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Antihepatotoxic Effect of Methanolic Extract of *Moringa oleifera* Leaves against Carbon Tetrachloride Induced Hepatotoxicity in Wistar Albino Rats

Ali, S. A. [1]*, Gameel, A.A[2]. Mohamed, A.H [3].

[1]Department of Veterinary Medicine and Surgery,College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum, Sudan
[2]Department of Pathology, Faculty of Veterinary Medicine, University of Khartoum, Khartoum, Sudan;[3]Faculty of Pharmacy, National Ribat University, Khartoum, Sudan

*Corresponding Author: Sumaia. A. Ali, Department of Veterinary Medicine and Surgery, College of Veterinary Medicine. Sudan University of Science and Technology, Sudan, P.O. Box 204, Hilat Kuku, Khartoum North, Sudan.

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Abstract

The present study was conducted to evaluate the antihepatotoxic effect of *Moringa oleifera* leaves methanolic extract against CCl_4 induced hepatotoxicity in rats. The methanolic extract was prepared and chemically screened. Rats were divided randomly into five groups each of five as follows; normal control, intoxicated rats group injected with CCl_4 in liquid paraffin 1:9 (v/v) at a dose rate of 0.2 ml kg^{-1} day^{-1} intraperitoneally (IP), standard hepatoprotective drug group given silymarin at a dose of 100 mg kg^{-1} dissolved in 5% *Acacia mucilage. M. oleifera* leaves methanolic extract groups were administered 200 and 400 mg kg^{-1} of the extract orally. CCl_4 was injected simultaneously with standard drug and the leaves extract for 10 days. Liver function test (serum aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP) and total bilirubin) were estimated at day 0, 5 and 10. The livers were sectioned and investigated histopathologically. The oral administration of *M. oleifera* leaves extract (200 - 400 mg kg^{-1}) and the standard hepatoprotective drug silymarin at a dose of 100 mg kg^{-1} significantly reduced the activities of AST, ALT, ALP and total bilirubin compared to CCl_4 – intoxicated rats. These results were further supported by histopathological findings of liver sections. Hepatic degenerative changes and lymphocyte infiltrations were reduced in animals treated with *M. oleifera* leaves extract as well as those receiving silymarin. Phytochemical screening of leaves exhibited the presence of alkaloids, saponins, flavonoids, tannins, sterols, glycosides and cumarins. The present results suggest that the methanolic extract of *M. oleifera* leaves could possess potential antihepatotoxic effect against CCl_4 – induced hepatotoxicity in rats.

Keywords: antihepatotoxic, *Moringa oleifera*, rats, CCl_4, phytochemical screening.

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Introduction

Liver is one of the most important organs in the metabolism of food, drugs, endogenous and exogenous substances. Problems related to liver such as acute or chronic inflammation, toxin-/drug-induced hepatitis and cirrhosis are well-known nowadays due to exposure to different environmental pollutants like chemicals, toxins, viruses …etc (Ansari and Jamil, 2011).

Because the liver plays an important role in detoxification and many other metabolic processes, a poorly functioning liver can broadly affect health. Signs of poorly functioning liver might include food or drug intolerance, chronic constipation, fat intolerance, poor digestion and may contribute to chronic skin disease, autoimmune disease, allergies and cancer. Depending on severity and signs, treatment of patients with subclinical liver dysfunction should include hepatoprotective and hepatorestorative herbs, especially if a history of liver damage or exposure to toxins or drug toxicity is reported. Hepatoprotective herbs given to minimize liver damage may include bupleurum, dandelion root, globe artichoke and milk thistle (Wynn and Fougère, 2007).

