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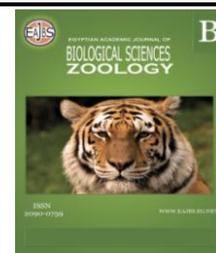


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Biochemical and Histopathological Effects of Colchicine and *Moringa oleifera* on Thioacetamide-Induced Liver Fibrosis in Rats

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ABSTRACT

Liver fibrosis is the progressive accumulation of connective tissue in the liver, which can be caused by chronic liver injury of various etiologies. *Moringa oleifera* is a pan-tropical species is used in nurturing both animal and human as an excellent nutritive supplement. Colchicine is a plant that effective against gouty arthritis and other forms of rheumatic diseases (rheumatoid arthritis, familial Mediterranean fever, Bechet's disease, etc. This study thrown light on the hepatoprotective effects of *Moringa oleifera* ethanolic leaves extract and colchicine on thioacetamide-induced liver fibrosis in rats. Liver function tests (serum ALT, AST, ALP, and Total protein) in addition to histopathological examination of the liver were performed. This study was carried out on a total number of 42 male albino rats were collocated into seven groups (6 rats per group). The experiment lasted for two months, through which all groups except for control and control positive were given *Moringa oleifera* ethanolic leaves extract and colchicine. Liver fibrosis was induced by intraperitoneal injection of thioacetamide (150 mg/kg) in groups II, V, VI & VII while other groups were injected with 0.9% normal saline of the same volume. Bodyweight was measured three times a week to detect the dose for each rat. The administration of thioacetamide leading a significant increase in serum liver enzyme level ALT, AST, ALP, and Total protein as compared to the control group, while treatment with colchicine and *Moringa oleifera* ethanolic leaves extract causing significant decreased serum liver enzyme level by ($P < 0.05$) than the induced hepatic group. In general, there is no fundamental difference between groups V, VI, VII. Also revealed that the injection of thioacetamide (150 mg/kg) induced liver fibrosis characterized by a drastic decrease in total protein as compared to the control group by ($P < 0.05$). The colchicine or *Moringa oleifera* ethanolic leaves extract was able to restore dysfunction. In general, the treatment with colchicine and *Moringa oleifera* showed good restore in biochemical function and histopathological of the liver. Also, we detected that there are no differences between them.

INTRODUCTION

The liver performs numerous functions, consisting of lipid, carbohydrate, and protein metabolism; storage, activation of vitamins; storage of minerals, glycogen, and triglycerides; extramedullary haematopoiesis; and synthesis of coagulant, anticoagulant, and several acute-phase proteins (Liu *et al.*; 2012). It also helps in digestion presses through the synthesis of bile acids and detoxification of many xenobiotics, in this case, the liver becomes susceptible to the toxicity from these agents as the metabolic products of detoxification reactions can be destructive the liver when in excess, the liver has a large functional reserve and the ability to regenerate, but if these toxics increased or liver exposure to viruses (B, C) or other chronic inflammatory agents the liver be injured and the processes of liver fibrosis begin (Friedman; 2003;Neil and John; (2007).

Fibrosis is strictly defined as pathological entities that are broadly defined by pathologists and hepatologists several decades ago hepatic fibrosis is a dynamic scarring process in which chronic inflammation stimulates the production and accumulation of collagen and extracellular matrix proteins (Christian *et al.*; 2013). The hepatic stellate cells are the primary cells responsible for producing these extracellular matrix proteins this dynamic process can also involve remodeling and regression of the fibrous tissue via the breakdown of matrix proteins by the protease enzymes matrix metalloproteinases causes only minor clinical symptoms or disturbance of liver cell function which leads to apoptosis (Hernandez-Gea &Friedman; 2011). The balance of the remodeling process occurs with the inhibition of the remodeling by tissue inhibitors of matrix metalloproteinases (Friedman; 2010).

Liver fibrosis is explained as an excessive extracellular matrix statement and is based on complex interactions between matrix-producing hepatic stellate cells and an abundance of liver-resident and infiltrating cells (Kumar *et al.* (2011). Biochemistry is closely related to molecular biology, the study of the molecular mechanisms by which genetic information encoded in DNA can result in the processes of life depending on the exact definition of the terms used, molecular immunocytochemistry biology can be thought of as a branch of biochemistry, or biochemistry as a tool with which to investigate and study molecular biology (Thakur *et al.*; 2008).

Experimental animal models are keys for our understanding of the mechanisms responsible for hepatic fibrogenesis, the most widely used is the rat, which is treated with different methods of liver fibrosis induction as carbon tetrachloride, diethyl nitrosamine, thioacetamide. Thioacetamide is a hepatotoxin causing centrilobular necrosis, which also induces apoptosis and periportal inflammatory cell infiltration in the rat liver. Thioacetamide elevates oxidative stress, enhancing free radical-mediated damage to proteins, lipids and DNA (Singh *et al.*; 2004).

