Research Article

Hepato Renal Protective Effect of Origanum Majorana against Adverse Effect of Ivermectin in Rabbits

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Abstract | The current study looked at the hepatic-renal protection of *Origanum majorana* "O.M" leaves extract in male rabbits against the negative effects of ivermectin. Twenty male rabbits were used and divided into four groups each group contain 5 rabbits as a following: Group one: rabbits which served as negative control were orally administered distilled water for 60 consecutive days. Group two: The rabbits in this group were given an aqueous extract of O.M. orally (200 milligrams per kilogram of body weight), once daily for 60 consecutive days. Group three: rabbits in this group received a subcutaneous therapeutic dose of ivermectin (200 micrograms per kilogram body weight), once monthly for 2 consecutive months. Group four: This group of rabbits was given an orally aqueous extract of O.M. (200 milligrams per kilogram of body weight) once daily for sixty consecutive days. and a subcutaneous therapeutic dose of ivermectin (200 micrograms per kilogram of body weight), once monthly for 2 consecutive months. The degree of hepatic protection was measured using liver enzymes activities (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), g-Glutamyl transferase (GGT) and total bilirubin, also the levels of Cholesterol, Triglycerides, HDL-cholesterol, LDL- cholesterol and VLDL-cholesterol were also estimated. The degree of renal protection was measured using creatinine, urea, sodium, potassium, also the levels of total protein, Albumin and Globulin were quantitated also the histo-pathological examination of the liver, kidneys were done. Results showed that the administration of O.M. extract restored the severely disrupted liver and kidney functions caused by ivermectin's unfavorable effect to almost normal levels as well as the co-administration of O.M. extract significantly reduced the histopathological effect of ivermectin on the liver and kidney. From our findings we concluded that the O.M. aqueous extract possessed a hepato-renal protective effect against ivermectin-induced adverse effects in rabbits.

Keywords | Hepato-renal, Ivermectin, Origanum Majorana, Rabbits

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INTRODUCTION

Avermectins have been first discovered as a macrocyclic lactone in the broth of a soil microorganism Streptomyces avermitilis. Avermectins consists of eight natural avermectin components A1a, A2b, A2a, A2b, B1a, B1b, B2a and B2b, the B series members were found very effective against helminthes and arthropods. Ivermectin [which its chemical structure is composed of a mixture of two compounds 22, 23 dihydroavermectin B1a (H2B1a), and 22, 23 dihydroavermectin B1b (H2B1b)], showed revolutionized antiparasitic control when being used in the treatment of animals and humans over the last decade (Steel, 1993; Shoop et al., 1995).

In humans, Ivermectin is used in the treatment of Onchocerciasis (river blindness), strongyloidiasis, Ascariasis, Trichuriasis, Filariasis, Enterobiasis, and Scabies (Bonerjee et al., 2009), while in animals it is used in the treatment and prevention of parasitic disease, it is even used as plant protection agents in the agricultural sector.

Although ivermectin appears to be a well-tolerated drug with no apparent adverse effect in mammals at pharmacological doses, (Castanha-Zanoli et al., 2012; Trailovic and Varagic, 2007) stated that the most prominent clinical signs of ivermectin poisoning in animals are CNS depression, coma and may be death. ivermectin induced pathological changes as neuronal degeneration and necrosis in pigeon brain tissue after sub chronic exposure to different doses of ivermectin for different periods (Ming et al., 2013). The repeated administration of different doses of ivermectin cause pathological changes in liver tissue of female rabbits as vacuolation of hepatocytes and fibrosis, the degree of affection depend on the given dose (Al-Jassim et al., 2015), ivermectin, when was given in therapeutic and double therapeutic doses to male rats, results in a significant decrease in total sperm count and mortality with pathological alternation in the liver, kidney, and testicles include blood vessels congestion, vascular and hydropic degeneration or necrosis and significant shift in liver and kidney functions (Elzoghby et al., 2015).

Traditional medical systems and some traditional medical procedures are now widely acknowledged in the world. Today, having a realistic approach and an analytical approach to testing drugs derived from herbs is essential.

As a result, Pharmacologists must receive a lot of knowledge from traditional healers concerning their treatments and the extraction of active principles for drug creation (Muthuraman et al., 2011).