Moringa oleifera is a member of the Moringaceae and it grows in northern and central Sudan. It is a multipurpose tree with most of its parts being used for a number of applications (Anwar *et al.,* 2007). Many studies demonstrated that the plant extracts have antihypertensive (Dangi *et al.,* 2002; Faizi *et al.,* 1995), anticancer (Guevara *et al.,* 1999; Costa-Lotufo *et al.,* 2005), hepatoprotective (Pari and Kumar 2002; Fakurazi *et al.,* 2008; Ali *et al.,* 2010), antimicrobial and antifungal (Caceres *et al.,* 1991; Chuang *et al.,* 2007), hypocholest-erolaemic (Ghasi *et al.,* 2000), potent antipyretic and anti-inflammatory activities (Anwar *et al.,* 2007; Caceres *et al.,* 1992) and regulate the thyroid hormone status (Tahiliani and Kar, 2000). The aqueous extract has been found to have significant wound healing properties (Rathi *et al.,* 2006). Additionally, pharmacological studies reported that this plant possesses antioxidant, hypolipidaemic and antiather-osclerotic activities and has therapeutic potential for prevention of cardiovascular diseases (Siddhuraju and Becker, 2003; Chumark *et al.,* 2008). However, no scientific investigations in Sudan have been reported regarding methanolic extract action on liver.

Hence, the present study was conducted to explore the antihepatotoxic effect of *M. oleifera* leaves methanolic extract against carbon tetrachloride induced liver damage in Wistar albino rats.

Materials and Methods

***Plant collection and identification*:** The leaves of *M. oleifera* were collected from trees growing in Khartoum state. The plant material was identified by botanists in Medicinal and Aromatic Plants Research Institute, Khartoum - Sudan, and a voucher specimen was deposited in the herbarium of the Institute.

***Extraction*:** Freshly collected leaves of the plant were dried in shed and pulverized to get coarse powders. Hundred gram of the powdered material of the plant was firstly defatted with chloroform (AR Analytical Rasayan) and then subjected to hot solvent extraction in a soxhlet apparatus (Quick fit, ex5/83. England) using methanol (AR Analytical Rasayan; 98%) at a temperature range of 60-80°C. The filtrates were evaporated to dryness under reduced pressure in a rotary evaporator (Buchi 011 Switzerland) (Harborne, 1984). The percentage yields of the extracts were calculated.

***Phytochemical screening*:** A preliminary phytochemical investigations for the presence and absence of phytoconstituents

were performed according to the method of Harborne (1984).

***Animals*:** Wistar albino strain rats of male sex were obtained from the Medicinal and Aromatic Plants, Traditional Medicine and Research Institute (MAPTMRI), National Center of Research (NCR), Khartoum, Sudan. The animals were kept in cages under standard condition in the Medicinal and Aromatic Plants Research Institute. They were allowed free access to standard laboratory feed and water *ad libitum*. The animals were allowed a week period prior to experimentation to acclimatize to laboratory conditions.

***Experimental Design*:** The animals were divided randomly into 5 groups, each of 5 animals.

Group 1: normal control: the animals received only the vehicle liquid paraffin (0.2 ml kg^{-1} day^{-1} 1P) for 10 days.

Group 2: CCl_4 control: the animals were intoxicated with 1:9 (v/v) mixture of CCl_4 (E. Merck, Darmstadt Art- 2222) in liquid paraffin (B.P Bell. Sons &CO. (Druggsts) LTD**.** England) at a dose rate of 0.2 ml kg^{-1} day^{-1} 1P for 10 days.

Group 3: intoxicated animals were simultaneously received standard drug silymarin (Simepar – mepha) suspended in 5% *Acacia mucilage* (100 mg kg^{-1} day^{-1}) for 10 days.

Group 4: intoxicated animals were simultaneously treated with methanolic extract of *M. oleifera* leaves at a dose of 200 mg kg^{-1} day^{-1} orally for 10 days.

Group 5: intoxicated animals were simultaneously treated with methanolic extract of *M. oleifera* leaves at a dose of 400 mg kg^{-1} day^{-1} orally for 10 days.

***Blood samples*:** The blood was collected from animals by retro-orbital puncture under anaesthesia at day 0, 5 and 10. Blood was allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 min and analyzed for various biochemical parameters.

***Assessment of liver function*:** The serum levels of alanine aminotranseferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and total bilirubin (TBIL) were measured by Mindray automatic analyzer for biochemistry (Mindray – BS-200 – China) using commercial kits supplied by Plasmatic Laboratory Ltd., U.K.

***Histopathological studies*:** At the end of the study, the animals were anaesthetized and decapitated, and post-mortem examination was performed. The livers were dissected quickly and samples were collected in 10% neutral buffered formal saline for histopathology.

***Statistical Analysis*:** Statistical analysis was performed using software Statistical Package for Social Sciences (SPSS) version 10.0. Complete factorial randomized design was used for the analysis of the data. The data were expressed as mean ± SDM (Mendenhall, 1971).

