Role of green tea and *Moringa oleifera* extracts to improve histological and physiological liver cirrhosis induced in mice.

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ABSTRACT

The present study aims to investigate the role of green tea and *Moringa oleifera* extracts in the improvement of histological and phisylogical cirrhotic liver induced experimentally in mice by CCl4. Eighty adult male albino mice weighing 25 ± 3 g were used and divided into 8 equal groups (10 mice / each); Group I: normal control mice group which received no treatment. Group II mice treated with olive oil only at a dose (1ml/kg/bw) twice a week for 6 weeks, Group III -IV: mice administered with green tea or *Moringa oleifera* extracts separately at a dose (600mg/kg/bw/d) or (400mg/kg/bw/d), respectively for one month, Group V: mice injected intraperitoneally (i.p.) by CCl4 at dose (1 ml/kg/bw) added to olive oil (1:1ml) twice a week for 6 weeks to induce cirrhosis, Groups VI -VIII: cirrhotic mice administered with green tea or *Moringa oleifera* extracts separately or co-administered together at the same doses (1:1ml) daily for a month. Histological study of the liver sections of control mice group demonstrated normal architecture of hepatocytes as well as seen in group treated with olive oil or administered with green tea or moringa, each alone. The cirrhotic liver showed loss of hepatic architecture, congested portal and central veins, pyknotic and karyolitic nuclei, hemorrhage in portal area, appearance of fat droplets, activation of Kupffer cells, dilation and thickness of bile ducts and infiltration of inflammatory leucocytes. The cirrhotic liver of mice administered with green tea showed improvement and regained most of normal hepatocytes than that administered with moringa or a mixture of both. By using Masson's trichrome stain, the liver sections of normal control mice demonstrated delicate collagen fibers around central vein as well as in the hepatic tissues of mice groups treated with either olive oil or administered with either green tea or moringa, each alone. The cirrhotic liver of mice group demonstrated intense thickness collagen fibers. Administration of green tea demonstrated improvement and recovery of the normal distribution

of collagen fibers more than that seen after using moringa or a mixture of both together. Physiological studies recorded highly significant increase of alanine amino transferase, aspartate amino transferase and alkaline phosphatase values in the cirrhotic mice group comparable to control one. Significant decrease of all enzymes were recorded after the administration of green tea, moringa or a mixture of both extracts to cirrhotic mice group, but the green tea was more effective than moringa or both together.

Keywords: Liver, Cirrhosis, Green tea, *Moringa oleifera*, Histology, ALT, AST, ALP, Mice.

Introduction

Liver cirrhosis is represented the final stage of liver fibrosis, and it is characterized by distortion of the liver parenchyma associated with fibrous septa and nodules formation as well as alterations in blood flow **(1)**, degeneration and necrosis of hepatocytes, dilation and congestion in blood sinusoids, extensive scar tissues (fibrosis) and cell death **(2)**. Liver function has begun to fail after liver damage. Liver cirrhosis leads to elevation of liver enzymes in to the blood that result from the damage of the liver cell membrane **(3).** The major cause of death in Egypt is primarily associated with liver cirrhosis **(4&5).**

Treatment for cirrhosis depends on the causes of the disease and whether complications are present. In the early stages of cirrhosis, the goals of treatment are to slow the progression of tissue scarring in the liver and prevent complications **(6).** There are many herbal therapies effect on liver cirrhosis and liver disease such as green tea and *Moringa oleifera.*

Green tea (*Camellia sinensis*) contains many compounds especially polyphenols. Epidemiological studies showed that the risk of a variety of diseases is reduced by polyphenolic compounds present in tea **(7).** Catechins are the main compounds in green tea; they consist of (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin-3-gallate, and (-)-epigallocation-3-gallate (EGCG) **(8).** EGCG considers the most biologically active constituent in green tea, and it is recognized as a component that provides the beverage with potential benefits for human health **(9).** Green tea catechins have benefit properties and are thought to act as an antioxidant in biological systems. Tea extracts or tea polyphenols have protective effects against liver fibrosis and liver cirrhosis, antimutagenic and anticarcinogenic inhibiting cancer cell proliferation and induction of apoptosis **(10).**

Moringa oleifera is suggested as a viable supplement of dietary minerals as K, Ca, P, Fe, and is a good source of protein, beta-carotene, amino acids, vitamins A, B & E, riboflavin, nicotinic acid, and various phenolics

compounds as zeatin, quercetinβ -sitosterol, caffeoylquinic acid and kaempferol. *Moringa* leaves extract is also essential phytochemicals **(11-14).** It is used as potential antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial agent. Moringa extracts improve the hepatotoxicity due to the reduction in the level of reactive oxygen species **(15).**

The present work was planned to study the role of green tea and *Moringa oleifera* extracts separately or co-administrated together in the improvement of histological and physiological liver cirrhosis of mice induced-experimentally by CCl4.

Materials and Methods

1. Animal selection and care:

Eighty adult male albino mice, 5 weeks old and weighing 25 ± 3 g were used in the present study and were obtained from Vacsera 51 Wezaret El Zeraa St. Agouza, Giza, Egypt. The animals were housed in plastic cages (10 per cage) for one week acclimatization under the same condition of temperature and natural dark- light cycle. Food and tap water were freely available to the animals throughout the experiment and all producers were in accordance with the approval of the Institution Animal Ethics committee of National Research Center of laboratory animals.