Medicinal plants have been used since ancient times for treating a wide variety of diseases as well as for hepatotoxicity (Heidarian and Rafieian-Kopaei; 2013). Owing to its lower costs and greater compatibility, herbal medicine has received great attention in recent decades (Azadbakht *et al.*; 2003). Herbs are rich in different compounds such as flavonoids, and polyphenols that can protect the liver against damage induced by chronic inflammatory agents (Galisteo *et al.*; 2000).

Moringa oleifera is cultivated worldwide due to its multiple utilities. Every part of *Moringa* is used for certain nutritional and medicinal purposes. Besides being a good source of protein, vitamins, oils, fatty acids, micro-macro mineral elements and various phenolic, it is also reported as anti-inflammatory, antimicrobial, antioxidant, anticancer, cardiovascular, hepatoprotective, anti-ulcer, diuretic, antiurolithiatic, and anthelmintic. Its multiple pharmaceutical effects are capitalized as a therapeutic remedy for various diseases in

the traditional medicinal system (Fozia; 2012). Further research on this charismatic healer may lead to the development of novel agents for various diseases. Phytochemicals are, in the strictest sense of the word, chemicals produced by plants. Commonly, though, the word refers to only those chemicals that may impact on health, or flavor, texture, smell, or color of the plants, but is not required by humans as essential nutrients. An examination of the phytochemicals of *Moringa* species allows examining a range of unique compounds. In particular, this plant family is rich in compounds containing simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates (Bennett *et al.*; 2003). For example, specific components of *Moringa* preparations that reported having hypotensive, anticancer, and antibacterial activity, it is also rich in many vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids (including β -carotene or pro-vitamin A) (Fahey *et al.*; 2001).

Moringa oleifera leaves can be eaten freshly cooked or stored as dried powder for several months the pods when young can be cooked; eaten like beans (National Research Council; 2006). Its oil and micronutrients have been reported to contain anti-microbial, anti-inflammatory and potent antioxidants (Saalu, *et al.*; 2011).

Furthermore, the leaf extract of *Moringa oleifera* regulates thyroid status (Tahiliani, *et al.*; 2000), maintenance of cholesterol levels (Ghasi *et al.*; 2000). The active anti-oxidant properties of *Moringa oleifera* known to contain specific plant pigments include carotenoids-lutein, chlorophyll, xanthine, alpha carotene and beta carotene. Other isolated phytochemicals of *Moringa oleifera* with antioxidant potential are quercetin, rutin, kaempferol and caffeoyquinic acids. Potent antioxidant vitamins- A, C, E (Siddhuraju and Becke; 2003) and essential micronutrients with antioxidant ability are selenium and zinc.

Acute Toxicity (LD₅₀) of the ethanol leaf extract of *Moringa oleifera* in mice was found to be more than 2600 mg/kg and less than 5000 mg/kg body weight. One animal died and the other remaining two animals in the group within 24 h of constant observation (Ugwu *et al.*; (2013).

Colchicine is a tri-cyclic plant alkaloid called autumn crocuses also known as methyl ether of colchicine. It is N-formyl -N-de-acetyl colchicine and 3-demethyl colchicine which during its metabolism it is oxidized to N-Acetyl-L-glutamic acid. Oral corticosteroids have an anti-inflammatory and anti-fibrotic effect (Seganish *et al.*; 2005, Finkelstein *et al.*; 2010). It is widely distributed and binds to intracellular elements. Colchicine is rapidly absorbed after oral administration from the gastrointestinal tract. Colchicine is primarily metabolized by the liver and de-methylated to major metabolites, which include 2-O-demethylcolchicine and 3-O-demethylcolchicine, and one minor metabolite, 10-O-demethylcolchicine (colchicine). According to in vitro studies, CYP3A4 metabolizes colchicine to 2- and 3-demethylcolchicine and is also excreted by the kidneys and enterohepatic recirculation and biliary excretion are routes for excreting of colchicine (Finkelstein *et al.*; 2010). Colchicine inhibits microtubule polymerization by way of binding to tubulin, probably the most primary constituent of microtubules. Availability of tubulin is essential to mitosis, and due to this fact colchicine successfully purposes as a mitotic poison or spindle poison. Since probably the most defining traits of cancer cells is a significantly higher mitosis, because of this cancer cells are significantly extra at risk of colchicine poisoning than customary cells (Nguyen *et al.*; 2005). However, the therapeutic worth of colchicine towards cancer is proscribed through its toxicity against standard cells. Apart from inhibiting mitosis, a procedure heavily dependent on cytoskeletal adjustments, colchicine additionally inhibits neutrophil motility and job, leading to a net anti-inflammatory impact. Colchicine additionally inhibits urate crystals (Raimond *et al.*; 2004).