Origanum spp. has been used as a spice and in ethno medicine for thousands of years (Fleisher and Fleisher, 1988). Its antifungal, antimicrobial, insecticidal, and anti-oxidant properties are all present (Kokkini, 1997; Baydar et al., 2004; Kulisic et al., 2004; Bakkali et al., 2008). Origanum spp. has been shown to have antispasmodic, antitumor, and analgesic properties (Elgayar et al., 2001; Puertas et al., 2002). It also causes the Inhibition of soybean lipoxygenase indicates that it has anti-inflammatory effects (Koukoulitsa et al., 2006).

This experiment was carried out to determine the effect of O.M. against the adverse effect of repeated therapeutic doses of ivermectin in rabbits.

MATERIALS AND METHODS

Materials

Ivermectin was obtained from the pharma swede company (Cairo, Egypt), as (paramectin® injection), each one ml containing 10 mg of ivermectin.

The fresh leaves of O.M. were gathered from a garden of the faculty of agriculture, Cairo University, Egypt. And undergo an extraction procedure according to (Roby et al., 2013). Briefly, the leaves of the plants were washed, dried in the shade for 20 days, and then powdered with an electric blender. The fine powder (250 g) was boiled in distilled water (1000 ml) for 90 minutes before being filtered through Whatman papers. To obtain a dry extract, the filtrated water extract was evaporated under low pressure and lyophilized. The dried residues were reconstituted in water to yield stock a solution containing 500 mg/ml. Filtration was used to sterilize the extracts. using Acrodisc (Gelman, 0.22 µm size) and then preserved in the refrigerator (4 °C) till be used.

Experimental rabbits

Twenty apparently healthy adult male New Zealand rabbits (weighing 1800-2000 g) were used. Rabbits were fed on concentrate pelleted ration and tap water which were provided ad libitum. Rabbits were housed in galvanized wire cages. Rabbits were accustomed to their surroundings for fourteen days before the beginning of the experiment. The experiment design and animal handling were approved by the research committee at the animal health research institute (AHRI), Agricultural research center (ARC).

Experimental protocol

At the beginning of the experiment, the rabbits were randomly grouped into four groups (each of 5 rabbits). Group one: Rabbits which served as the control was orally administered distilled water for 60 consecutive days; Group two**:** rabbits in this group received orally aqueous extract of O.M. (200 mg/kg b. wt.), once daily for 60 consecutive days and served as positive control group; Group three: rabbits in this group received a subcutaneous therapeutic dose of ivermectin (200 µg/kg b. wt.) (EMA, 2014), once monthly for 2 consecutive months; Group four: rabbits in this group received orally aqueous extract of Origanum (200 mg/kg b.wt.), once daily for 60 consecutive days and a subcutaneous therapeutic dose of ivermectin (200 µg/kg b. wt.), once monthly for 2 consecutive months.

Sampling

At the end of the experiment, blood samples for biochemical analysis were taken from the studied rabbits' ear veins using a syringe and allowed to clot at room temperature. and then centrifuged (15 min, 3000rpm) then the obtained serum

samples were stored at -20˚C until the determination of biochemical parameters.

After humane end of the used rabbit by authentication (decapitation) which has been done according to protocol authorized by The Institutional Animal Care and Use Committee (ARC-IACUC)/Agricultural Research Center (ARC/AH/22/03), the liver and the 2 kidneys were collected and preserved in 10% formalin till histo-pathological examination.