Results

Phytochemical screening: Phytochemical screening of the powdered material of *M. oleifera* revealed the presence of alkaloids, saponins, flavonoids, tannins, sterols, glycosides and cumarins. The plant material was devoid of anthraquinone constituents.

Effect of M. oleifera leaves extract on liver function test: Rats intoxicated with CCl_4 (group 2) showed a significant ($P< 0.001$) increase in the levels of serum AST, ALT, ALP and total bilirubin concentration at day 5 and 10 of the experiment suggesting the occurrence of severe hepatocellular damages as compared to normal animals. Serum enzymes values in the animals treated concurrently with *M. oleifera* leaves methanolic extract at a dose of 200 and 400 mg kg^{-1}, (groups 4 and 5) were significantly decreased especially at day 10

($P < 0.05$) when compared to rats receiving CCl_4. There were no significant differences in serum enzymes activities between rats in the normal control group and intoxicated rats treated with standard drug silymarin. The concentration of bilirubin and the levels of ALP and ALT in rats receiving 200 mg kg^{-1} *M. oleifera* leaves methanolic extract were comparable to those receiving 100 mg kg^{-1} standard drug silymarin (Table 1).

Table 1: Effect of *M. oleifera* leaves methanolic extract on CCl_4 induced hepatotoxicity in rats.

Group	Bilirubin (mg/dl) (Mean ± SD.)			ALP (U/I) (Mean ± SD.)		
	Day 0	Day 5	Day10	Day 0	Day 5	Day 10
Normal control	0.12 ± 4.5	0.09 ± 1.3	0.13 ± 4.1	343.2 ± 29.7	316.2 ±33.4	322.4 ± 69.2
CCl_4 control	0.14 ± 4.0	0.38 ± 9.1	1.26 ± 0.2	393.6 ± 75.4	681.4 ± 160.3	1104.2 ± 187.8
CCl_4 + Silymarin	0.11 ± 5.6	0.23 ± 2.7	0.21 ± 3.1	330.2 ± 40.7	422.4 ± 89.8	440.4 ± 47.5
CCl_4 +200 mg kg^{-1}	0.11 ± 8.9	0.34 ± 9.4	0.40 ± 0.1	249.2 ± 80.0	601.0 ± 112.3	477.6 ± 125.8
CCl_4 +400 mg kg^{-1}	0.16 ± 4.1	0.17 ± 5.5	0.61 ± 0.2	302.0 ± 98.6	758.0 ± 148.3	829.8 ± 117.7
Main effects						
Dose						
Normal control		0.11 ± 3.8^{c}			327.3 ± 45.6^{c}	
CCl_4 control		0.59 ± 0.5^{a}			726.4 ± 332.1^{a}	
CCl_4 + Silymarin		0.18 ± 6.4^{bc}			397.7 ± 76.9^{c}	
CCl_4 +200 mg kg^{-1}		0.28 ± 0.2^{b}			442.6 ± 181.0^{c}	
CCl_4 +400 mg kg^{-1}		0.31 ± 0.2^{b}			629.9 ± 267.5^{b}	
Sig		***			***	
Days						
Day 0		0.13 ± 4.3^{b}			323.6 ± 80.3^{b}	
Day 5		0.24 ± 0.1^{b}			555.8 ± 198.7^{a}	
Day 10		0.52 ± 0.4^{a}			634.9 ± 314.9^{a}	
Sig		***			***	
Interaction Dose&Day						
Sig		***			***	