From this point on a given the importance of the liver as an important organ of the body and the wall threat that fibrosis poses, liver fibrosis was chosen to shed light on it,

trying to understand it and then find a treatment or at least control it. So, this study throws light on the effect of herbal plants and synthetic in the treatment of induced hepatic fibrosis through the determination of the following parameters, liver function tests (serum ALT, AST, ALP, total protein) and histopathological examination of liver.

MATERIALS AND METHODS

Animals:

This study was carried out at the Faculty of Veterinary Medicine of Alexandria. A total of 42 male albino rats weighing 150 ± 20 g and about 90 ± 10 days old were purchased from the Medical Research Institute of Alexandria University, Egypt. The animals were kept as 6 rats in metal cages under environmental controlled conditions with optimum temperature (23 ± 2), humidity (55 ± 5), and dark/light cycle (12h) and they were given a basal diet which consisted of corn 66.3%, SBOM 19.8 %, C. gluten 7.40%, fat 1.60 %, Dical. Phos. 1.89%, limestone 1.24%, lysine 0.40%, meth 0.09%, mineral vitamin premix 0.3%, salt 0.40% and drinking water ad libitum. The international ethical guidelines for the care and use of laboratory animals were performed to handle the animals and the experimental procedures were approved by the Experimental Animal Use and Ethics Committee at the Faculty of Veterinary Medicine, Alexandria University, Egypt. All animals were housed for two weeks before the experiment for acclimatization and to ensure normal growth and behaviour.

Chemicals and Reagents:

Thioacetamide was purchased from Biotechnology chemical Co., Egypt and the molecular formula C_2H_5NS . Molecular Weight is 75.13 (Fig.1).

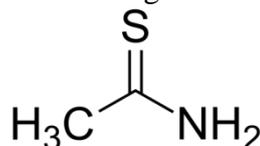


Fig. 1: The structure of Thioacetamide

Colchicine was purchased from a pharmacy (El Nasr pharmaceutical chemicals Co.). The molecular Formula: $C_{22}H_{25}NO_6$ and the Molar mass: 399.437 g/mol (Fig. 2).

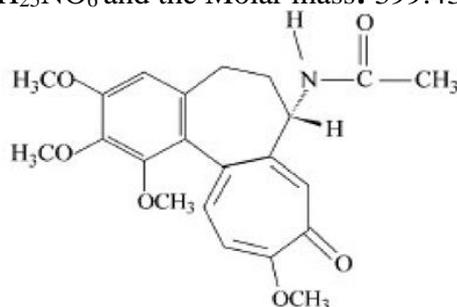


Fig. 2: The structure of Colchicine

Moringa oleifera was purchased from a specialized company for growing up *Moringa oleifera* in Alexandria Farmers Association for Rural Development. Kits for ALT, AST, ALP, and Total protein were purchased from Biosystems Co., Egypt.

Methods:

Preparation of *Moringa oleifera* Leaves Extract:

Moringa oleifera leaf extract was prepared according to the method previously described by (Nilanjan *et al.*; 2012) with some modifications. Leaves of *Moringa oleifera* were purchased from Alexandria Farmers Association for Rural Development. Leaves were thoroughly washed in distilled water and dried in a vacuum oven at 50°C for 10 h. Clean,

dry leaves were crushed and 5 g of the powder was mixed with 50 mL of 90% ethanol. The mixture was stand in a container for about 24h and filtered afterward. The resulting filtrate was evaporated in a rotary evaporator to remove alcohol (adjustment bath: 40-45 °C, rotation: 50 rpm, pressure: ~15 psi, condenser: 4 °C) to avoid denaturation of the active ingredients. The alcohol reresidue of the sample was weighed (~500 mg) and dissolved in 100 mL distilled water to constitute the final extract solution (5 mg/mL).

Experimental Design:

