Effect Of Moringa Oleferai leaves and Oils On Liver Disorder

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ABSTRACT:

Liver diseases are among the prominent causes of chronic disease states and results in a significant global health problems. Well-established causes of liver diseases include viral infection, alcohol, obesity, insulin resistance, and autoimmune, metabolic, and genetic disorders. Irrespective of the underlying cause the pathophysiologic principles of liver injury usually involve inflammation, necrosis, apoptosis, oxidative stress, and much more. *Moringa oleifera* as one of protective plants possesses many pharmacological properties such as anti-cancer, anti-diabetic, anti-inflammatory and antioxidant. These properties might be due to phenolic and flavonoids.

Our experiment divided fourty rats (160±5 g) randomly into eight groups as follow; negative control (4 rats) were fed on basal diet. Injected positive group (6 rats) with CCl_4 was fed on basal diet. Treated group (6 rats) were fed after injection with *Moringa oleifera* leaves for four weeks. Treated group (6 rats) were fed after injection with *Moringa oleifera* oil for four weeks. Group (3 rats) was drinking ethanol after injection with for four weeks. Group (3 rats) was drinking hexane after injection with for four weeks. Group of 6 rats was fed on leaves and oil (1:1) for four weeks. Group of 6 rats was fed on basal diet with Hepaticum after injection for four weeks.

Diet supplemented with different amounts from moringa leaves and oil enhanced cholesterol, triglycerides and liver enzymes as ALP, AST and ALT. Moreover, LDL and vLDL were decreased in the last two groups, besides many enhancements have been noticed in the experiment.

Key Words: ***Moringa oleifere*** **leaves ,** ***Moringa oleifere*** **oil, Liver diseases** Serum lipid profile.

Introduction

Important metabolic pathways are regulates by liver so hepatic disorder is accompanied with distortion metabolic functions (**Wolf, 1999**). Liver exposes to many xenobiotics and toxin absorptions, so it considers a key organ of metabolism. Scientists usually actively search for save and protective way against a huge variable diseases to avoid the deleterious effects. From a long time, medicinal plants were widely got attention in the global due to their low cost and availability. Also, their minimal side effects encourage in focusing on their safe therapeutic efficiency (**Khalil et al., 2020**). Carbon tetrachloride proved to induce acute and subacute liver injury in rats. **Sergazy *et al*. (2023)** evaluated hepatoprotective effect of lingonberry, cranberry and blueberry polyphenols on CCl_4. **Peugnet-Gonzalez *et al.* (2023)** showed antioxidant and hepatoprotective efficacy for watercress extract against liver fibrosis induced by CCl_4 in rat.

Our research concentrate on relieving the health problems related to liver damage and hepatocellular carcinoma using valuable medicinal plants and compare their potential with standard drugs. *Moringa oleifera* leaves and oil showed role in attenuating the toxic and oxidative stress induced by carbon tetrachloride injection. As been dicussed with many researches (**Mackowiak, 2013** and **Kumar *et al*., 2011**), natural plant parts were effective in liver function and renal function as well as antioxidant status.

Material and Methods

Moringa oleifera. Leaves and oil were purchased from Agricultural Research Center, Giza, Egypt. Cleaned fresh leaves using running then distilled water were dried at 25°C. The dried plant materials were then pulverized and extracted with ethanol (98%). Extraction (250g plant powder/ 1 liter ethanol) was applied according to **Breuer and Devkota (1990)** at room temperature using magnetic stirrer for 48 hours. The supernatant was concentrated using rotary evaporator at 40^{o}C. After that, the crude extracts were weighted and stored in refrigerator. Oil in hexane (1:1 solution) was injected orally to rats daily for the experimental time. Thirty male rats between 165 and 180 g were purchased from Agricultural Research Center, Giza, Egypt. They were housed individually in cages for specific eight groups, and the mean weight of each group was 174 ± 2 g.