Group	ALT (U/I) (Mean ± SD.)			AST (U/I) (Mean ± SD.)		
	Day 0	Day 5	Day10	Day 0	Day 5	Day 10
Normal control	45.2 ± 5.7	40.6 ±7.6	43.8 ± 6.3	155.2 ± 34.6	161.2 ± 39.8	165.6 ± 22.6
CCl_4 control	48.4 ± 3.6	1281.6 ± 138.8	1295.6 ± 133.9	163.6 ± 32.0	1512.2 ± 262.7	2499.0 ± 515.8
CCl_4 + Silymarin	47.2 ± 9.2	98.4 ± 20.4	140.6 ± 50.4	138.8 ± 33.6	216.0 ± 85.3	197.8 ± 42.5
CCl_4 +200 mg kg^{-1}	34.0 ± 9.5	381.2 ± 222.4	424.4 ± 71.4	113.8 ± 5.0	845.2 ± 186.9	804.0 ± 35.1
CCl_4 +400 mg kg^{-1}	62.0 ± 9.1	841.4 ± 78.7	590.4 ± 133.3	156.0 ± 40.5	783.4 ± 118.2	840.8 ± 96.9
Main effects						
Dose						
Normal control		43.2 ± 6.4^{d}			160.7 ± 31.0^{c}	
CCl_4 control		941.9 ± 668.2^{a}			1391.6 ±1038.2^{a}	
CCl_4 + Silymarin		95.4 ± 49.3^{cd}			184.2 ± 63.9^{c}	
CCl_4 +200 mg kg^{-1}		279.9± 219.8^{c}			587.7 ± 361.9^{b}	
CCl_4 +400 mg kg^{-1}		497.9 ± 346.3^{b}			593.4 ± 332.0^{b}	
Sig		***			***	
Days						
Day 0		47.4 ± 11.6^{b}			145.5 ± 34.1^{b}	
Day 5		528.6 ± 493.7^{a}			703.6 ± 523.1^{a}	
Day 10		539.0 ± 534.1^{a}			901.4 ± 892.7^{a}	
Sig		***			***	
Interaction Dose&Day						
Sig						

a,*b*: means within the same column followed by different superscripts are significantly ($p< 0.05$) different.
*** Significant at ($p< 0.001$). N: 5 rats per group.

Histopathological findings: The control group showed normal hepatic cellular architecture (Figure 1). The liver sections of rats intoxicated with CCl_4 showed massive centrilobular degenerative and necrotic changes associated with cellular infiltration, haemorrhage and congestion of blood vessels (Figure 2), while those of rats treated with silymarin exhibited slight vacuolar degeneration with sinusoidal dilatation (Figure 3). Distinct lobulations and slight degenerative changes were seen in the liver sections of rats treated with 200 mg kg^{-1} methanolic extract of *M. oleifera* leaves, mainly hepatocyte swellings with loss of cellular outlines, cytoplasmolysis and vacuolar degeneration and some areas showed mononuclear cell infiltration of mainly lymphocytes (Figure 4). In rats treated with 400 mg kg^{-1} methanolic extract of *M. oleifera* leaves diffuse vacuolar degenerative and necrotic changes were observed; cytoplasmolysis and chromatolysis were frequent (Figure 5).

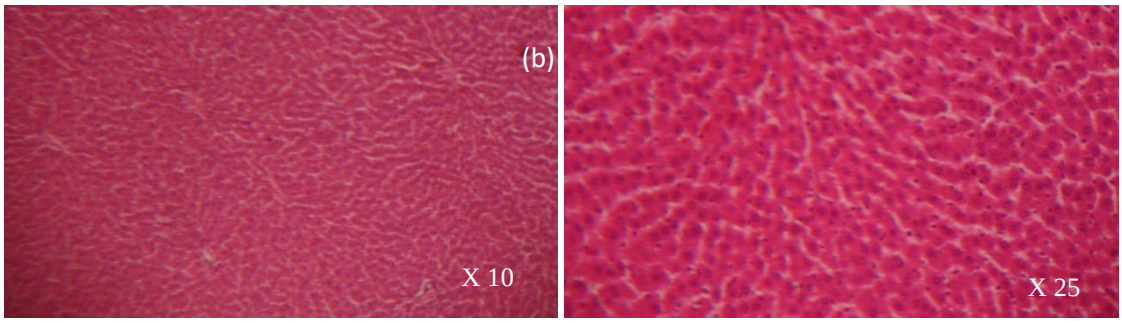

Figure 1 (a,b): section of the liver tissue of control animl normal hepatic archtecture (H & E stain)

