Effect of *Peganum Harmala* Methanol Extract on Liver and Kidney of Mice Administered MTX Drug

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Abstract

This research was designed to evaluate the role of *Peganum harmala* methanol seed extract in amelioration the biochemical side effect of liver and kidney in mice administered MTX drug. The biochemical detections for both kidney functions (urea and creatinine) and liver enzymes (GOT, GPT and ALP) were investigated. This detection was carried on 30 mice through two stages. In stage I, two doses (75 and 100) mg/kg of *P. harmala* seed extract and a single dose of MTX 50 mg/kg were used. The tested materials were dosed as a single dose (0.1 ml) per a day for 6 days, and then mice were sacrificed on day 7 for subsequent assesments. In stage II, interaction between MTX drug and two doses of extract were carried out. Mice were given MTX dose on day one, while the extract was given in day two till day 6, then mice were sacrificed on day 7 for laboratory evaluation. Results revealed that mice treated MTX showed a significant increase in liver enzymes and kidney function; in contrast, biochemical parameters demonstrated the amelioration effect of extract through decreasing these parameters.

Keywords: *Peganum harmala*, Biochemical side effects, liver enzymes, mice.

Introduction

Peganum harmala is recognized as one of the most widely spread and usable medicinal plants in herbal medicine in many countries around the world. It is known as "harmal" in Saudi Arabia and as "African Rue" in some African and Australian regions [1]. *P. harmala* seeds has been reported to be widely used in treatment of several diseases, for instant, seeds powder is used as anti-thelmintic and protozoacidal agent, and as treatment of asthma, jaundice, eczema, and malaria [2]. The compounds of the seeds were reported to have anti-inflammatory effects, and an analgesic effects. The seeds also known to be used by women as flooder of milk during lactation period, and to regulate the menstruation cycle [3]. *P. harmala* seeds contain tens of chemical compounds including: flavinoids, amino acids, polysaccharids, volatile compounds and several kinds of alkaloids compounds. These alkaloids are known to be the most active and effective compounds, and most of the therapeutic properties of the harmala seeds are believed to be due the effects of these compounds. Alkaloids of harmala seeds are classified into three main groups: - First group is called Harmala indole alkaloids (i.e. Harmaline, Harmane, Harmol, Harmalol, Harmine & Harmalan), Second group is called quinazoline alkaloids which including vasicine and deoxyvasicine [4]. Harmala Indole alkaloids (i.e. Harmaline and its derivatives) have many important physiological and pharmacological properties, therefore it attracted the attention of many researchers, these pharmacological properties were reported by [5] including; Potent ability to inhibit activity of type A-monoamine oxidase and Na/K active transport, anti-oxidative action, vasorelaxative action, acting as serotonin agonists, ability to induce hallucinogenic effects (at high doses). Its seed extracts are known to contain β-carbolin alkaloids, anthroquinons, and a small quantity of flavonoid glycosides. There are reports that alkaloids in *P. harmala* seed are mainly responsible for differentpharmacological activities including antibacterial effects in addition to vasorelaxant, antihemosporidian, anticancer, antinociceptive, antitumor or antineoplastic, and anti-protozoal effects [6],[7].

Materials and Methods

Extract preparation

A quantitiy of 250g of *P. harmala* seed was bought from a local market, grinded and extracted with (500 ml) of methanol for 6 hrs by Soxhelt apparatus at 40–60 °C. The extract was filtered, and evaporated to dryness at 40 ºC and stored until use [8]. A small amount

of extract was obtained which was 12.0 g (4.8% of the original weight). This crude extract was used to prepare the required doses (75 and 100 mg / kg) depending on the lethal dose (LD 50) which was 425 mg / kg as mentioned by [9].

Experimental designs of this study were partitioned into four parts as follows:

Experimental Design

Albino male mice, weighing 25-40 g were used throughout the study. In this part, the biochemical effects of two doses of *P. harmala* methanol extract and MTX were investigated. Animals were divided into four groups designated as A, B, C and D. Each group consisted of 5 animals and treated as follows:

Group A: The animals were treated with distilled water (negative control of the extract).

Group B: The animals were treated with MTX at a dose of 50 mg / kg (positive control).

Group C: This group was subdivided into two subgroups as follow:

C1: Treated with a dose of 75 mg/kg of the extract.

C2: Treated with 100 mg / kg of the extract. Followed by treating both subgroups with 50 mg / kg of MTX for pre-treatment study.

Group D: This group was used to study the post-treatment effect of *P. harmala* Extract. The animals were treated with a dose of 50 mg / kg of MTX and subdivided into two subgroups as follow:

D1: Treated with 75 mg / kg of the extract.

D2: Treated with 100 mg / kg of the extract.

The tested materials were injected orally as a single dose (0.1 ml) per day for six days. Then, the mice were sacrificed on day 7 for laboratory assessment.