Biological Experiment. Fourty adult male Sprague–Dawley rats, they were divided into 8 groups. Upon arrival, the animals were given 2 weeks acclimation period, during which they were fed a standard rat pellet diet and water ad libitum. Rats were housed in wire cages under the normal condition at 25^{o}C ±5, with 12 hr light and 12 hr dark. The basal diet was prepared according to the following formula mentioned in table 1. The food was withdrawn on the day before the injection but allowed free access of water. Silymarin as standard protection was administered to rats through the Hepaticum drug (Medical Union Pharmaceuticals Company). Carbon tetrachloride was obtained from Merck Limited, India.

Biological experiment. The eight biological groups were divided as follow;

Group (1): Four rats were fed on basal diet only as a (-) control

Solution of CCl_4 in paraffin oil was prepared in equal volumes (1:1). Single i.p. injection 2ml/kg bw (**Janbaz and Gilani, 1995**) was administrated for groups 2-8 before offering basal diet by 2 hours.

Group (2): Six rats were kept without any treatment as (+) control.

Group (3): Six rats were orally gavage injected with 100 mg/kg daily *Moringa oleifera* leave extract after CCl_4 injection for four weeks.

Group (4): Six rats were orally injected with 100 mg/kg daily *Moringa oleifera* oil in hexane for four weeks

Group (5): Three rats were orally injected with ethanol as vehicle solvent for four weeks.

Group (6): Three rats were orally injected with hexane as vehicle solvent for four weeks

Group (7): Six rats were orally injected with *Moringa oleifera* leaves extract and oil solution in ratio 1:1 w/w for four weeks

Group (8): Six rats were orally injected with 100 mg/kg b.w. daily Hepaticum commercial drug for four weeks as standard and natural medical agent.

Food consumption and body weight were determined and the biological experiment was lasted for 4 weeks. The animals were sacrificed, the blood was collected in the end of the biological experiment from the orbital plexus. Blood was allowed to clot and then centrifuged at 3000 rpm for 15 min, and serum kept at $20^{O}C$ until required.

Biochemical analysis methods. Body weight gain, food efficiency ratio and food intake with organs/ body weight% were measured according to (**Champman et al., 1959**). Blood hemoglobin concentration using Drakbin's solution, hematocrit ratio into a heparinized microcapillary tube and red blood cell were determined **(Drabkin, 1949).**

Glutamic-oxaloacetic transaminase (AST), glutamic-pyruvic transaminase (ALT) (**Reitman and Frankel, 1957**) and alkaline phosphatase (**Belfield and Goldberg, 1971**) were colorimetrical measured. Triglycerides TG, cholesterol CHL and HDL were enzymatic colorimetrically determined in rat serum using the enzymatic colorimetric (**Richmond, 1973; Fassati and Prencipe, 1982**). LDL was calculated (Friedewald et al., 1972) (mg/dl)

Reagent kits were purchased from Gomhoria company. Basal diet was introduced in special feed cups to avoid scattering of feed also water was provided to the rats by glass tubes through the wire case.

Statistical Analysis. Results of biochemical analysis and biological evaluation of each group were statistical analyzed (mean standard deviation and one way ANOVA test) using SPSS package and compared with each other using the suitable test [Least Significant Differences (L.S.D)] (**(SPSS software, version 6.4 2008),**). The levels of significance were accepted with $p < 0.05$.

Results and Discussion

Moringa oleifera is a valuable natural source among other medicinal plants. It is a cruciferous plant belongs to genus Moringa under family Moringaceae. *Moringa oleifera* was mentioned to be among 13 cultivars of Moringa which has health purposes (**Ma et al., 2020**).

Effect of *moringa oleifere* leaves and oil on cholesterol chl and triglycerides TG

The Effect of feeding *Moringa oleifere* leaves and oil was presented in table 1. Control group showed a level of 70.00, and TG 24.15 mg/dl. Significant increase showed for injured group comparing to control group. Compared to CCl_4 group, group hepaticum drug was superior and the highly significant improvement for chl (65 mg/dl). While, TG value (10.0 mg/dl) showed the highly significant improvement in hepaticum the standard drug. In general view, leaves extract has chl and TG values better than that for ethanol the vehicle solvent. For both chl and TG values, oil/hexane has lower value or much healthier than the hexane.

