INTRODUCTION:-

Liver diseases and particularly hepatotoxicity are now receiving a supreme importance; due to the large population suffering of hepatic disorders all over the world. From the attempts to use the natural products to the intense use of drugs the patients are out there waiting the expected help so as to be well again. Depending on a wide research bases and a powerful huge advance in the medical field technology, hundreds of studies are made yearly for developing the art of using both the natural and the synthetic agents to enhance and enlarge the agents available to be used in such conditions. So, the current study is focusing on the use of the in-vitro method for the purpose of providing a new important tool to be added along the well documented and trusted studies to help minimizing the suffering of the patients especially that the use of that kind of studies could enable the researchers of the vast and wide screening of the new promising compounds, we thought in a new natural product to be screened. Liver toxicity could be induced in the expermintal field by usage of either chemicals such as (CCl4, Alcohol) or biologically such as the use of (LPS), and the models used could be in-vivo, in-vitro in addition to the ex-vivo.

Precision-cut liver slices (PCLS) from various species have been widely used to study the pathology of disease as well as the absorption, distribution, metabolism, elimination and toxicity (ADME-Tox) of drugs. Successful cryopreservation of PCLS would allow the creation of a

tissue bank with PCLS from various species, which would be particularly valuable for enabling the use of human PCLS because of the scarcity of human material.

Liver slices are used in various research fields, e.g. in toxicology, pharmacology, and metabolism of xenobiotics. In these studies the incubation conditions (five incubation systems) of the liver slices are essential for maintenance of the viability and function of the cells in the slice.

Silymarin is a polyphenolic flavonoid derived from milk thistle (Silybum marianum), which has anti-inflammatory, cytoprotective, and anticarcinogenic effects, that suppress the TNF-induced production of ROS and lipid peroxidation and improve the antioxidant status in blood and liver**.**

Also reports about the use of silymarine as a liver protectant agent had been documented, Worldwide, researches continue to show interest in milk thistle derivatives as a potential treatment for several diseases as evidenced by the over 12,000 related scientific publications produced on this subject within the last 10 years**.** Curcumin is a bright yellow compound found in turmeric, which is derived from the rhizomes of the plant Curcuma longa Linn, a perennial herb of the Zingerberaceae family. Curcumin In various chronic illnesses in which inflammation is known to play a major role, curcumin has been shown to exhibit therapeutic potential. The current study is focusing on the protective effect could be achieved on the stimulated PCLS by the two natural products Silymarine and Curcumin.

ABSTRACT

Hepatotoxicity is now receiving a supreme importance. LPS is the most potent activator of macrophages. The aim of this study is to demonstrate the prophylactic activity of silimarin and curcumin against LPS-induced hepatotoxicity in rats. The liver slices had been divided into 4 groups; normal, control (LPS), LPS+silimarin and LPS+curcumin (N=12). After 24 hours, analysis of NO levels, LDH activity, AST levels, ALT levels, GSH levels, TNF-α, IL-1β and IL-10 levels were carried out. . Nitric Oxide in the control group showed a high significant elevation in comparison to the normal group, while group 3 and 4 showed a high significant decrease compared with the control group. LDH activity in the control group showed a high significant elevation in comparison to the normal group, while group 3 and 4 showed a high significant decrease compared with the control group. AST and ALT in the control group showed a high significant elevation in comparison to the normal group, while group 3 and 4 showed a high significant decrease compared with the control group. In control group a significant reduction in GSH level was observed; where in group 3 and 4 a significant increase was noticed in comparison to the control group. Control group showed a high significant elevation in TNF-**α** level in comparison to normal group, where in group 3 and 4 a high significant decrease was detected in comparison to the control group. Levels of IL-1β in the control group indicated a high significant elevation compared to the normal group, while in group 3 and 4 a high significant decrease in the IL-1β levels were detected in comparison to the control group. IL-10 levels in the control group indicated a high significant elevation compared to the normal group, while in group 3 and 4 a high significant decrease in the IL-10 levels were detected in comparison to the control group. In conclusion, silimarin and curcumin showed a hepatoprotective activity against hepatotoxicity induced by LPS.

AIM OF WORK

Liver toxicity and/or dysfunction are of a high incidence, globally occurring disease, ranging from mild and simple inflammation (hepatitis) to more serious conditions, cirrhosis, fibrosis and even liver failure which could be fatal and a prophylaxis is required to prevent the progression of the deteriorated hepatic functions.