Methods of biochemical analysis

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were quantitatively estimated according to the method described by (Reitman and Frankel, 1957; Young, 1997), Alkaline Phosphatase (ALP) was quantitatively estimated according to the method described by (Tietz, 1995), ɣ-Glutamyltransferase (ɣGT) (Szase, 1974), total bilirubin, (Tietz, 1995), Triglyceride (Wahlefeld, 1974). cholesterol (Watson, 1960). Albumin (Doumas et al., 1997). Total protein was estimated according to (Tietz,1994). Globulin level are estimated mathematically by subtracting albumin amount from total proteins amount, albumin: globulin ratio was calculated by dividing albumin amount by globulin amount (Tietz,1994). High-density lipoprotein (HDL) according to (Peace and Kaplan, 1987), serum urea level according to (Wybenga et al., 1971). Serum creatinine level according to (Tietz, 1986). Potassium was estimated according to (Hoeflmayr, 1979; Tietz, 1976) and sodium was estimated according to (Trinder, 1951; Tietz, 1976), very low density lipoprotein (VLDL) cholesterol was calculated by dividing the amount of triglycerides by 5 and low density lipoprotein (LDL) cholesterol was calculated by subtracting high density lipoprotein (HDL) cholesterol and very low density lipoprotein (VLDL) cholesterol from total cholesterol amount (Friedewald et al., 1972).

Statistics analysis

The obtained data from biochemical analysis were computerized and evaluated for statistical significance, standard error estimation, and variance according to ANOVA test and by using SPSS 20. Duncans test of homogeneity was used to estimate the similarities for each group separately. All the results were expressed as a mean ±SE.

Method of histo-pathological examination

Hepatic and renal tissue specimens from different investigated groups which have been preserved in 10% formalin will be examined according to (Suvarna et al., 2012). Another set of tissue sections on the positively charged slide for IHC. Tissue sections were investigated using primary antibody of PECAM-1 and Avidin-biotin-peroxidase complex kit (Vectastain ABC Kit Standard, VectorLaboratories), were used according to the manufacturer's instruction protocol.

RESULTS AND DSICUSSION

Biochemical analysis

Table 1 showed that repeated therapeutic subcutaneous injection of ivermectin significantly increased the serum levels of Aspartate aminotransferase, Alanine aminotransferase, alkaline phosphatase, g-Glutamyl transferase, and total Bilirubin in group three as compared with group one.

The data also showed no significant differences in Aspartate aminotransferase, Alanine aminotransferase, alkaline phosphatase, g-Glutamyl transferase and total Bilirubin levels between group four and group one. The data illustrated also did not show any significant differences between group two as compared with the control group.

Table 2 showed that repeated therapeutic subcutaneous injection of ivermectin non-significantly decreases the cholesterol, triglycerides and non-significantly increase HDL-Cholesterol serum levels between group three and group one, the data also showed no significant difference in cholesterol and HDL-cholestrol levels and a significant difference in Triglycerides levels between group four and group one. The data illustrated also did not show any significant differences between group two as compared with group one (the control group).

In Table 3 there was a significant increase in creatinine, urea and potassium levels and a non-significant increase in sodium level in group three as compared with group one while there were no significant differences in all parameters between all other groups and group one (control group).

Table 4 showed that therapeutic subcutaneous injection of ivermectin significantly increased the serum level of total protein and albumin between group three and group one while no significant difference was observed in of total protein and albumin level between all other groups as compared to group one.

Histopathological study

Macroscopic investigation did not detect noticeable changes in the livers and kidneys of the different investigated groups.

Microscopic investigation revealed the following histopathological changes; both of control negative group1 and group 2 exhibited normal histological architectures in both hepatic and renal tissues. Group 3 revealed moderate dilation of peri-glomerular space with noticeable number of fragmented glomeruli, while others appeared collapsed

Table 1: Effect of O.M. aqueous extract of 200 mg/kg and/or ivermectin (200 µg/kg) on AST, ALT, ALP, GGT and Total Bilirubin concentration in serum of rabbits (Liver function test).

Parameters/ groups	Aspartate aminotransferase (AST) (U/L)	Alanine aminotransferase (ALT) (U/L)	alkaline phosphatase (ALP) (U/L)	g-Glutamyl transferase (GGT) (U/L)	Total Bilirubin (mg/dl)
Group one	16±0.32^{ab}	8±0.56^{ab}	137±4.49^{a}	8.5±0.22^{a}	0.54±0.027^{ab}
Group two	17±0.55^{b}	7±0.45^{a}	138±5.61^{a}	8.7±0.35^{a}	0.56±0.031^{ab}
Group three	19±0.84^{c}	11±0.52^{c}	157±5.56^{b}	10.8±0.66^{b}	0.68±0.019^{c}
Group four	16±0.71^{ab}	8.5±0.34^{b}	138 ±3.44^{a}	8.6±0.40^{a}	0.57±0.021^{b}

* Results are expressed as means ± SE (n =5), Anova test; * Values with different superscript litters within different rows differed significantly at (P≤0.05) according to Duncans test.