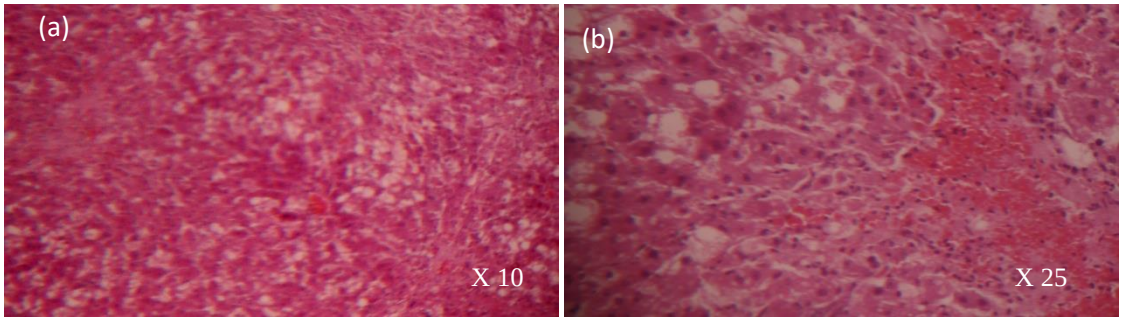

Figure 2 (a,b): section of the liver tissue of animal treated with CCl_4 showing massive centrilubular degenerative and necrotic changes, congestion and haemorrhage (H & E stain).

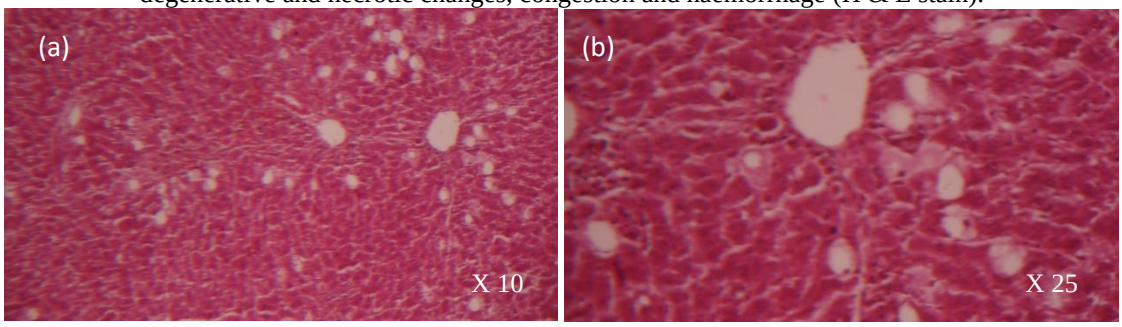

Figure 3 (a,b): section of the liver tissue of silymarin treated animals showing slight vacuolar degeneration and sinusoidal dilatation (H & E stain).

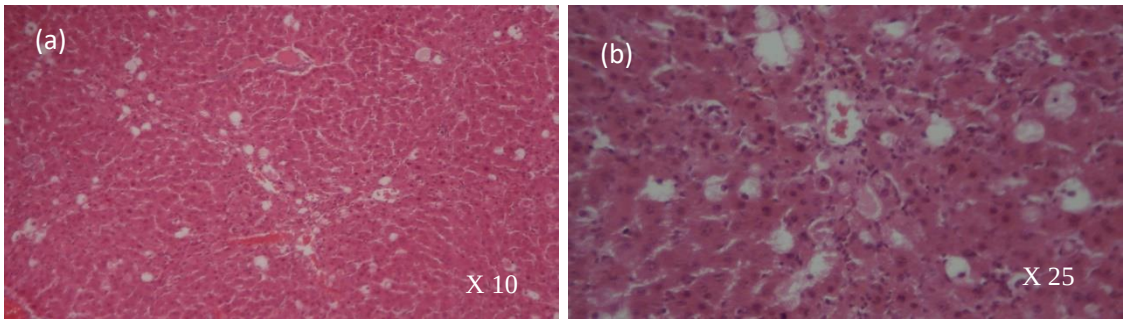

Figure 4 (a,b): liver section of intoxicated rat treated with 200 mg kg $^{-1}$ methanolic extract, showing limited vacuolar degeneration with sinusoidal dilatation (H & E stain).