2. Induction of cirrhosis:

CCl4 and olive oil were received from Vacsera 51 Wezaret El Zeraa St. Agouza, Giza, Egypt used to induce liver cirrhosis. CCl4 at a dose 1ml/kg/bw was added to olive oil (1:1ml) and was injected intraperitoneally (i.p.) to mice twice a week for 6 weeks according to **Sakaida *et al*. (16).**

3. Treatment :

a) Green tea (GT) extract was received from local pharmacy and administered orally by (gastric tube) to mice every day at a dose 600 mg/kg/bw according to **Thomas and Thomas (17)** for one month, **b)** *Moringa oleifera* (MO) extract was received from Vacsera 51 Wezaret El Zeraa St. Agouza, Giza, Egypt and given orally to mice at a dose 400 mg/kg/bw/d for one month **(18)**, and **c)** mixture of GT and MO extracts (1:1ml) were administered to mice at same previous doses daily for one month.

4. Experimental design:

Eighty mice were divided into 8 equal groups (10 mice/each), **Group I:** normal control mice group received no treatment. **Group II:** mice injected (i.p.) with olive oil only at a dose 1 ml/kg/bw twice a week for 6 weeks. **Groups III-IV:** mice received orally GT or MO extracts separately at the previous mentioned doses for one month. **Group V:** liver cirrhosis of mice group which induced by CCl4 at a dose 1 ml/kg/bw added to olive oil (1:1ml) injected i.p. twice a week for 6 weeks. **Group VI-VIII:** cirrhotic mice administered with GT or MO extracts or together orally at the same doses mentioned before.

At the end of experimental period, mice were sacrificed after 5 hrs and the blood were collected from the reto-orbital plexus from all mice groups for physiological study and the liver specimens were carefully removed and fixed in 10% neutral buffered formalin for histological study.

a. Histological preparation:

The fixed liver specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin wax, sections of 5 microns thickness were cut using rotary microtome and mounted on clean slides. The parrafin sections were stained with haematoxyline and eosin (H&E) **(19)** for histological study, and with Masson's trichrome to demonstrate collagen fibers **(20)** in the hepatic tissues under light microscope.

b. Physiological estimations:

The collected blood sera were centrifuged at 2500xg for 15 minutes at 30°C to measure alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) in the blood sera by colormetric method by using Biomed Kit according to **Tiez *et al*. (21).**

Statistical analysis:

Statistical analysis data was expressed as mean and standard error and carried out by one-way analysis of variance (ANOVA). Significant differences in means were set at $P<0.001$.

Results

1) Histological observations: -

a- Haematoxylin & Eosin (H&E):

- **Control mice groups (groups I-IV):-** Sections of the liver of normal control mice (group I) stained with H&E showed normal architecture of hepatocytes with normal cytoplasm and nuclei. Hepatocytes are polyhedral in shape, arranged in single-cell cords or plates and they are linked together via intercellular adhesion complexes. All the hepatocytes seem to be apparently homogeneous by light microscopy. Normal central veins and normal Kupffer cells that located in the blood sinusoids are seen as well as that observed in the liver mice treated with olive oil or administered with either GT or MO extracts separately (groups II-IV) **(Fig1).**
- **Cirrhotic mice group (group V):** The cirrhotic liver sections could be induced by the injection of mice with CCl4 added to olive oil (1:1ml) at a dose (1ml/kg/bw) twice a week for 6 weeks showed loss of hepatic architecture with the appearance of pyknotic and karyolitic nuclei, congested portal and central veins, hemorrhage and congestion of central veins and portal areas, fat droplets accumulation, dilated and thickness of bile duct, infiltration of inflammatory leucocytes and activations of Kupffer cells **(Figs. 2&3).**

- **Cirrhotic mice administrated with GT extract (group IV):** The cirrhotic liver of mice administered with GT extract at a dose (600mg/kg/bw/d) for one month illustrated many obvious improvements and regained approximately normal structure of the hepatocytes with normal homogenous cytoplasm, intact nuclei and appearance of normal central veins in most liver tissues. **(Fig. 4).**

- **Cirrhotic mice administrated with MO extract (group V):** Moderate improvements were demonstrated in the hepatocytes, cytoplasm, blood sinusoids and central veins of cirrhotic mice administered with MO extract at a dose 400mg/kg/bw/d for one month. Some degeneration cytoplasm, necrotic cells, dilated blood sinusoids with activated kupffer cells were still appeared **(Fig. 5).**

- **Cirrhotic mice administrated with a mixture of GT and MO extracts (group VI):** Minimized improvements in the hepatic architecture of cirrhotic mice administered with a mixture of GT and MO extracts (1:1ml) at the same doses explained previously for one month were observed. Hepatocytes, blood sinusoids, kupffer cells and central veins appeared with abnormal structure; there were degeneration of cytoplasm with pyknotic and karyolitic nuclei in addition to necrotic areas in many lobules of hepatic tissues **(Fig. 6).**

In brief, the administration of cirrhotic mice with GT extract showed a lot of improvements and regained of hepatocytes architecture to normal structure more than MO extract or a mixture of both together.