A total of 42 male albino Rats were classified into the following 7 groups each group contains 6 rats as follows: Group I, rats were kept on normal basal diet and water ad libitum and were given 0.9% NaCl solution (1ml/rat) given by intraperitoneal injection 3 times /a week for 8 weeks + given distal water by stomach tube 3 times/a week for 8 weeks. Group II (Thioacetamide treated group): rats received intraperitoneal injection of Thioacetamide dissolved in 0.9 NaCl solutions a dose of 150 mg/kg body weight three times a week for 8 weeks for induction of liver fibrosis according to (El-Kersh *et al.*; 2016). Group III (Colchicine): The rats were treated with colchicine that was given by the stomach tube in a dose of (50 µ/kg days) dissolved in distal water for 8 weeks according to (Vaidya *et al.*; 2018). Group IV (*Moringa olifera* treated group): rats were treated with *Moringa oleifera* ethanolic leaves extract that was given by stomach tube in a dose of 400 mg/kg body weight once daily for 8 weeks according to (El-bakry *et al.*; 2016). Group V (Thioacetamide and Colchicine treated group): rats received a simultaneously intra-peritoneal injection of Thioacetamide in a dose of 150 mg/kg body weight three times a week together with colchicine that given by stomach tube in a dose of (50 µ/kg days) for 8 weeks. Group VI (Thioacetamide and *Moringa oleifera* treated group): rats received simultaneously intraperitoneal injection of Thioacetamide in a dose of 150 mg/kg body weight three times a week together with *Moringa oleifera* ethanolic leaves extract that given by stomach tube in a dose of 400 mg/kg body weight once daily for 8 weeks. Group VII (thioacetamide, Colchicine and *Moringa oleifera* ethanolic leaves extract-treated group): rats received simultaneously intraperitoneal injection of thioacetamide in a dose of 150 mg/kg body weight three times a week together with *Moringa olifera* ethanolic leaves extract that given by stomach tube in a dose of 400 mg/kg body weight once daily and colchicine that given by stomach tube also in a dose of (50µ/kg day) for 8 weeks.

Blood Samples Collection:

At the end of the experiment, the animals were fasted overnight then weighed then the blood collected from the orbital plexuses of the eye under anaesthesia into clean tubes then left to be clotted, the animals then scarified by decapitation; all blood samples centrifuged at 3000 rpm for 10 min at 4°C to obtain cleared and non-hemolyzed sera and plasma then samples were transferred to Eppendorf tubes and stored at 20°C until analysis.

Tissue Samples Collection:

After decapitation, livers were removed from rats of all groups, washed with saline then blotted with filter paper and was fixed in 10 % paraformaldehyde solution for histopathological examination.

The international ethical guidelines for the care and use of laboratory animals were performed to handle the animals and the experimental procedures were approved by the Experimental Animal Use and Ethics Committee at the Faculty of Veterinary Medicine, Alexandria University, Egypt.

Biochemical Analysis:

Determination of serum alanine aminotransferase (ALT) activity (U/L) was determined by colorimetric method according to Young, (1990). Determination of serum Aspartate aminotransferase (AST) activity (U/L) was determined by the colorimetric method according to Young, (1990). Determination of serum alkaline phosphatase (ALP) activity

(U/L) was performed according to Jackson, et al method (1996). Determination of serum Total protein concentration (g/dl) was determined by the colorimetric method according to (Bauer, 1982).

Histopathological Examination:

At the end of experiment, the liver of each animal was fixed in 10 % formalin solution for one week. After that, the liver tissues were dehydrated in ascending series of alcohol. The samples were cleared in xylene and immersed in paraffin. The paraffin blocks were sectioned at 5 μ m thickness and mounted on clean glass slides. The ordinary haematoxylin and eosin stain was used and then read under an optical microscope (Drury and Wallington, 1980)

Statistical Analysis:

The distribution of each variable was assessed with the Shapiro-Wilk test, and variance homogeneity among groups was checked with Levene's test. When parametric assumptions were valid, we used univariate analysis of variance (ANOVA) with Tukey's test as a post-hoc. Otherwise, the nonparametric alternatives (Kruskal-Wallis test and Dunn's post-hoc test) were performed. A probability value of 0.05 or less was considered statistically significant. All analyses were performed using the SPSS statistical software package (version 22, IBM Corp., Armonk, NY, USA).

RESULTS

Biochemical Results:

Effect of *Moringa olifera* ethanolic leaves extract and colchicine on serum liver enzyme activities in thioacetamide-induced liver fibrosis in rats. The injection of thioacetamide-induced liver fibrosis characterized by a drastic increase in serum level of SGPT, SGOT, and ALP as compared to the control group. Administration of colchicine in group III and *Moringa olifera* ethanoilic extract in group IV have no significant changes in liver enzymes compared to control group while these changes are significant as compared to thioacetamide groups. Treatment of hepatic rats with colchicines and administration of *Moringa olifera* with thioacetamide showed a significant decrease in serum levels of SGPT, SGOT, ALP and bilirubin that returned slightly to the normal group levels. The co-administration of colchicine and *Moringa olifera* (400 mg/kg), with thioacetamide-induced rat liver fibrosis revealed that a significant decrease in serum liver enzymes and bilirubin as compared to their respective control groups (Figs.3, 4, 5 &6).



Fig.3: Effect of *Moringa olifera* ethanolic leaves extract and colchicine's on serum SGPT (ALT) in induced thioacetamide liver fibrosis in rats.