Liver disease afflicts over 10% of the world population. This include chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma, which are the most health-threatening conditions drawing considerable attention from medical professionals and scientists. Patients with alcoholism or viral hepatitis are much more likely to have liver cell damage and cirrhosis, and some may eventually develop hepatocellular carcinoma, which is unfortunately a fatal malignancy without cure. Although treatment options exist for most of the liver diseases, many types remain incurable and the emergence of drug resistance is pervasive. Thus, novel treatment approaches are essential to improve outcome. Nearly half of the agents used in liver therapy today are either natural products or derivatives of natural products. The term "natural products" is usually associated with secondary metabolites produced by an organism, which in most cases function as defense mechanisms against microorganisms, insects and competing plants. A variety of natural products, mostly from plant source, contain several active components and have been used for thousands of years by a significant fraction of the population, and are still used in healthcare in many countries or regions of the world. Natural products have generated a rich source of structurally diverse substances with a wide range of biological activities, which could be useful for the development of alternative or adjunctive therapies.

Recently, more attention has been given towards finding natural, pure, safe and novel compounds, which have the ability to modify the immune system.

The current study aims at investigation of the effect of certain natural products such as (Silymarine and Curcumin) against lipopolysaccharide induced hepatotoxicity in rats; using the rat liver slices as a model for the hepatotoxicity.

SUMMERY

liver diseases represent significant number of population all over the world, an easy research methodology should be available to enlarge the options available, but the ease of research must not be on the expense of accuracy and precision, the current study utilized a methodology in which a great results could be obtained easily in accurate and precise way, PCLS model representing a tunnel between the in vivo sophisticated methods and the in vitro methods. Finding a highly effective research methodology is of great importance so as to accelerate the rate of enhancing human life against the fast growing disease attacks.

As the present study showed; the actions of both natural agents against LPS-induced liver injury can be summarized as follows:

SILYMARIN:

-Reduced Glutathione levels

The use of LPS induced significant decreased levels of GSH in comparison to the control group by one third, while Silymarin resulted in a significant protection of that level to be only 70% of the normal.

- Tumor Necrosis Factor Alpha (TNF-α) levels

The use of LPS induced significant increase levels of TNF-α in comparison to the control group by 2 folds, but Silymarin resulted in a significant decrease to 1 fold of the control.

- Levels of Nitric Oxide (NO)

The use of LPS induced significant increase of NO levels in comparison to the control group by 3 folds of the control, while Silymarin resulted in a significant decrease to just one fold of the control.

- Lactate Dehydrogenase Enzyme Activity (LDH)

The use of LPS induced significant increase of LDH activity in comparison to the control group by 3 folds, the use of Silymarin along with same dose of LPS resulted in a significant decrease to only one fold of the control.

- Alanin Amino Transferase (ALT) levels

The use of LPS induced significant increase in of ALT levels in comparison to the control group by 4 folds, but Silymarin resulted in a significant decrease to only 2 folds of the control.

- Aspartate Amino Transferase (AST) levels

The use of LPS induced significant increase of AST levels in comparison to the control group by 3.5 times the control, while the use of Silymarin along with same dose of LPS resulted in a significant decrease to 1.6 times the control.

- Interleukin one Beta (IL-1β) levels

The use of LPS induced significant increase of IL-1β levels in comparison to the control group by 2.3 folds, but the use of Silymarin along with same dose of LPS resulted in a significant decrease to 1.7 times control.

- levels of Interleukin ten (IL-10)

The use of LPS induced significant increase of IL-10 levels in comparison to the control group by 3.5 folds, while the use of the Silymarin along with same dose of LPS resulted in a significant decrease to 2 folds.

CURCUMIN:

-Reduced Glutathione level

The use of LPS induced significant decreased level of GSH level in comparison to the control group by one third, while the use of the tested natural agent Curcumin resulted in a significant recovery up to 50% of the control levels.

- Tumor Necrosis Factor Alpha (TNF-α)

The use of LPS induced significant increase of TNF-α levels in comparison to the control group by 2 folds, while the use of the tested natural agent Curcumin resulted in a significant decrease to 1.7 times the control.

- Levels of Nitric Oxide (NO)

The use of LPS induced significant increase of NO levels in comparison to the control group by 3 folds of the control, while the use of the tested natural agent Curcumin resulted in a significant decrease to 2 folds.