Table (1): Effect of administered *Moringa oleifere* leaves and oil on cholesterol and triglycerides (mg/dl).

Groups	Cholesterol mg/dl	Triglycerides mg/dl
Group1	$70.00^{c} \pm 4.38$	$24.15^{b} \pm 19.17$
Group2	$95.33^{a} \pm 2.73$	$190.0^{a} \pm 1.37$
Group3	$70.00^{c} \pm 3.29$	$40.00^{c} \pm 15.34$
Group4	$84.00^{b} \pm 13.15$	$135.00^{a} \pm 10.95$
Group5	$80.00^{b} \pm 2.19$	$120.50^{ab} \pm 22.46$
Group6	$85.50^{b} \pm 3.83$	$137.50^{ab} \pm 6.57$
Group7	$70.00^{c} \pm 12.05$	$37.50^{d} \pm 0.55$
Group8	$65.00^{d} \pm 2.19$	$10.0^{c} \pm 2.74$
F	9.574	21.357
Sig.	0.000	0.000

Values in table are mean of mentioned numbers as mentioned in materials and methods ± SD, similar letters mean nonsignificant and different mean significant

These results were in agreement with that mentioned by **Hassan et al. (2021).** They discussed that therapeutic effect for moringa leaves may due to numerous phenolic compounds and flavonoids. Besides, they reported that leaves protect the kidney from damage caused by melamine, leaves showed hypoglycemic attributes and attenuate the risk of diabetes caused by alloxan by releasing insulin.

Effect of *moringa oleifere* leaves and oil on lipid profile

The Effect of feeding *Moringa oleifera* leaves and oil on high, low and very low density lipoprotein (HDL, LDL and vLDL respectively) was studied. Positive control or CCl_4 significantly decreased HDL and increased both LDL and vLDL comparing to negative control. All the treated groups and even vehicle groups were above value of HDL for CCl_4 and lower values of LDL and vLDL for CCl_4 group. The most interesting result for HDL was that for

hepaticum and the equal mixture of leaves and oil which recorded 45 mg/dl. This unsignificant result was very closed to that for control group. In the same time, hepaticum group recorded 2 mg/dl for vLDL and leaves extract administration showed 8 mg/dl. That was significant improvement comparing to positive control.

Table (2): Effect of administered *Moringa oleifere* leaves and oil on lipid profile (mg/dl).

Groups	HDL mg/dl	LDL mg/dl	vLDL mg/dl
Group1	$47.67^{a} \pm 1.37$	$17.50^{b} \pm 0.55$	$4.83^{e} \pm 1.10$
Group2	$30.00^{c} \pm 1.10$	$27.00^{a} \pm 16.43$	$38.0^{a} \pm 1.37$
Group3	$44.00^{a} \pm 2.19$	$18.00^{ab} \pm 6.57$	$8.0^{c} \pm 3.83$
Group4	$38.50^{b} \pm 1.64$	$18.50^{ab} \pm 1.64$	$27.0^{c} \pm 3.83$
Group5	$34.50^{bc} \pm 6.02$	$21.50^{ab} \pm 0.55$	$24.00^{bc} \pm 1.10$
Group6	$33.0^{c} \pm 3.29$	$25.00^{ab} \pm 0.89$	$27.5^{b} \pm 0.00$
Group7	$45.0^{a} \pm 6.57$	$17.5^{b} \pm 4.93$	$7.5^{d} \pm 3.29$
Group8	$45.0^{a} \pm 3.29$	$18.0^{ab} \pm 6.57$	$2.0^{d} \pm 0.55$
F	57.530	12.420	2.898

Values in table are mean of mentioned numbers as mentioned in materials and methods ± SD, similar letters mean nonsignificant and different mean significant at ($p < 0.05$).

Effect of *moringa oleifere* leaves and oil on kidney functions

Table 3 showed significant increase in urea, creatinine and uric acid reached to 34, 0.8 and 3.05 mg/dl respectively comparing to negative control. Equal mixture of leaves extract and oil/hexane proved to be the most valuable group reached 23.5, 0.5 and 2.05 mg/dl respectively. Hepaticum was the following group after the mixture and gave significant values 25.15, 0.55 and 2.12 mg/dl comparing to positive control treated with CCl_4. In the same trend, leaves extract showed prospective results and significant records such as 26.33, 0.55 and 2.25 mg/dl comparing to positive control.