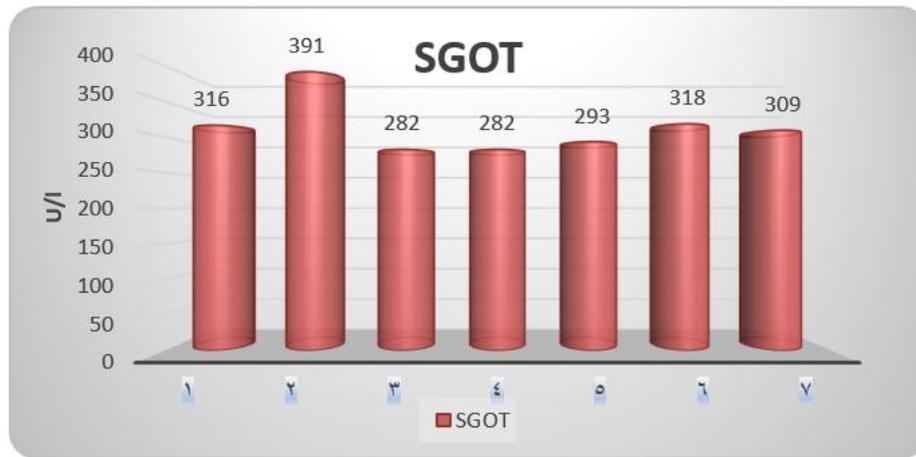


Fig.4: Effect of *Moringa olifera* ethanolic leaves extract and colchicine's on serum SGOT (AST) in induced thioacetamide liver fibrosis in rats.

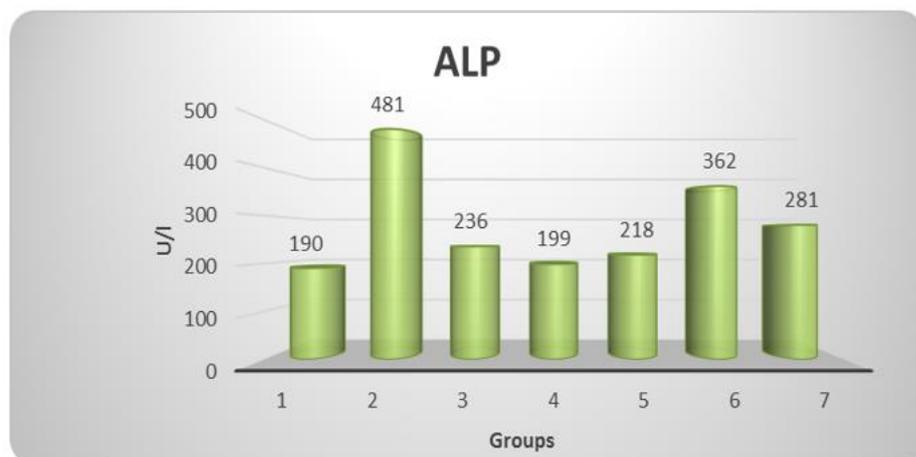


Fig.5: Effect of *Moringa olifera* ethanolic leaves extract and colchicine on serum ALP in induced thioacetamide liver fibrosis in rats.