- Lactate Dehydrogenase Enzyme Activity (LDH)

The use of LPS induced significant increase of LDH activity in comparison to the control group by 3 folds, while the use of the tested natural agent Curcumin resulted in a significant decrease to 2 folds.

- Alanin Amino Transferase Activity (ALT) levels

The use of LPS induced significant increase levels of ALT in comparison to the control group by 4 folds, while the use of the tested natural agent Curcumin resulted in a significant recovery by 3 folds.

- Aspartate Amino Transferase Activity (AST) levels

The use of LPS induced significant increase levels of AST in comparison to the control group by 3.5 times, while the use of the tested natural agent Curcumin resulted in a significant recovery to 2.5 folds.

- Interleukin one Beta (IL-1β) levels

The use of LPS induced significant increase of IL-1β levels in comparison to the control group by 2.3 folds, Curcumin resulted in a significant decrease to 1.7 folds.

- levels of Interlukin ten (IL-10)

The use of LPS induced significant increase IL-10 levels in comparison to the control group by 3.5 folds, while Curcumin resulted in a significant decrease by 2 folds.

conclusion

the current study proved that the effectiveness of the usage of pure natural agents in the prophylaxis against hepatotoxicity induced by LPS is of great importance. The study concluded that the effectiveness of Curcumin is of a significant importance to be used effectively as hepatic supporting agent. Further studies are required to establish whether Curcumin will be able to show an activity when it is administered after an infection established, with the possibility of mixing Curcumin to current used medication in one dosage form.

استمارة معلومات الرسائل التى تمت مناقشتها نموذج رقم (F-750-03-02)

الكليـة : الصيدلة **القسم:** الأدوية والسموم

1- الدرجة العلمية : ماجستير ☐ دكتوراه ☑

2- بيانات الرسالة:

عنوان الرسالة باللغة العربيـة **:**

تأثيرات بعض المواد الطبيعية ضد التسمم الكبدي المحدث بواسطة متعدد السكر الدهني في الجرذان

عنوان الرسالة باللغة الأجنبية:

Effects of certain natural products against lipopolysaccharide- induced hepatotoxicity in rats

التخصص الدقيق : أدوية وسموم

تاريخ المناقشة: 2015/9/6

3- بيانات الطالب:

الاسم :سامح شعبان علي جاد الجنسيـة: مصري النـوع : ذكر

العنوان : فيلا 40ج ممر رقم 1 ش صبري شريف-الاف مسكن رقم التليفون: 01207355765

جهـة العمـل:جامعة أكتوبر للعلوم الحديثة والاداب رقم الفاكس:البريد الإلكتروني:sameh.shaaban@msa.eun.eg

4- <u>المشرفون على الرسالة:</u>

الاسم	القسم	الكلية	الجامعة
1- أ. د./ **عزالدين الدنشارى** أستاذ	الأدوية و السموم	كلية الصيدلة	جامعة القاهرة

2- أ. د./ **أمانى ابراهيم البرايرى** أستاذ و رئيس قسم الأدوية و السموم كلية الصيدلة جامعة أكتوبر للعلوم الحديثة و الاداب

5- مستخلص الرسالة (Abstract)

5-1 باللغة العربية :

من المعروف أنه كلما اتسمت البحوث بالفاعلية والسهولة كلما زادت معدلات اتمام البحوث المختلفة التي من شأنها تحسين المستوي الصحي والمعيشي للانسان, في وقت تتزايد فيه الأخطارالتي تتعلق بالصحة والحياة البشرية ككل, ولكن لابد أن لا تأتي هذه الفاعلية والسهولة علي حساب الدقة ودرجة التقارب في النتائج عند إعادة ذات التجربة مرات مختلفة, وإلا تحولت السهولة والبساطة إلي تبسيط مبالغ فيه يطغي علي الهدف الاساسي المنشود كما ذكرنا من قبل.

هذه الدراسة تم عملها لإثبات امكانية استخدام الكركم كدواء فعال ومؤثر تأثيراً إيجابياً في الوقاية من التسمم الكبدي المحدث بواسطة متعدد السكر الدهني، هذا وقد أستخلص من هذه الدراسة أن استخدام الكركم ذو فائدة في منع التسمم الكبدي المحدث كيميائياً.