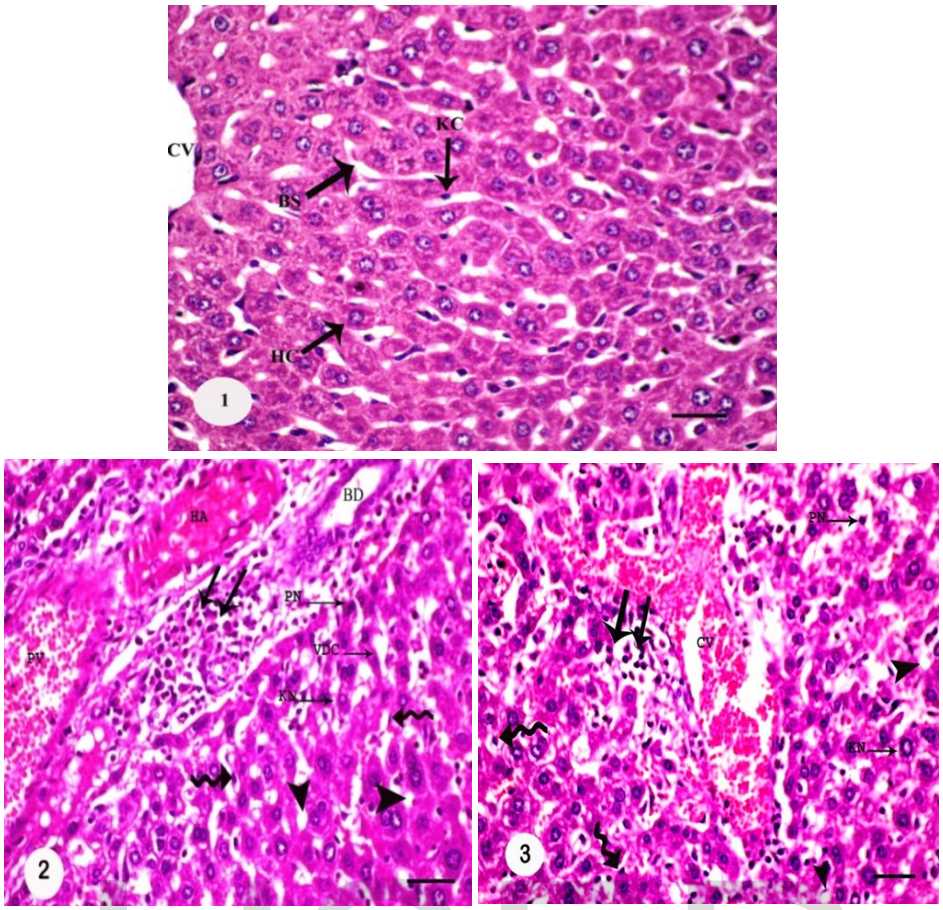


Fig.(1): Section of the liver of a normal control mouse showing normal architecture of hepatocytes (HC), central vein (CV) and blood sinusoids (BS) with normal Kupffer cells (KC). H&E, Bar =6.25µm.

Figs.(2&3): Sections of the liver of mice which injected with CCl4 at a dose 1ml/kg/bw twice a week for 6 weeks showing loss of hepatic architecture with the appearance of pyknotic (PN) and karyolitic nuclei (KN), congested portal and central veins (PV&CV respectively), hemorrhage in portal area (HA), appearance of fat droplets (arrowheads), dilated and thickness of bile duct (BD), infiltration of inflammatory leucocytes (double arrow) and activation of Kupffer cells (zigzag arrows). H&E, Bar = 6.25 µm.