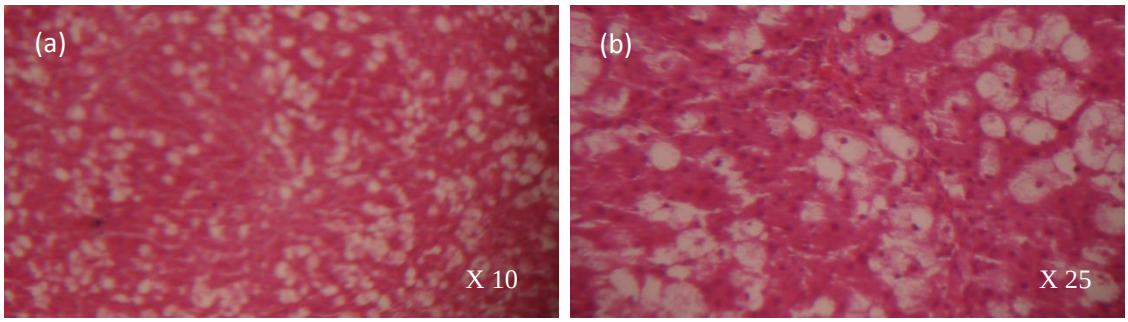

Figure 5 (a,b): liver section of intoxicated rat treated with 400 mg kg $^{-1}$ methanolic extract, showing vacuolar degenerative and necrotic changes (H & E stain).

Discussion

M. oleifera is a highly valued plant and it has an impressive range of medicinal uses and high nutritional values (Anwar *et al.*, 2007). In recent years many studies have shown that the different parts of this plant possess wide range of physiological, biochemical and pharmacological effects due to the properties of its constituents (Caceres *et al.*, 1992; Tahiliani and Kar, 2000; Siddhuraju and Becker, 2003; Chumark *et al.*, 2008). This study was carried out to investigate the antihepatotoxic effect of *M. oleifera* leaves aqueous and methanolic extracts against CCl_4 induced hepatotoxicity in Wistar albino rats.

Liver damage induced by CCl_4 is commonly used as model for the screening of hepatoprotective drugs (Ahsan *et al.*, 2009). Carbon tetrachloride is metabolically activated by cytochrome P450 enzymes in the endoplasmic reticulum to form trichloromethyl free radical (CCl_3•) and trichloromethyl peroxyl (CCl_3O_2) free radicals, which combine with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation and finally to cell apoptosis and necrosis (Abraham *et al.*, 1999; Weber *et al.*, 2003).

In the present study, the animals intoxicated with CCl_4 revealed high levels of serum AST, ALT, ALP and bilirubin suggesting severe hepatic damage.

The serum enzymes levels of rats treated with silymarin showed almost normal values indicating the protective effect of the reference drug silymarin. Concurrent treatment with *M. oleifera* leaves extracts

seem to stabilize the plasma membrane and protect the hepatocytes from the toxic effect of CCl_4, which indicate that *M. oleifera* has antihepatotoxic effect. This is in agreement with the findings of Diallo *et al.* (2009); Fakurazi *et al.* (2008); Pari and Kumar (2002). The hepatoprotective effect of *M. oleifera* leaves is more pronounced in the low dose of methanolic extract compared to rats treated with standard drug silymarin as indicated by the levels of ALT, ALP and bilirubin. These findings were found to be similar to the results of aqueous extract of *M. oleifera* leaves but the aqueous extract is more effective than the methanolic extract (Ali *et al.*, 2010). The protection effect is less effective or marginal at higher dose of methanolic extract, but it exhibited a significant decrease in enzyme markers when compared to CCl_4 control. It is evident that high dose of the plant methanolic extract decreased the serum enzymes levels but not to the control values.

Histopathological studies also confirmed the antihepatotoxic effect of *M. oleifera* leaves extracts. Hepatocellular changes that observed in rats intoxicated with CCl_4 were significantly masked by administration of *M. oleifera* extracts especially at lower dose. The hepatocellular changes were observed in rats treated with lower doses of the plant extract were mainly hepatocyte swelling with limited degenerative changes and sinusoidal dilatation, similarly to the changes observed in animals treated with silymarin.

Furthermore, the preliminary phytochemical study revealed the presence of alkaloids, saponins, flavonoids, tannins, sterols, glycosides and cumarins which is in agreement with the findings of Manjunatha, *et al.*, (2008). Several studies reported a number of phytoconstituent with protective effect on liver due to antioxidant properties such as flavonoids, triterpenoids and sterols. Presences of those compounds in *M. oleifera* suggest that they are responsible for the observed protective effects (Gupta *et al.*, 2004; Manjunatha, *et al.*, 2008). Moreover, *M. oleifera* is known to be a source of antioxidants due to its total phenolic, vitamin A and vitamin E contents (Anwar *et al.*, 2007; Diallo *et al.*, 2009) which reduce lipid peroxidation.