Table 2: Effect of O.M. aqueous extract of 200 mg/kg and/or ivermectin (200 µg/kg) on Cholesterol, Triglycerides, HDL-Cholestrol, LDL-Cholestrol, VLDL-Cholestrol concentration in serum of rabbits (lipid profile).

Parameters/ groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-Cholestrol (mg/dl)	LDL-Cholestrol (mg/dl)	VLDL-Cholestrol (mg/dl)
Group one	172±5.15^{a}	105.4 ±2.73^{b}	58.2± 1.02^{a}	92.72	21.08
Group two	167±3.8^{a}	102 ± 2.81^{ab}	59 ± 5.92^{a}	87.6	20.4
Group three	165±7.58^{a}	99 ± 4.23^{ab}	67 ± 5.24^{a}	78.2	19.8
Group four	161±5.51^{a}	92 ± 5.69 ^{a}	59 ± 2.59^{a}	83.6	18.4

* Results are expressed as means ± SE (n =5), Anova test; * Values with different superscript litters within different rows differed significantly at (P≤0.05) according to Duncans test.

Table 3: Effect of O.M. aqueous extract (200 mg/kg), mg/kg B. Wt., and ivermectin (200 µg/kg) on creatinine, urea, sodium and potassium concentration in serum of rabbits (kidney function test).

Parameter/ groups	Creatinine (mg/dl)	Urea (mg/dl)	Sodium (mmol/l)	Potassium (mmol/l)
Group one	1.47±0.107^{a}	50.5±2.17^{a}	155±1.00^{ab}	5.35±0.11^{a}
Group two	1.50±0.169^{a}	51±0.95^{a}	154.4±1.29^{a}	5.35±0.14^{a}
Group three	1.94±0.167^{b}	56.8±1.32^{b}	156 ± 1.03^{b}	6±0.38^{b}
Group four	1.45±0.0481^{a}	45.5±1.75^{a}	154 ± 0.93^{a}	5.3±0.1^{a}

* Results are expressed as means ± SE (n =5), Anova test; * Values with different superscript litters within different rows differed significantly at (P≤0.05) according to Duncan´s test.

Table 4: Effect of O.M. aqueous extract of 200 mg/kg and/or ivermectin (200 µg/kg) on total protein, albumin, globulin concentration and albumin: globulin ratio in serum of rabbits.

Parameters/ groups	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	Albumin globulin ratio
Group one	7.9 ± 0.47^{b}	3.5±0.237^{b}	4.4	0.8
Group two	7.58 ± 0.09^{b}	3.30±0.11^{b}	4.28	0.77
Group three	6.2 ± 0.25^{a}	2.5±0.217^{a}	3.7	0.68
Group four	7.8 ± 0.26^{b}	3.4±0.06^{b}	4.4	0.77

* Results are expressed as means ± SE (n =5), Anova test; * Values with different superscript litters within different rows differed significantly at (P≤0.05) according to Duncan´s test.

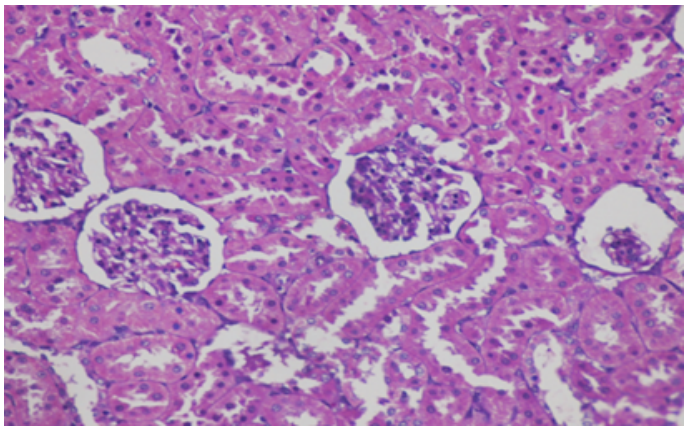

Figure 1: Renal tissue of Ivermectin treated group revealed; moderate dilation of peri- glomerular space, fragmentation and even degeneration of some glomerular tufts. H&E X 400.