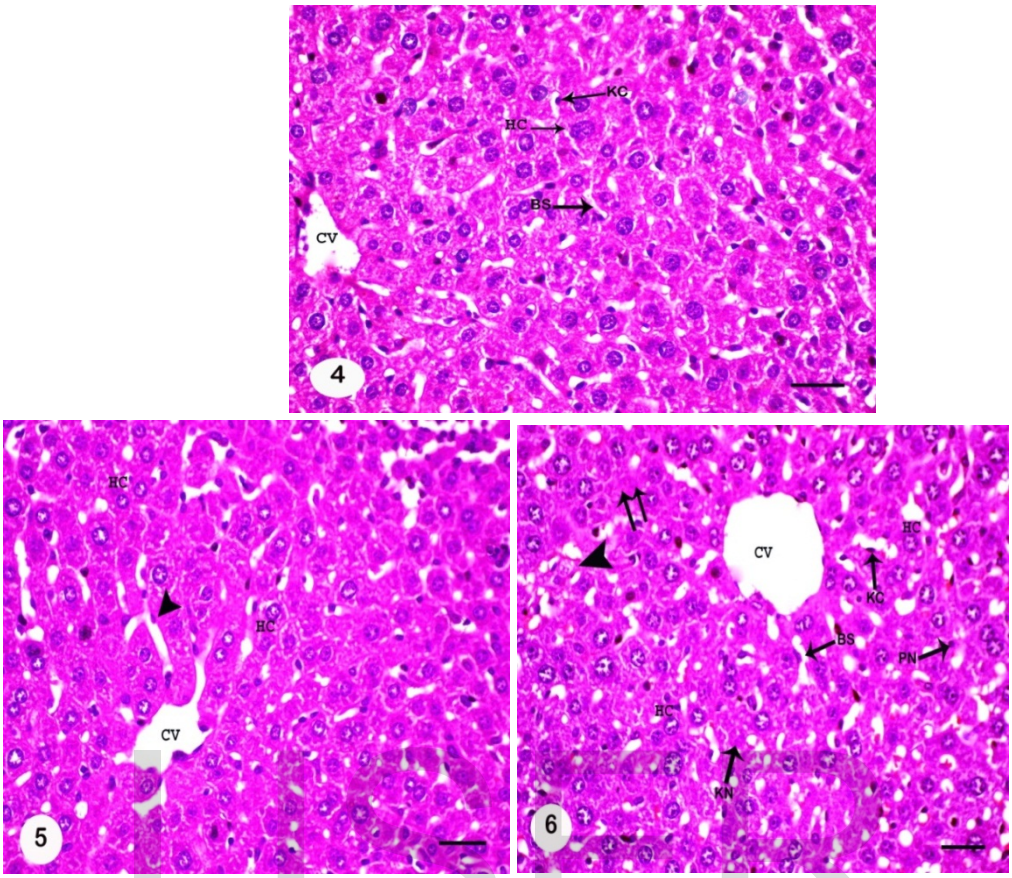


Fig.(4): Section of the cirrhotic liver of a mouse received GT extract at a dose 600mg/kg/bw/d for one month showing an obvious improvement and regain approximately normal structure of the hepatocytes (HC) with normal homogenous cytoplasm and intact nuclei in most liver tissue, normal blood sinusoids (BS) with normal Kupffer cells (KC) and normal central veins (CV). H&E, Bar = 6.25 µm.

Fig.(5): Section of the cirrhotic liver of a mouse administered with MO extract at dose (400mg/kg/bw/d) for one month showing moderate improvement in hepatocytes (HC) and central vein (CV). Dilated blood sinusoids with activated Kupffer cells (arrowheads) are still demonstrated. H&E, Bar = 6.25 µm.

Fig.(6): Section of the cirrhotic liver of a mouse administered with a mixture of GT and MO extracts for a month showing minimized improvements in hepatocytes (HC), blood sinusoids (BS), Kupffer cells (KC) and central veins (CV). Degeneration of cytoplasm (arrowhead) with pyknotic (PN) and karyolitic (KN) nuclei are still demonstrated (double arrows). H&E, Bar = 25µm.

b- Masson's trichrome:-

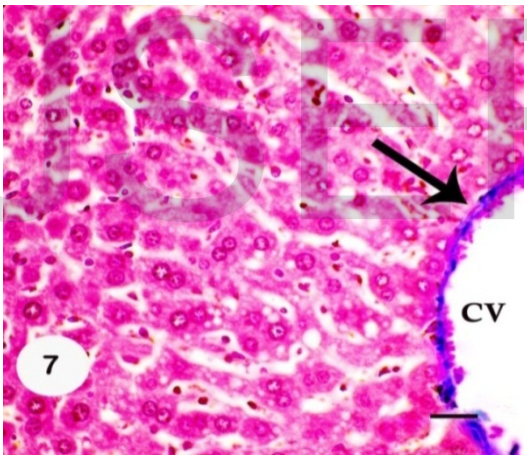
The collagen fibers or fibrotic tissues can be demonstrated as a blue colour by Masson's trichrome stain. Liver sections of normal control mice (group demonstrated normal distribution of delicate collagen fibers around central veins as well as that observed in the

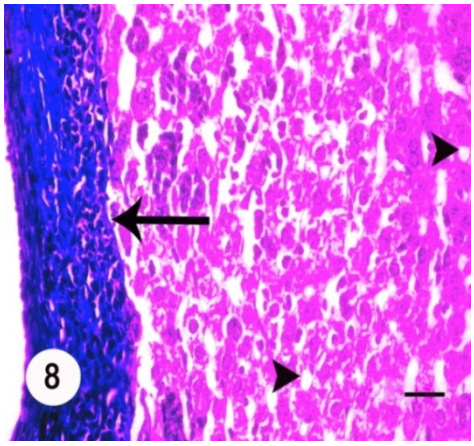
liver mice treated with olive oil or administered with either GT or MO extracts separately (groups II-IV) **(Fig.7).**

The cirrhotic liver of mice induced by CCl4 (group V) demonstrated highly thickness of collagen fibers peripheral to the hepatic nodules **(Fig.8)** and highly intense distribution of collagen fibers were seen at the necrotic portal areas and around the thickness of blood vessels **(Fig. 9).**

The administration of the cirrhotic mice with GT extract (group VI) or MO extract (group VII) demonstrated the liver sections with approximately normal delicate distribution of collagen fibers around central veins similar to control one **(Figs. 10&11).** While the administration of the cirrhotic liver with a mixture of GT and MO extracts (group VIII) demonstrated nearly normal delicate distribution of collagen fibers peripheral to central vein **(Fig.12).**

In brief, administration of cirrhotic liver mice with either GT or MO extracts or a mixture of both together demonstrated approximately normal fine distribution of collagen peripheral to central vein similar to normal liver.