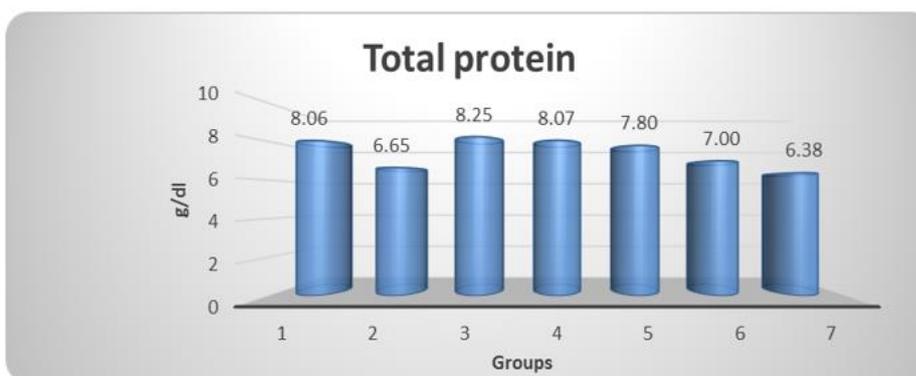


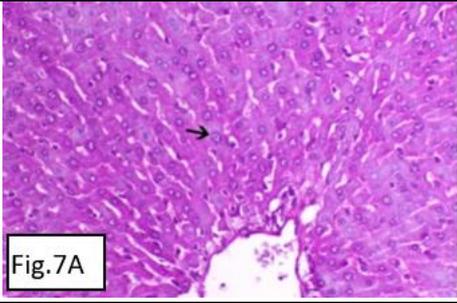
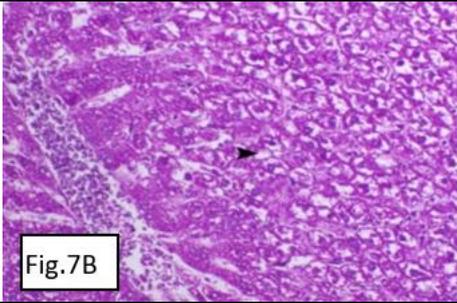
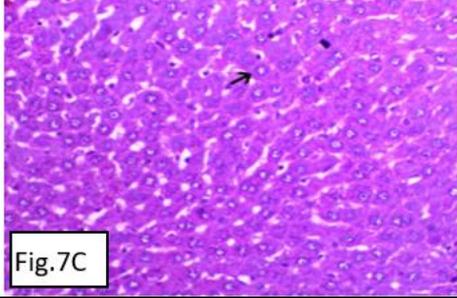
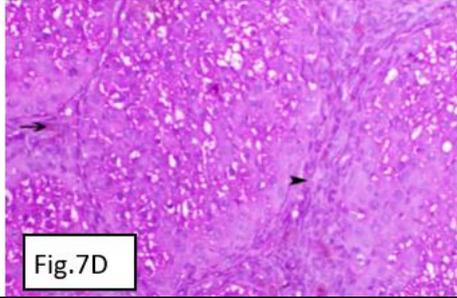
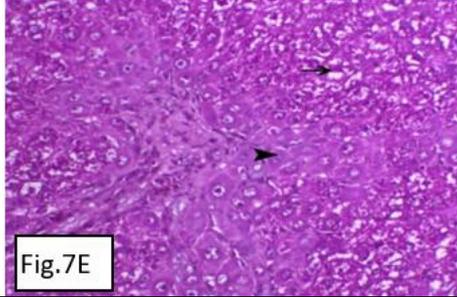
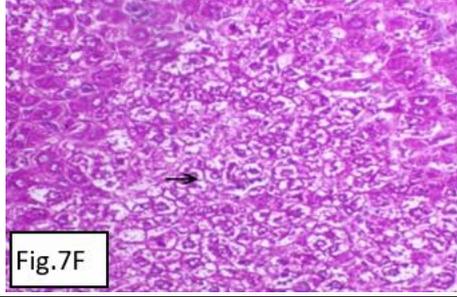
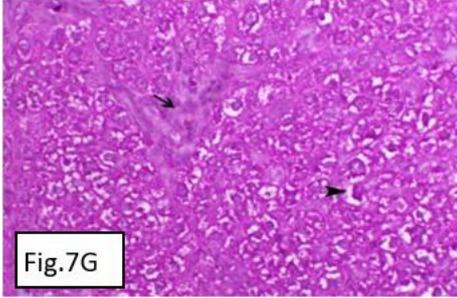
Fig.6: Effect of *Moringa olifera* ethanolic leaves extract and colchicine's on serum total protein in induced thioacetamide liver fibrosis in rats. Columns are means and bars are standard error of means. Groups with C differ significantly ($P < 0.05$) compared to the Control group; groups with T differ significantly ($P < 0.05$) compared to the Thioacetamide group.

The depicted data revealed that the injection of thioacetamide-induced liver fibrosis characterized by a drastic decrease in total protein as compared to the control group. Administration of colchicine's and *Moringa oleifera* ethanolic leave extract in groups II and III respectively showed non-significant changes in total proteins as compared to the control group. The resulted data from the treatment of hepatic rats with colchicine's in group V illustrated that significant increase in total protein as compared to liver induced fibrosis group. The treatment by *Moringa oleifera* in group IV resulted that a significant increase in total protein as compared to the thioacetamide treated group. And these results approximate the control group level. While co-administration of colchicine and *Moringa olifera* with thioacetamide.

Histopathological Results:

The gross appearances of livers from the normal rats in Group 1, II, III appeared reddish with smooth surfaces and without signs of nodules, and histology showed normal architecture. Livers from the fibrotic rats of Group IV appeared congested with numerous micro- and macro-nodules.

Microscopic examination of liver sections from the control rats' group that fed basal diet shows the normal hepatic architecture, where the hepatocytes are arranged in cords around the central veins and alternate with blood sinusoids. Each hepatic cell possesses a limiting membrane, centrally placed one or two large nuclei and prominent nucleoli (Fig. 7A). The liver of rats treated with colchicine showed disturbance of the normal hepatic lobule. The hepatocytes in the midzonal and periportal area showed hepatic vacuolation (Fig.7B). Administration of the ethanolic extract of leaves *Moringa oleifera* revealed the normal structure of the hepatic lobule. The hepatocytes appeared more or less like normal (Fig. 7C). In the case of liver from thioacetamide treated group, examination of sections showed lobular hepatic fibrosis associated with bile stasis. Most of the hepatocytes were swollen and showed hydropic degeneration, but some are shrunken and necrotic were noticed. Thick fibrous septa were also shown (Fig.7D). Histopathological investigation of sections of liver from thioacetamide and colchicine treated group pre-treatment showed preservation, and organization of hepatocyte plates and sinusoids. Moderate midzonal hepatic vacuolation was found (Fig. 7E). Examination of liver sections from thioacetamide and ethanolic extract of leaves *Moringa oleifera* administered rats showed moderate periportal hepatic vacuolation, and periportal hepatic regeneration (Fig. 7F). On the other hand, liver sections from thioacetamide, colchicine and ethanolic extract of leaves *Moringa oleifera* administered rats showed a mild degree of periportal fibrosis that associated with a moderate degree of hepatic vacuolation (Fig. 7G).