Laboratory Methods

Biochemical tests

Biochemical tests included the measurement of GOT (AST), GPT (ALT) and ALP enzymes. Urea and creatinine as renal tests were estimated in the serum of the tested mice. All biochemical tests were carried out by Reflotron Plus Instrument (Roch).

Results and Discussion

Effects of *P. harmala* extracts on liver enzymes (AST, ALT and ALP)

The effect of MTX and *P. harmala* on AST, ALT and ALP levels was shown in Table (1).

Table (1)
Effect of MTX and P. harmala seed methanolic extracts on mouse liver enzymes activity.

Group		*GOT (AST) IU/ml (m±SE)*	*GPT (ALT) IU/ml (m±SE)*	*ALP IU/ml (m±SE)*
Negative Control		*A 214.91±3.71	A 70.662±4.499	A 62.288±4.790
Positive Control (MTX) (50 µg/kg)		B 282.05±14.86	B 91.608±4.123	B 72.620±7.475
Pre-treatment	*100 mg/kg*	A 220.01±2.04	A 72.294±3.449	A 64.932±4.699
	75 mg/kg	A 223.22 ±4.26	A 73.168 ±6.053	AB 68.518±2.698
Post-treatment	*100 mg/kg*	A 226.41 ±6.51	A 73.358±3.260	AB 68.124±3.332
	75 mg/kg	A 228.81±6.73	A 74.830±4.191	AB 69.324± 2.182

**** Different letters (A, B) represent variation to compare between rows.***

Results exhibited that there was a significant increase (P≤0.05) in AST activity in all groups of mice administered MTX drug reached 282.05 IU/mL as compared with the negative control (214.91 IU/mL). Also a significant difference in ALT was shown after MTX administration reached 91.608 IU/mL as compared with the negative control

(70.662 IU/mL). In pre-treatment with *P. harmala* extract, there was a significant differences in AST activity in all groups of mice treated with extract, (220.01 and 223.22) IU/mL for both 100, 75 mg/kg respectively as compared with the positive control (282.05IU/mL). Result indicated that *P. harmala* extract cause a significant differences in the activity of ALT all groups of mice treated with extract.The activity of ALT reached 72.294 and 73.168 IU/mL for both doses respectively in comparison with positive control (91.608 IU/mL). Regarding post-treatment, there was a significant difference in AST activity all groups of mice treated with extract as compared with the positive control (282.05 IU/mL). The activity of AST reached 226.41 and 228.81 IU/mL for both doses 100 and 75 mg/kg respectively. Also, there was a significant differences of these doses on ALT activity that reached (73.358 and 74.830) IU/mL as compared with the positive control (91.608 IU/mL). Results showed that ALP activity was significantly higher (72.620 IU/ mL) in mice treated with MTX drug than negative control (62.288 IU/mL). Regarding pre-treatment with *P. harmala* extract, ALP activity showed a significant differences in all groups of mice treated with extract for both doses 100 and 75 µg / kg at which the activities of ALP reached (64.932 and 68.518) IU/mL respectively as compared with the positive control (72.620 IU/mL). In post-treatment ALP activities showed a significant differences in both doses (100 and 75 µg / kg) at which the activities of ALP were (68.124 and 69.324) IU/mL respectively as compared with the positive control (72.620IU/mL). Results revealed that treatment with MTX causes a significant increase in AST, ALT activity. This might be due to cytotoxic effect of MTX drug on liver. It is known that treatment with MTX resulted in increase the permeability of liver cell membrane, causing the movement of high quantity of these enzymes to blood [10]. A similar result was obtained by Al-Motabagani, [11], who reported that MTX therapy causes serious side effects like hepatic toxicity and acute liver failure. Song [12] pointed that the agents that have cytotoxic effects cause lysosomal lysis which are damaged in all the organules inside the cell and lead to death of paranchymal cell which causes an increase in the serum levels from AST and ALT.On the same time most of chemical compounds and MTX have one of these compounds lead to inhibit the activity of the detoxification enzymes such as superoxide dismutase (SOD) and glutathione-S-transferase (GST) that scavenging free radicals from the cell [13]. Results showed that treatment with *P. harmala* extract had no effect on the enzymes level. This could be due to the presence of flavonoids which are known for their excellent antioxidative capacity in various model systems [14]. In addition *P. harmala* have in their content active compounds such as vitamin A, B, C, E, and K, with high mineral content such as sodium iron, and calcium. These compounds have a strong antioxidant activity against reactive oxygen species (ROS), and the hepatoprotective activity [15]. Qi *et al*., [16] reported that flavonoids compounds have a hepatoprotection function and has been used for clinical treatment of liver. Novikoff [17] reported that alkaloids compounds rapidly induce liver cells against toxicity. Also, Raj 18], reported that alkaloid compounds were able to normalize the biochemical levels which were altered due to intoxification.