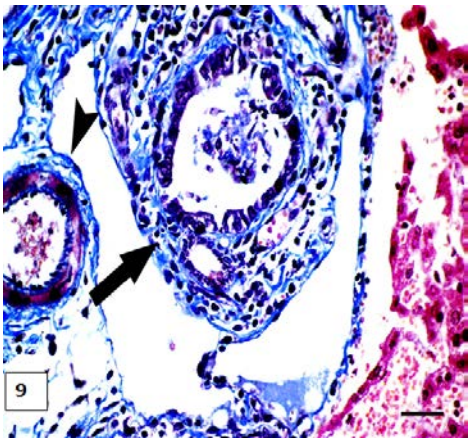
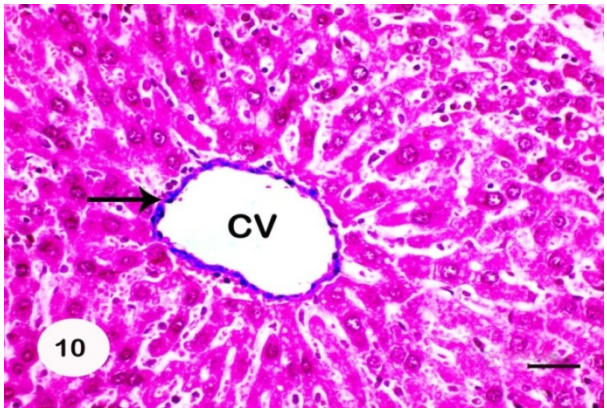


Fig.(7): Section of the liver of a normal control mouse showing normal distribution of delicate collagen fibers around central vein (arrow). Masson's trichrome, Bar=6.25 µm.

Figs.(8&9): Sections of the liver of mice injected with CCl4 at a dose 1ml/kg/bw twice a week for 6 weeks showing: **Fig(8):** highly thickness of collagen fibers peripheral a nodule (arrow), note, fat droplets are cleared (arrow head), and **Fig.(9):** highly intense distribution of collagen fibers at the necrotic portal area (thick arrow), and around the thickness of blood vessel (arrow head). Masson's trichrome, Bar = 6.25µm.



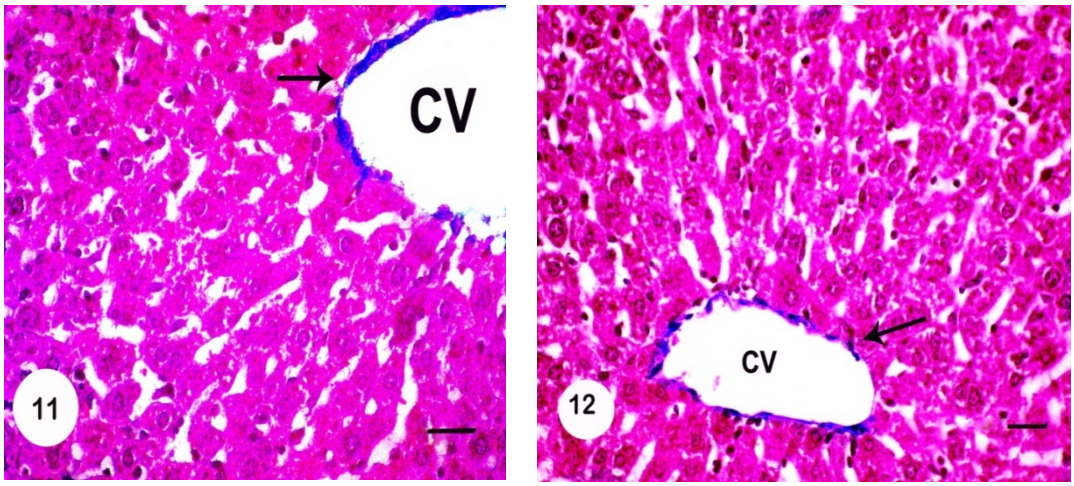


Fig.(10): Section of the cirrhotic liver of a mouse administered with GT extract showing normal fine distribution of collagen fibers around central vein (arrow). Masson's trichrome, Bar=6.25 µm.

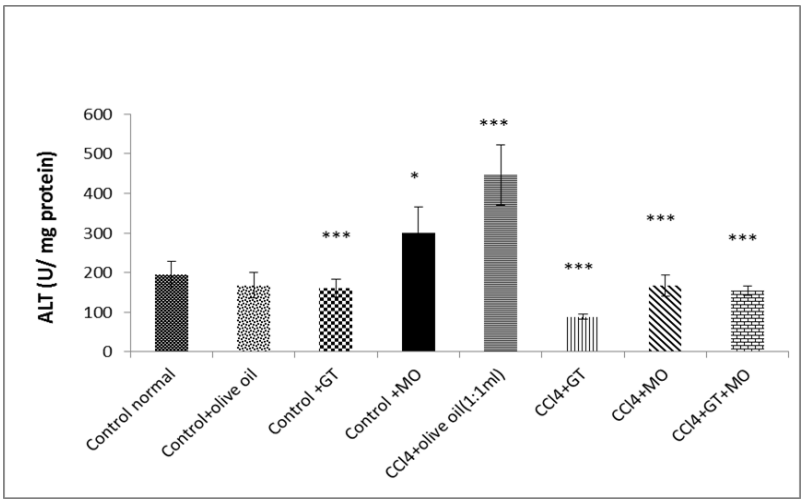
Fig.(11): Section of the cirrhotic liver of a mouse administered with MO extract showing approximately normal fine collagen fibers peripheral to central vein (arrow). Masson's trichrome, Bar=6.25 µm.

