2018; Yejjer, Kolsi, Aich, Al-Rashed, Borjini, & Ben Aissia, 2017; Öztop, Abu-Nada, Varol, & Chamkha, 2011 and Aich, Kolsi,Aydi, Al-Rashed, Messaoudene, & Borjini, 2017). All these studies have dealt with conventional pure fluids. In this last decade more interest has been given to nanofluids. Al-Rashed, Kolsi,Kalidasan, Malekshah, Kanna, & Borjini, (2017a) investigated the entropy generation of carbon nanotube/water nanofluid convection with a focus on the effect of an inserted conductive Ahmed body. The entropy generation is increased proportionally with the Raleigh number; also, the inclination is found to be an effective parameter on irreversibility control.

However, the beneficial feature of natural convection is considerably affected by applying a magnetic field that produces a Lorentz force opposing the buoyancy force and reducing the heat transfer rate (Mehryan, Ghalambaz,

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التحكم المغناطيسي في إنتاج اللاانعكاسية عبر الحمل الحراري النانوي داخل تجويف شبه منحرف ثلاثي الأبعاد

رجب بن محمد الصائغ[1*]

(قدم للنشر في 1440/09/03 هـ؛ وقبل للنشر في 1441/01/06 هـ)

ملخص: تتناول الدراسة الحالية تحليلًا للتحكم في توالد الإنتروبي عبر المجال المغناطيسي داخل فضاء شبه منحرف ثلاثي الأبعاد مملوء بمائع نانوي. يتم تسخين الجانب الأيسر المائل ويتم فتح الجانب الأيمن بالكامل للسماح بدخول السائل النانوي البارد بينما يفترض أن الجوانب الأخرى معزولة تمامًا. استنادًا إلى طريقة العناصر المنتهية، تم إجراء هذا الاستقصاء حسب المتغيرات التالية: عدد رايلي ($10^3 \leq Ra \leq 10^5$) ، زاوية ميلان المجال المغناطيسي التي تتراوح بين (0° - 90°)، تركيز المائع النانوي بين (0 - 0.05) ورقم هارتمان ($0 \leq Ha \leq 100$). أثبتت النتائج أن زيادة تركيز الجسيمات النانوية يرافقه زيادة في إجمالي الإنتروبيا المنتجة سواء مع أو بدون تسليط الحقل المغناطيسي. كما تبين أنه بالنسبة لجميع زوايا الميل وجميع قيم رقم هارتمان، فإن زيادة قيمة عدد رايلي تؤدي إلى زيادة إجمالي الإنتروبيا المنتجة والتي تهيمن عليها اللاإنعكاسية الناتجة عن التبادل الحراري بشكل أساسي في حين أن تأثير تبديد اللزوجة يمكن إهماله. إضَافَةً إلى ذَلِك، فإن استخدام الحقل المغناطيسي الخارجي يؤدي إلى إخماد الانتقال الحراري ويقلل من إجمالي معدل إنتاج الإنتروبيا. كما أن الحد الأدنى من إنتاج اللاانعكاسية يحدث عند درجة ميلان 90° بينما يكون الحد الأقصى عند 60°. هذا و يمكن استخدام نتائج هذه الدراسة لتقليل إنتاج الإنتروبيا في أنظمة الطاقة الحرارية حيث يجب تجنب زاوية ميلان 60° في حين أن زاوية الميلان المثلى للحقل المغناطيسي هي 90°.

كلمات مفتاحية: أنابيب الكربون النانوية ، توالد الإنتروبي، الحمل الحراري الحر، هيدروديناميكية الحقل المغنطيسي، فضاء شبه منحرف مفتوح، ثلاثي الأبعاد.

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Magnetic Control of Irreversibility Production Due to Nanofluid Free Convection in a 3D Open Trapezoidal Cavity

Rajab Al-Sayegh[1*]

(Received 08/05/2019; accepted 05/09/2019)

Abstract: This study analyzes how entropy production can be controlled via a magnetic field in a 3D open trapezoidal enclosure filled with nanofluid. The inclined left side is heated and the right one is fully opened to allow the entrance of cold nanofluid while the other sides are assumed to be perfectly insulated. Based on the finite element method, the investigation was carried out according to the following variables: Rayleigh number ($10^3 \leq Ra \leq 10^5$), magnetic field inclination angle in the range ($0° \leq \alpha \leq 90°$), nanofluid concentration ($0 \leq \varphi \leq 0.05$) and Hartmann number ($0 \leq Ha \leq 100$). The results indicated that the increase in the nanoparticles-concentration increases the total produced entropy with and without the application of the magnetic field. It was also found that for all inclination angles at all Ha values, the increase in Ra values resulted in an increase in total produced entropy, which is mainly predominated and controlled by heat transfer irreversibility while viscous dissipation had negligible effects. Furthermore, the application of the external magnetic field resulted in convective heat transfer suppression and diminished the total entropy production rate. Moreover, the minimum of irreversibility production occurred for $\alpha=90°$ while its maximum occurred with a magnetic field inclination $\alpha=60°$. The results of the present study can be used to minimize entropy production in thermal energy systems. Indeed, in the current case study, an inclination angle $\alpha=60°$ must be avoided whereas the optimum inclination for the magnetic field is $\alpha=90°$.

Keywords: CNT suspension; Entropy generation control; Free convection; Magnetic field; MHD; Nanofluid, 3D Open trapezoidal enclosure.

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14. The bibliography, list of all the sources used in the process of researching, must be added in alphabetical order using the author's last name according to the APA reference style (6th edition) in 12-font size for Arabic and 9-font size for English.

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    Citation from books:

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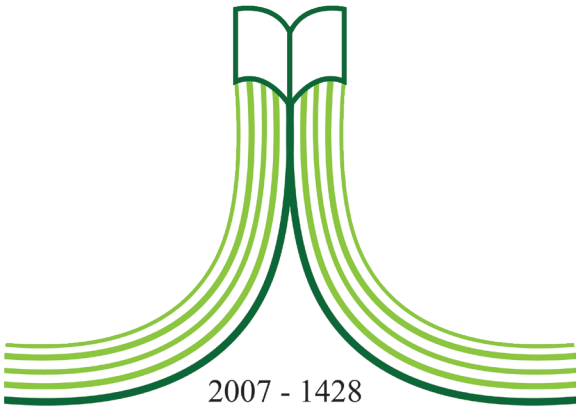

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