| | |
|---|---|
|  <p>Fig.7A</p> |  <p>Fig.7B</p> |
| <p>Fig. (7A): A section of liver of control group showing a normal hepatocyte arranged in cords around the central vein (arrow),</p> | <p>Fig. (7B): A section of liver of colchicine group showing disturbance of the normal hepatic lobule. The hepatocytes in the midzonal and periportal portal area showed hepatic vacuolation (arrowhead),</p> |
|  <p>Fig.7C</p> |  <p>Fig.7D</p> |
| <p>Fig. (7C): A section of liver of ethanol extract of leaves <i>Moringa olifera</i> administered rat showing a normal structure of the hepatic lobule. The hepatocytes appeared more or less like normal (arrow),</p> | <p>Fig. (7D): A section of liver of thioacetamide treated group showing lobular hepatic fibrosis (arrowhead) associated with bile stasis. Most of the hepatocytes are swollen and hydropic, but some are shrunken and necrotic were noticed. Thick fibrous are shown (H&E, X 200).</p> |
|  <p>Fig.7E</p> |  <p>Fig.7F</p> |
| <p>Fig. (7E): A section of liver of thioacetamide and colchicine treated group pretreatment showing preserved and organized hepatocyte plates and sinusoids (arrowhead). Moderate midzonal hepatic vacuolation (arrow) is found,</p> | <p>Fig. (7F): A section of liver of thioacetamide and ethanol extract of leaves <i>Moringa olifera</i> administered showing moderate periportal hepatic vacuolation (arrow), and periportal hepatic regeneration (arrowhead),</p> |
|  <p>Fig.7G</p> | |
| <p>Fig. (7G): A section of liver of thioacetamide, colchicine and ethanol extract of leaves <i>Moringa olifera</i> administered showing mild degree of periportal fibrosis (arrow) associated with moderate degree of hepatic vacuolation (arrowhead) (H&E, X200).</p> | |

DISCUSSION

Liver fibrosis is a scarring mechanism of the liver skin to the permanent development of an extracellular matrix. Without successful treatment at an early stage, reversible liver fibrosis turned to irreversible cirrhosis leading to liver failure, this progressive scarring results in liver cirrhosis, which is the foremost health burden leading to death worldwide. Hepatocyte apoptosis and liver fibrosis are major features of a wide range of chronic liver injuries, including metabolic, viral, cholestasis and genetic disease. The failure of bile salt excretion in cholestasis leads to retention of hydrophobic bile salts in the hepatocytes and causes apoptosis and necrosis then fibrosis (Friedman; 2010).

The current management of liver fibrosis consists mainly of treatment of its complication or attempts to control its etiological factors. Nowadays, interesting to use herbal plants for treating hepatic fibrosis as using the *Moringa olifera* for treatment or as prophylaxes for liver fibrosis that may be due to it contains antioxidant agents (Ghasi *et al.*; 2000). The horseradish shape tree may be used as extraction from leaves (leaves aqueous extract, leave ethanolic extract) or roots extract. Also, the use of colchicine as antioxidant to leave the harmful full effects of oxidative stress led to liver fibrosis.

This study was designed to gain more information about the hepatoproticve effect of *Moringa olifera* ethanolic leaves extract and colchicine on induced thioacetamide liver fibrosis and its relationship with apoptosis in rats. In the present study, we have indicated that the hepatic rats had elevated liver assessment of liver functions that can be made by estimating the activities of serum AST, ALT, and ALP compared with control, the SGPT is primarily localized to the liver but SGOT present in the heart, skeletal, kidney, brain and liver tissues. SGOT is present in mitochondria and cytosol of liver cells but SGPT is found only in cytosol. These enzymes originally present in higher concentrations in the cytoplasm when there is hepatopathy so, these enzymes leak into the blood stream in conformity with the extent of liver damage (Venukumar and Latha; 2004) as due to thioacetamide induces hepatocyte damage following its metabolism to thioacetamide sulphene and sulphone, through a critical pathway that includes cytochrome-mediated biotransformation that leading to cellular leakage and loss of functional integrity of the cell membrane in the liver. This result was in coordination with those obtained by Yong *et al.* (2019) who used an intraperitoneal injection (i.p.) of thioacetamide (150 mg/kg/day) for four weeks to study the effect of estrogen deficiency on induced liver fibrosis in ovariectomized female rats. Also, this result run in parallel with that obtained by Salunkhe and Patil; (2017) who found that thioacetamide significantly increased AST, ALT, and ALP that were restored by Silymarin treatment caused a significant reduction in serum levels of AST, ALT, ALP and total protein contents.