Fig.(12): Section of the cirrhotic liver of a mouse administered with a mixture of GT and MO extracts demonstrating nearly normal delicate distribution of collagen fibers peripheral to central vein (CV) (arrow). Masson's trichrome, Bar=6.25µm.

2) Physiological observations:

A- Effect of the two herbal extracts on ALT activity of the liver cirrhosis-:

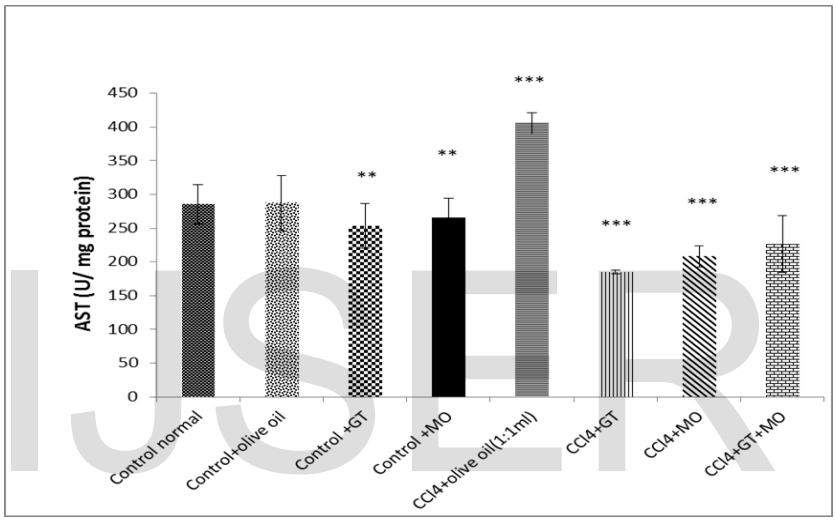
The present study revealed that cirrhotic group showed significant increase in serum ALT activity as compared to normal control group (***P<0.001), significant decrease in GT or MO extracts separately or a mixture of GT+ MO extracts administered cirrhosis groups as compared to cirrhotic group (***P<0.001), significant decrease in group received GT extract as compared to cirrhotic group (***P<0.001) and significant decrease in group received MO extract as compared to cirrhotic group (*P<0.05) **(Graph 1).**



Graph 1:- Effect of GT and MO extracts on ALT activity of liver cirrhosis induced in mice by CCl4. Significant at ∗P<0.05, ∗∗P<0.01 and ∗∗∗P<0.001.

B- Effect of the two herbal extracts on AST activity of the liver cirrhosis:-

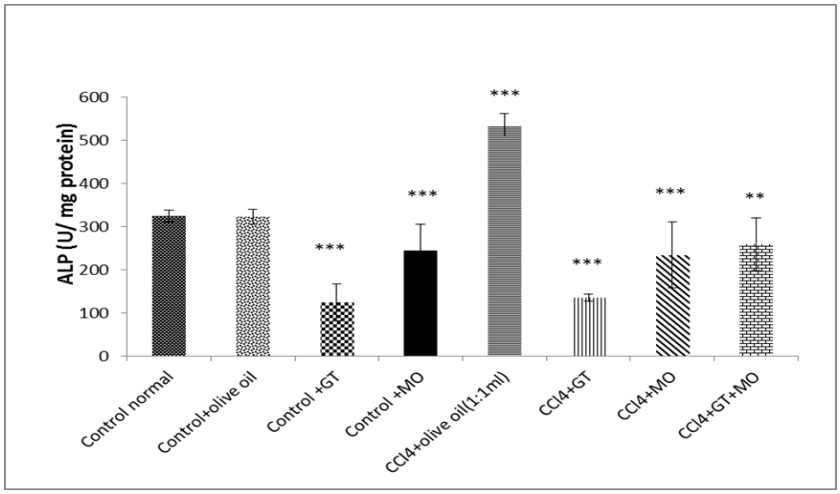
The present study revealed that cirrhotic group showed significant increase in serum AST activity as compared to normal control group (∗∗∗P<0.001), significant decrease in groups administered with GT or MO extracts separately or a mixture of GT+MO extracts to cirrhosis groups as compared to cirrhotic group (∗∗∗P<0.001) and significant decrease in groups received GT or MO extracts separately as compared to cirrhotic group (∗∗P<0.0) **(Graph 2).**



Graph 2: Effect of GT and MO extracts on AST activity of liver cirrhosis induced in mice by CCl4. Significant at ∗P<0.05, ∗∗P<0.01 and ∗∗∗P<0.001.

C-Effect of the two herbal extracts ALP activity of the liver cirrhosis :-

The present study revealed that cirrhotic group showed significant increase in serum ALP activity as compared to normal control group (∗∗∗P<0.001), significant decrease in GT or MO extracts separately administered to cirrhosis groups as compared to cirrhotic groups (∗∗∗P<0.001), significant decrease in groups received GT or MO extracts separately as compared to cirrhotic group (∗∗∗P<0.001) and significant decrease in a mixture of GT+MO extracts administered to cirrhosis group as compared to cirrhotic group (∗∗p<0.01) **(Graph 3).**



Graph 3: Effect of GT and MO extracts on ALP activity of liver cirrhosis induced in mice by CCl4. Significant at ∗P<0.05, ∗∗P<0.01 and ∗∗∗P<0.001.