The possible mechanism of *M. oleifera* extracts as hepatoprotective may be due to the ability of the several phytoconstituents to activate microsomal enzymes thereby accelerating the excretion of CCl_4 or by inhibiting lipid peroxidation induced by CCl_4 by activated free radical scavengers (Gupta *et al.*, 2004; Diallo *et al.*, 2009; Pattanayak and Priyashree, 2008).

In conclusion, the results of this study indicate that *M. oleifera* leaves methanolic extract may processes a potential hepatoprotective activity against CCl_4 induced liver injury in rats especially at low dose. Further studies are needed to study different types of extracts and to determine the exact phytoconstituent(s) responsible for the hepatoprotective effect of *M. oleifera* leaves.

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الأثر المضاد للتسمم الكبدى للمستخلص الميثيلى لأوراق نبات الرواق ضد رابع كلوريد الكربون المسبب للتسمم الكبدى فى الجرذان

سمية عوض الكريم على[1]، أحمد عبد الرحيم جميل[2]، عبد الوهاب حسن محمد [3]

1. **قسم طب وجراحة الحيوان، كلية الطب البيطرى، جامعة السودان للعلوم والتكنولوجيا**
2. **قسم الامراض كلية الطب البيطرى جامعة الخرطوم**
3. **كلية الصيدلة، جامعة الرباط الوطنى.**

المستخلص

أجريت هذه الدراسة لتقييم الفعالية الوقائية للمستخلص الميثانولى لاوراق نبات مورينقا اوليفرا ضد تسمم الكبد بواسطة رابع كلوريد الكربون فى الجرذان. تم تحضير المستخلص الميثانولى وتم مسحة كيميائيا. تم تقسيم الجرذان عشوائيا الى خمسة مجموعات، كل مجموعة بها خمسة جرذان كالاتى: المجموعة الضابطة، المجموعة المسممة والتى تم حقنها بخليط من رابع كلوريد الكربوان وزيت البرافين بنسبة 9:1 بجرعة 0.2 مل كجم $^{-1}$ فى الغشاء البريتونى. مجموعة العقار المعيارى الواقى للكبد و تم اعطاؤها عقار السليمارين بجرعة 100 ملجم كجم $^{-1}$ المذاب فى 5% من الصمغ العربى. مجموعتى مستخلص نبات الرواق والذى تم اعطاؤه بجرعتى 200 و400 ملجم كجم $^{-1}$ فمويا. تم حقن رابع كلوريد الكربون بالتزامن مع اعطاء العقار المعيارى ومستخلص الاوراق لمدة عشرة ايام. تم تقييم وظائف الكبد (serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) والبلروبن الكلى) فى اليوم الصفرى، الخامس والعاشر. تم عمل الفحص النسيجى المجهرى لشرائح للكبد. اعطاء مستخلص أوراق مورينقا اوليفرا (200 و 400 ملجم كجم $^{-1}$) وكذلك الدواء الواقى للكبد سليمارين (100 ملجم كجم $^{-1}$) فمويا قلل باعتدال من مستوى انزيمات AST, ALT, ALP و البلروبن الكلى بالمقارنة مع الجرذان المسممة برابع كلوريد الكربون. دعمت هذه النتائج بنتائج الفحص المجهرى لشرائح الكبد والتى أظهرت أن التغيرات التنكسية فى الكبد وارتشاح الخلايا اللمفية كان أقل فى الجرذان التى أعطيت مستخلص مورينقا اوليفرا وكذلك التى أعطيت عقار السليمارين. أظهر المسح الكيميائى للاوراق عن وجود القلويدات، الصابونينات، الفلافونويدات، التانينات، الستيرويدات، الجليكوسيدات والكيومارينات. تدل نتائج هذه الدراسة على أن مستخلص أوراق مورينقا اوليفرا ربما كان له أثر فعال ضد تسمم الكبد المحدث برابع كلوريد الكربون فى الجرذان.