Table (3): Effect of administered *Moringa oleifere* leaves and oil on kidney function (mg/dl).

Groups	Urea	Creatinine	Uric acid
Group1	$21.55^{d} \pm 1.26$	$0.50^{d} \pm 0.00$	$1.20^{e} \pm 0.33$
Group2	$34.00^{a} \pm 0.00$	$0.80^{a} \pm 0.00$	$3.05^{a} \pm 0.05$
Group3	$26.33^{bc} \pm 1.37$	$0.55^{cd} \pm 0.05$	$2.25^{cd} \pm 0.16$
Group4	$30.25^{ab} \pm 7.94$	$0.59^{bc} \pm 0.02$	$2.40^{c} \pm 0.00$
Group5	$31.80^{a} \pm 6.24$	$0.60^{bc} \pm 0.00$	$2.70^{b} \pm 0.33$
Group6	$32.90^{a} \pm 1.20$	$0.65^{b} \pm 0.05$	$2.75^{b} \pm 0.05$
Group7	$23.50^{cd} \pm 0.55$	$0.50^{d} \pm 0.11$	$2.05^{d} \pm 0.05$
Group8	$25.15^{cd} \pm 1.26$	$0.55^{cd} \pm 0.05$	$2.12^{d} \pm 0.12$
F	4.983	0.000	0.000
Sig.	0.000	0.000	0.000

Values in table are mean of mentioned numbers as mentioned in materials and methods ± SD, similar letters mean nonsignificant and different mean significant at ($p < 0.05$).

Comparing leaves extract and oil/hexane, moringa leaves as expected showed enhancing in uric acid value followed by oil. Urea values showed better results with leaves supplements and followed by oil submitted group. In another view, creatinine value for moringa leaves extract was much better than oil treatments.

Abou-Zeid et al. (2021) attributed that creatinine increased for its filteration entirely by the glomerulus and minimal reabsorbed by renal tubules. In the time, urea is freely filtered through the glomeruli and reabsorbed with water in the proximal tubules.

Effect of *Moringa oleifere* leaves and oil on liver enzymes

Comparing to negative control, the three liver enzymes (Table 4) have significant increased values reached 258.45, 67.75 and 324.15 U/l for AST, ALT and ALP respectively. Hepaticum group succeeded to have significant enhancement comparing to carbon tetrachloride. These values were 117.7, 51.65 and 194.6 U/l for AST, ALT and ALP respectively. Equal mixture of leaves and oil/hexane approved to have significant values compared with positive control. Except of hepaticum and mixture groups, leaves extract showed high effect in protecting against liver enzymes among other groups.

Table (4): Effect of administered *Moringa oleifere* leaves and oil on liver enzymes (U/l).

Groups	AST	ALT	ALP
Group1	155.30 ^{cd} ± 9.97	51.2 ^{b} ± 7.50	182.3 ^{c} ± 30.23
Group2	258.45 ^{a} ± 27.88	67.75 ^{a} ± 10.19	324.15 ^{a} ± 131.07
Group3	180.85 ^{bcd} ± 55.37	53.15 ^{b} ± 12.54	223.55 ^{bc} ± 11.23
Group4	194.70 ^{bc} ± 31.99	53.15 ^{b} ± 9.37	241.95 ^{bc} ± 13.42
Group5	210.67 ^{b} ± 2.25	56.33 ^{b} ± 1.37	254.00 ^{b} ± 3.58
Group6	217.35 ^{b} ± 38.83	58.00 ^{b} ± 7.45	258.00 ^{b} ± 22.79
Group7	158.00 ^{cd} ± 28.81	53.15 b ± 5.09	203.45 ^{bc} ± 23.50
Group8	117.70 ^{e} ± 7.12	51.65 ^{b} ± 1.04	194.60 ^{bc} ± 19.06
F	0.000	0.000	0.000
Sig.	0.000	0.000	0.000

Values in table are mean of mentioned numbers as mentioned in materials and methods ± SD, similar letters mean nonsignificant and different mean significant at ($p < 0.05$).