Discussion

Liver disease results from many causes change in the liver structure and function due to scar tissue replace normal tissue and block the blood flow through the liver. In this case, it called liver fibrosis progressed to liver cirrhosis **(22).**

In the present work, the liver cirrhosis is induced by using CCl4 at a dose 1 ml/kg/bw twice a week for 6 weeks similar to **singh *et al*. (23) and Olatosin *et al*. (24).** Many changes were seen in hepatic tissues as loss of hepatic architecture with cytoplasmic degeneration, pyknotic and karyolitic nuclei, congested and dilated central veins and portal veins, fat droplets accumulation, appearance of nodules separated by thick fibers, infiltration of inflammatory leucocytes and activation of Kupffer cells.

Similar results of liver changes were seen in the liver cirrhosis induced by CCl4 **(25)** as well as seen by **Elsakka *et al*. (26)** and **Wang *et al*. (27)** who found that the fibrous septa and nodules were appeared in area with hepatic injury induced by CCl4. Lipid accumulation were also demonstrated around capillary because of the problems in lipid metabolism caused by CCl4 free radicals **(28).**

CCl4-induced liver injuries are mediated through the activation of cytochrome P450 to produce reactive intermediates such as trichloromethyl free radical (CCl3and trichloromethyl peroxy radical (CCl3OO . These radicals can bind to cellular molecules (nucleic acids, proteins, and lipid), forming alkoxy and peroxyl radicals to produce lipid peroxide, causing damage to cell membrane

and changes in enzyme activity **(29).** Therefore, the inhibition of production and activation of free radicals is considered a very important factor for the prevention of CCl4-induced liver injury **(30).**

The present findings showed that the administration of green tea (GT) extract at a dose 600mg/kg/bw or *Moringa oleifera* (MO) extract at a dose 400mg/kg/bw extract or a mixture of both together for one month to cirrhotic mice improved and regained the architecture of hepatocytes with approximately normal cytoplasm and nuclei, central veins, blood sinusoids, Kupffer cells and the disappearance of fat droplets. However, GT extract was more effective than the MO extract or a mixture of both together to improve and regain the hepatocytes architecture to normal form.

In accordance, **Noori *et al*. (31)** and **Chunga *et al*. (32)** illustrated that GT treated liver cirrhosis induced by CCl4 markedly prevented alternations in liver damaged tissues, reduced sever of liver inflammation, steatosis and lipid accumulation histologically. The animals with fatty liver disease treated with GT extract at various doses showed normal lobular structure with no fatty change around central vein and improvement of liver steatosis **(16).** Moreover, **Safer *et al*. (33)** recorded that GT improved liver injury and disappeared many histological abnormalities of liver such as hepatocyte destruction, vacuolated cytoplasm, large fatty cells and inflammation.

GT extract contains the polyphenol epigallocatechin-3-gallate (EGCG) that improved fibrotic group by decreasing the fibrotic areas **(34)** and decreased liver necrosis, inflammation, hypertrophy and hemorrhage **(35).** Additionally, **Elgawisha *et al*. (36)** reported severe hepatic fibrosis induced by CCl4 in hamster, and it was substantially reduced by the administration of GT extract.

Polyphenols in GT prevent oxygen free radical-induced hepatocytes damage, prevents lipopolysaccharide-induced liver injury through inhibition of inducible nitric oxide synthase and tumor necrosis factor-α expression and inhibits carcinogen or toxin induced liver oxidative DNA damage **(37).** GT contains catechins which have antioxidant properties that have protective effects against liver fibrosis and cirrhosis in rats **(10).**

The administration of MO extract in the present study reduced the damage in the histological architecture of the liver cirrhosis that induced in mice by CCl4 and most of the hepatocytes appeared normal **(15).** Similar observations were seen by maintaining the structure integrity of the hepatocellular membrane in liver injury treated with MO extract and exhibited a remarkable restoration in the histological profile **(38).**

Additionally, in agreement with many authors, the rats treated with MO extract after induction of hepatic injury by CCl4 have recovered most normal area of hepatic tissue compared to group of hepatic injury **(39&40).**

Also, **Olatosin *et al*. (24)** recorded that MO seed oil prevented hepatotoxicity of CCl4 and the histological structure feature of necrosis .

MO has hepatoprotective activity against oxidative stress **(11&41).** MO contains flavonoids which are phytochemicals that responsible for antioxidant activities **(15).** The antioxidants of MO prevent oxidative damage of a tissue indirectly by enhance natural defense of cell and/or prevent reactive species generated during liver injury **(42).**

The present results demonstrated that GT extract was more effective than MO extract after administration to cirrhotic mice probably may due to GT contains polyphenols (antioxidant) about 90% **(43)** while MO has (65.1-66.8%) phenolic compounds that act as antioxidants **(44).** Antioxidants prevent oxidative damage of a tissue indirectly by enhancing natural defenses of cell and/or directly by scavenging the free radical species **(42).** However, the administration of high level of antioxidants than usual has a negative impact on health **(45)**. Thus, it may be propably in the present work, a mixture of GT and MO has less improvement in the cirrhotic liver mice.