This work showed that the treatment of hepatic rats with colchicine (group V) and administration of *Moringa olifera* ethanolic leaves extract 400 mg/kg with thioacetamide 150 mg/kg (group VI) also co-administration between them showed a significant decrease in serum levels of ALT, AST, and ALP it returned slightly to the normal groups levels that regarded to colchicine is an anti-inflammatory and anti-fibrotic medication as colchicine is inhibition of the migration of granulocytes into the inflamed area and decreased metabolic and phagocytic activity of granulocytes. Further, colchicine is an anti-mitotic and anti-fibrotic agent. Colchicine retards the microtubule-mediated transport of pro-collagen and enhances collagenase activity (Rambaldi and Gluud; 2005) and that may help to revel hepatic cell integrity.

Administration of *Moringa olifera* ethanolic leaves extract enhanced activities of serum ALT, AST, and ALP. These findings in coordination with that of (El-bakry *et al.*; 2016) who founded that *Moringa olifera* ethanolic leaves extract administration ameliorates

the effects of CCl₄-intoxication on serum activities ALT, AST, and ALP. This improvement in liver function biomarkers may be related to the improvement effect of *Moringa olifera* ethanolic leaves extract on hepatic tissue architecture due to its anti-inflammatory and antioxidant activities these results suggested that *Moringa oleifera* extract reduced toxicity due to elimination of the toxic products of thioacetamide in rats

The present investigation showed that the injection of thioacetamide-induced hepatic fibrosis by a drastic decrease in serum level of total proteins compared to the control group and this find is because one of the synthetic functions of the liver is protein synthesis, and with the destructive effect of thioacetamide on liver cells, therefore, level of total protein production in the blood decreases, also may be due to increase of protein catabolism and decrease of food utilization too. That result coordinated with Salama *et al.* (2013) & Salunkhe and Patil; (2017) who found that serum TP and Alb were sharply decreased due to protein oxidation by thioacetamide administration. While our result showed that treatment with colchicine or *Moringa oleifera* ethanolic extract leading to improvement in synthetic liver function that improved in significant increase in total protein and albumin that in agreement with Buraimoh *et al.* (2011) who reported that ethanolic leave extract of *Moringa oleifera* has an appreciable ability to prevent damage of the liver. Also the anti-inflammatory effect of *Moringa* was confirmed by the decrease in globulin level in serum and by the decrease in inflammatory cells infiltrations in histopathological analysis (Hamza; 2010).

In the development of liver fibrosis, massive hepatocellular death is considered to be the core event. In this study, we showed via histopathological examination of liver specimens with H&E staining that massive bridging and centrilobular fibrosis/apoptosis the histopathological observation included marked congestion in portal vessels and central vein associated with inflammation in lymphocytes and plasma cells, also focal hepatocyte degenerative changes. Meanwhile, the administration of *Moringa oleifera* ethanolic leaves extract and/or colchicine showing gradual enhancement in the histopathological alternations include a decrease of inflammatory response and congestion indicating a reduction in hepatic damage resulted from thioacetamide administration. These findings are in agreement with Wang *et al.* (2019), and Li *et al.* (2002), which reported the hepatoprotective effect of ethanolic extract of *Moringa oleifera* on paracetamol-induced liver damage in rats. Sadek; (2014), and Sadek; *et al.* (2017) reported that histopathological appearances were extraordinarily enhanced in the liver of rats that were treated with *Moringa olifera* ethanolic extract against diethyl nitrosamine induce hepatocellular carcinoma. Also, Selvakumar and Natarajan; (2008) said that immune-histochemically studies revealed that liver fibrosis was retracted by the *Moringa* plant.

Lastly, that is clear from our results that, *Moringa olifera* leaves extract and colchicine were able to ameliorate liver fibrosis as they improved function of the liver, reduced severity and incidence of morphologic changes of fibrosis and normalized biochemical and immunoenzymatic parameters in rats receiving thioacetamide. So they possess the ability to give significant protection to the liver of albino Wistar rats against thioacetamide-induced liver fibrosis. Moreover, the results showed that there are no significant differences between colchicine and *Moringa olifera* ethanolic extract.

So, this study recommended that using either neutral plant as *Moringa olifera* for the healthy and hepatic person or both *Moringa olifera* and colchicine for the hepatic patient as they can control the liver fibrosis and keep the liver healthy condition.

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