وبذلك من الممكن الاستفادة من نتائج هذا البحث في التطبيقات الإكلينيكية لكل من الحرشف البري والكركم وذلك في الوقاية من التسمم الكبدي والأمراض التي تصيب الكبد.

(الكلمات الدالة: الوقاية, الكركم, الحرشف البري, متعدد السكر الدهني)

2-5 باللغة الأجنبية :

Hepatotoxicity is now receiving a supreme importance. LPS is the most potent activator of macrophages. The aim of this study is to demonstrate the prophylactic activity of silimarin and curcumin against LPS-induced hepatotoxicity in rats. The liver slices had been divided into 4 groups; normal, control (LPS), LPS+silimarin and LPS+curcumin (N=12). After 24 hours, analysis of NO levels, LDH activity, AST levels, ALT levels, GSH levels, TNF-α, IL-1β and IL-10 levels were carried out. . Nitric Oxide in the control group showed a high significant elevation in comparison to the normal group, while group 3 and 4 showed a high significant decrease compared with the control group. LDH activity in the control group showed a high significant elevation in comparison to the normal group, while group 3 and 4 showed a high significant decrease compared with the control group. AST and ALT in the control group showed a high significant elevation in comparison to the normal group, while group 3 and 4 showed a high significant decrease compared with the control group. In control group a significant reduction in GSH level was observed; where in group 3 and 4 a significant increase was noticed in comparison to the control group. Control group showed a high significant elevation in TNF-**α** level in comparison to normal group, where in group 3 and 4 a high significant decrease was detected in comparison to the control group. Levels of IL-1β in the control group indicated a high significant elevation compared to the normal group, while in group 3 and 4 a high significant decrease in the IL-1β levels were detected in comparison to the control group. IL-10 levels in the control group indicated a high significant elevation compared to the normal group, while in group 3 and 4 a high significant decrease in the IL-10 levels were detected in

comparison to the control group. In conclusion, silimarin and curcumin showed a hepatoprotective activity against hepatotoxicity induced by LPS.

(Key Words: Prophylaxis, silimarin, curcumin, lipopolysaccharide (LPS), Nitric Oxide (NO), aspartate transferase (AST), reduced glutathione (GSH), alanine amino transferase (ALT), tumor necrosis factor alpha (TNF-α) lactate dehydrogenase (LDH), Inter leukin 1 beta (IL-1β) and inter leukin (IL-10))

6- أهم النتائج التطبيقية التى تم التوصل إليها:

هذه الدراسة تم عملها لإثبات امكانية استخدام الكركم كدواء فعال ومؤثر تأثيراً إيجابياً في الوقاية من التسمم الكبدي المحدث بواسطة متعدد السكر الدهني، هذا وقد أستخلص من هذه الدراسة أن استخدام الكركم ذو فائدة في منع التسمم الكبدي المحدث كيميائياً.

وبذلك من الممكن الاستفادة من نتائج هذا البحث في التطبيقات الإكلينيكية لكل من الحرشف البري والكركم وذلك في الوقاية من التسمم الكبدي والأمراض التي تصيب الكبد.

7- ما هى الجهات التى يمكن أن تستفيد من هذا البحث:

7-1 المراكز البحثية

7-2 شركات الأدوية حيث ثبت أن نبات الكركم من الممكن استخدامه للوقاية من التسمم الكبدي

8- <u>هل توجد علاقة قائمة بإحدى هذه الجهات :</u> **نعم** ☐ **لا** ☑

9-<u>هل توافق على التعاون مع جهات مستفيدة من خلال الجامعة :</u>

لا ☐ **(لماذا**)

نعم ☑

(أ) لتطبيق البحث : ☑

(ب) لاستكمال البحث: ☐

(ج) أخري : ☐ **(تذكر**)

10- <u>هل تم نشر بحوث مستخرجة من الرسالة فى مجلات أو مؤتمرات علمية</u>

10-1 جارى النشر British Journal Of Pharmacology And Toxicology 2015

2-10 تم النشر في مؤتمر 6th International Scientific Conference of Faculty of Pharmacy, Cairo Uinversity

11- هل سبق التقدم لتسجيل براءات اختراع

لا

12- هل توافق على إعطاء البيانات المذكورة فى هذه الاستمارة لجهات أخرى

نعم	

توقيع الطالب: توقيع المشرفين:

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وكيل الكلية للدراسات العليا و البحوث : التاريخ :