Collagen fibers are the main structural protein in the extracellular space in the various connective tissues in animal bodies. They are the most abundant protein in mammals. Collagen fibers are making up from 25% to 35% of the whole-body protein content depending upon the ratio within other tissues. Collagen fibers are also found in the wall of blood vessels and inter-follicular blood capillaries **(46).**

The present results revealed in the cirrhotic mice group an obvious increase of fibrotic tissues peripheral the nodules, around the central veins, at the necrotic portal area and around thickness blood vessels. After administration of cirrhotic mice with GT or MO or both together, it was seen that the liver sections demonstrated approximately normal delicate distribution of collagen fibers.

Similarly, **Fujii *et al*. (47)** declared a significant level of fibrosis in the cirrhotic rats induced by CCl4 indicated by the extensive accumulation of collagen fibers showed cirrhosis and nodular formation with fibrosis. CCl4 initiates fibrogenic pathway by the effect of its free radical and oxidative stress that activate hepatic stellate cells (HSCs) **(48).** HSCs are the main source of collagen synthesis during hepatic fibrosis. Activation of HSCs is associated with the accumulation of extracellular matrix, including types I and III collagens. During liver fibrogenesis and oxidative stress which are a key mechanism in chronic liver damage and fibrosis, the chronic inflammation and reactive oxidative species play a mean role in the activation of HSCs. CCl4 hepatotoxicity depends upon its metabolism by cytochrome P-450, which generates highly reactive trichloromethyl free radicals, leading to lipid peroxidation and membrane damage **(25).**

The administration of GT extracts to cirrhotic group was resulted a decrement of collagen fibers **(33&49).** Cirrhotic hamsters that were administered with GT extracts showed less thick fibrotic tissues which resulted in less destruction of the liver architecture **(36).** An obvious improvement of fibrotic tissues in hepatotoxic liver induced by ethanol or CCl4 and demonstrated similar to normal after administration with GT extract. GT extract have antioxidant, hepatoprotective and anti-inflammatory effects that inhibitebd HSCs activations and fibro-genesis and prevent liver fibrosis and cirrhosis **(50).**

Moreover, the administration of MO to fibrotic liver of rats has significally reduced the score of liver fibrosis by anti-oxidation, hepatoprotective and anti-inflammatory effects of moringa **(41).** MO extracts has antioxidant property that protected against CCl4-induced toxicity and fibrosis. MO contains important bioactive compounds including glucosinolates, isothiocyanates, thiocarbamates and flavonoids. These compounds prevent ROS activity and regenerate membrane-bound antioxidants, then reduce activation of HSCs and prevent collagen accumulation **(51).**

The present results recorded a highly significant increase in activities the of AST, ALT and ALP serum levels in cirrhotic liver mice induced by CCl4. The activities of these enzymes were significantly decreased in cirrhotic mice after administered of GT or MO extracts or a mixture of both together, but GT extract was more significant effective than MO extract or co-administration of both.

Many studies have shown a correlation between AST/ALT ratio and presence of liver cirrhosis **(52&53).** The results of **Sreelatha and Padma (15)** recorded that CCl4 raised the serum level enzymes like ALT, AST and ALP in rats which are indicator to liver injury. The elevation in the serum liver marker enzymes result from the hepatotoxic effect of CCl4 due to its active metabolite, trichloromethyl radical and the free radicals caused structural integrity damage of the liver cell membrane and hence a leakage of the cellular enzymes into the blood **(3).** Moreover, these enzymes were significant raised in viral hepatitis and cirrhosis patient as compared to control **(53).** Liver function has begun to fail after liver damaged in chronic liver disease **(17&18).**

A decrement of ALT serum in cirrhotic rats administered with GT was recorded **(31).** Moreover, GT also improved the value of ALT, AST and ALP due to preventing of intracellular enzyme leakage resulting from cell membrane of hepatocytes diseases in comparable to CCl4 group **(33&54).**

Conversely, other researchers reported that ALP levels increase in CCl4 + GT treated group compared to CCl4 group **(31).** Many herbal supplements contain compounds such as GT carry potentially severe side effects including hepatotoxicity, this affected liver functions levels (AST, ALT and ALP) indicated hepatocellular injury **(55).**

Oral administration of MO extract to cirrhotic liver of mice in the present results showed a significant protective action made evident by its effect on the levels of AST, ALT and ALP. Similar results were recorded in the administration of MO leaves extract to CCl4 induced rats that decreased the activity of liver function enzymes (AST, ALT and ALP) **(40).** Moreover, MO leaves have hepatoprotective activity and alleviating activity of AST, ALT and ALP enzymes in liver damaged of Wistar rats group **(38).** MO showed hepatoprotective activity in subchronic treatment which may be due to protection against oxidative stress **(13&40).** High antioxidative compounds present in MO leaves complement the nutritive role by counteracting reactive species generated during liver injury in nonalcoholic fatty liver diseases **(56).**

The physiological data of ALT, AST and ALP serum levels activities confirmed the histological results in the current study. These enzymes recorded elevation in the blood sera of cirrhotic mice due to damage and destruction of liver cells and cell membrane. However, these enzymes were decreased in blood sera of cirrhotic liver mice after given with GT or MO or mixture of these two herbs extract, because they have antioxidant properties that were more in GT extract.

In conclusion, administration of either GT or MO or co-mixture of them to mice with liver cirrhosis illustrated the recovery and improvements of hepatic tissues and functions but GT extract alone was more effective than MO extract alone or a mixture of both to improve liver cirrhosis.

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