Effects of *P. harmala* extracts on kidney functions

The effect of MTX and *P. harmala* on urea and creatinine levels was shown in Table (2).

Table (2)
Effect of MTX and P. harmala seed methanolic extracts on mouse kidney functions.

Group		Urea Mg/dl (m±SE)	Creatinine Mg/dl (m±SE)
Negative Control		A 17.394±1.678	A 0.50800±0.06380
Positive Control (MTX) (50 µg/kg)		B 22.602±3.473	B 0.66600±0.04827
Pre-treatment	100 mg/kg	A 18.466±1.668	AB 0.57200±0.07430
	75 mg/kg	AB 19.836±2.362	A 0.56000±0.06205
Post-treatment	100 mg/kg	A 18.998±1.108	A 0.51200±0.07981
	75 mg/kg	A 18.102±1.632	A 0.51200±0.05541

Results exhibited that there was a significant in the urea level in all groups of mice administered MTX drug reached (22.602 mg/dl) in comparison with negative control (17.394 mg/dl). Also a significant increase (P≤0.05) in creatinine level was shown after MTX treatment and reached to (0.66600 mg/dl) in comparison with the negative control (0.50800 mg/dl). In pre-treatment, *P. harmala* extract show a significant variations (P≤0.05) in all groups of mice treated with extract, the level of urea reached (18.466 and 19.836) mg/dl for both (100, 75) mg/kg respectively in comparison with positive control (22.602mg/dl). Results indicated that there was a significant variation (P≤0.05) in level of creatinine in all groups of mice trated with extract. The level of creatinine reached (0.57200 and 0.56000) mg/dl for both doses respectively in comparison with positive control (0.66600 mg/dl). Regarding post-treatment, results showed that there was a significant decrease in all groups of mice treated with extract (P≤0.05). The level of urea has reached (18.998 and 18.102) mg/dl for both doses (100 and 75) mg/kg respectively as compared with positive control (22.602 mg/dl). Also, there was a significant effect (P≤0.05) of these doses (100 and 75) mg/kg on creatinine level that reached to (0.51200 and 0.51200) mg/dl for both doses respectively as compared with the positive control (0.66600 mg/dl). The increase in the levels of Urea and Creatinine after MTX treatment is considered as an indicator of renal functions failure due to the toxicity of MTX. Chelab and Majeed [19] reported that methotrexate cause damage and sever toxicity in renal system of mice. Results showed that methanolic extract of *P. harmala* has the ability to normalize the urea and creatinine regarding pre and post–treatment, this activity may be due to the presence of many active compounds in the extract of *P. harmala* like alkaloids and flavinoids. mahajan *et al*., [20], reported that Alkaloids impaired the kidney and liver functioning. Wang *et al*., [21], reported that alkaloids have been showed to exert protective effects on the renal function. Long *et al*., [22] reported that flavonoids compounds prevents nephrotoxicity and improves kidney functions and promotes kidney primary epithelial tubular cell regeneration. Also, De *et al*., [23] also reported that flavonoids mixture significantly lowered plasma creatinine and urea concentration, booth indicating a better postoperative kidney function.

Conclusions

The results of this study indicated that treatment with methanol *P. harmala* extract caused a significant improvement in liver and kidney function in mice administered MTX drug, since this extract modulated the liver

enzymes and kidney function regarding both pre and post – treatment.

References

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الخلاصة

صممت الدراسة الحالية لتقييم دور المستخلص الميثانولي لبذور نبات الحرمل في تحسين التأثيرات الجانبية على كبد وكلية الفئران المعالجة بعقار MTX المضاد للأورام، حيث أجريت بعض الفحوصات الكيموحيوية على وظائف الكلية (اليوريا والكرياتنين) وأنزيمات الكبد (GOT, GPT, و ALP). أجريت الدراسة على 30 فأرة بيضاء من خلال مرحلتين: شملت المرحلة الأولى دراسة تأثير جرعتين من مستخلص النبات (75 و 100) ملغم/ كغم وجرعة 50 ملغم/كغم من عقار MTX. جرعت الفئران بالمستخلص لمدة ستة أيام وفي اليوم السابع ضحي بالحيوانات لغرض أجراء التقييمات المختبرية. في المرحلة الثانية أجري تداخل بين عقارMTX والمستخلص النباتي، وفي هذا التداخل جرعت الحيوانات في اليوم الأول بجرعة واحدة من عقار MTX، أما في اليوم الثاني وحتى اليوم السادس فقد أعطيت الحيوانات جرعات من المستخلص. أظهرت النتائج أن معاملة الحيوانات بعقار MTX قد تسبب في زيادة معنوية في أنزيمات الكبد ووظائف الكلية، وعلى العكس، اظهرت المعاملة بالمستخلص النباتي دوراً في تحسين المعاملات الكيميائية للكبد والكلية من خلال فحص مستويات هذه المعاملات.

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