Our results agreed with **Hamza (2010)** who found that moringa reduced liver damage as liver fibrosis and decreased CCl_4 induced elevation of serum aminotransferase. He attributed the mechanism for the antioxidant properties and anti inflammatory effect.

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دراسة كيميائية وبيولوجية وهستولوجية لتأثير أوراق وزيت المورينجا اوليفيرا علي أمراض الكبد في فئران التجارب

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الملخص العربي:

تعد أمراض الكبد من بين الأسباب البارزة لحالات الأمراض المزمنة وتؤدي إلى مشاكل صحية عالمية كبيرة. تشمل الأسباب الراسخة لأمراض الكبد العدوى الفيروسية والكحول والسمنة ومقاومة الأنسولين واضطرابات المناعة الذاتية والتمثيل الغذائي والعوامل الوراثية. بغض النظر عن السبب الكامن وراء المبادئ الفسيولوجية المرضية لإصابة الكبد عادة ما تنطوي على التهاب، نخر، وموت الخلايا المبرمج، الإجهاد التأكسدي، وأكثر من ذلك بكثير. وقد تم اقتراح العديد من الاستراتيجيات للحد من مضاعفات أمرض الكبد منها المورينجا أوليفيرا باعتبارها واحدة من النباتات الواقية حيث تمتلك العديد من الخصائص الدوائية مثل المضادة للسرطان، المضادة للسكري، المضادة للالتهابات ومضادات الأكسدة. قد تكون هذه الخصائص بسبب احتوائها علي العديد من المركبات النشطه بيولوجيا ويعد من اشهرها الفينول والفلافونويد

تم تقسيم 40 فأرًا ألبينو بوزن 160 ± 5 جرام إلى 8 مجموعات علي النحو التالي؛ تم تغذية مجموعة الكنترول السلبية (4 الفئران) على النظام الغذائي القاعدي. فقط لمدة اربعة اسابيع وتم تغذية المجموعة الإيجابية (6 الفئران) مع حقن ccl4 على النظام الغذائي القاعدي فقط لمده اربعة اسابيع. تم تغذية المجموعة الثالثة (6 فئران) بعد الحقن بccl4 بمستخلص أوراق المورينجا أوليفيرا لمدة أربعة أسابيع. وتم تغذية المجموعة المعالجة الرابعة (6 فئران) بعد الحقن بزيت المورينجا أوليفيرا لمدة أربعة أسابيع. وتم تغذية المجموعة الخامسة(3 فئران) بالإيثانول بعد الحقن لمدة أربعة أسابيع. وتم تغذية المجموعة السادسة (3 فئران) بالهكسان بعد الحقن لمدة أربعة أسابيع. وتم تغذية (6الفئران)على خليط من الأوراق والزيت معا(1:1) لمدة أربعة أسابيع. وتم تغذية مجموعة الثامنة (6 الفئران)على النظام الغذائي القاعدي مع السيليمارين بعد الحقن لمدة أربعة أسابيع. وجد أنه عند تغذية الفئران بنسب مختلفة من أوراق المورينجا وزيت المورينجا والخلط بينهما أدى ذلك إلى انخفاض في نسبة الدهون (الدهون الثلاثية، الكولسترول الكلي، LDL، VLDLووانزيمات الكبد مثل(ALP، AST وALT.) إلى جانب العديد من التحسينات التي قد لوحظت في التجربة وذلك بسبب الارتفاع مركبات النبات النشطة ومضادات الأكسدة فيه. ويمكن اعتبار أن أوراق المورينجا اوليفيرا وزيت المورينجا اوليفيرا فعالة في علاج امراض الكبد ومضاعفاتها لدي فئران التجارب.

الكلمات المفتاحية: أوراق المورينجا أوليفيرا، زيت المورينجا أوليفيرا، أمراض الكبد دهون الدم.