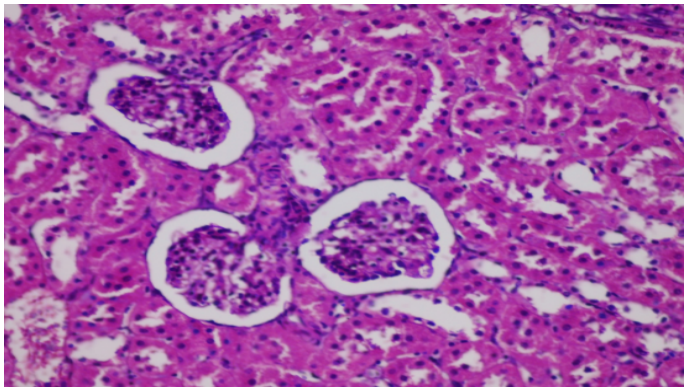

Figure 2: Renal tissue of Ivermectin-origanium treated group revealed; dilation of peri-glomerular space, H&E X 400.

and degenerated (Figure 1). Group 4 revealed restoration in the reno-pathological changes including peri-glomerular dilation and diminish in the incidence the glomerular tuft degeneration (Figure 2).

Regarding to Platelet endothelial cell adhesion molecule-1 (PECAM-1); group 1 and 2 showed expression of PECAM-1 in the glomerular tufts membrane (Figure 3a and b). Group 3 manifested loss of PECAM-1 expression in a significant number of glomerular tufts associated with increase in the tubular epithelial reactivity for PECAM-1 (Figure 3c). In group 4, restoration of PECAM-1 expression at a significant degree in the glomerular tufts and to some degree in the interstitial endothelial (Figure 3d) was detected.

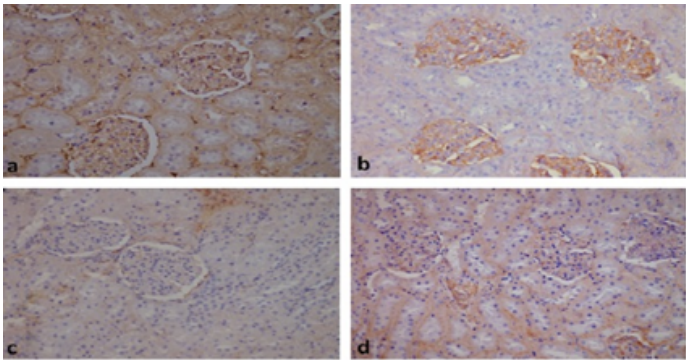

Figure 3: PECAM-1 expression showing; normal pattern of expression in both of glomerular tufts and tubular epithelia in control negative group 1a and group 2b. Loss of expression in both glomerular tufts and Tubular epithelia in Ivermectin treated group (group 3c) and significant restoration of expression in group 4d. HRP/ PECAM-1 X 400.

Hepatic parenchyma showed normal histological criteria in control negative group 1. Group 2 showed mild degree of hydropic degeneration of hepatocytes radiated from central vein and associated with dilation of hepatic sinusoids in some areas (Figure 4). Group 3 showed marked dilation of hepatic sinusoids associated with distortion of hepatic cords in addition to fibrosis of portal area (Figure 5), some areas showed massive hydropic degeneration of hepatocytes. Group 4, despite dilation of hepatic sinusoids, the hepatic cords preserve its integrity (Figure 6).

PECAM-1 expression was absent in group 1 (Figure 7a), group 2 revealed minute expression of PECAM-1 at the margin of some dilated hepatic sinusoids (Figure 7b). Group 3 revealed intense expression of PECAM-1 at the margin of the markedly dilated hepatic sinusoids (Figure 7c). Meanwhile; weak expression of PECAM-1 in investigated hepatic parenchyma of group 4, even at the margin of scarcity dilated hepatic sinusoids (Figure 7d).

Dermatological diseases are among the most common clinical entities in household pets and fur-bearing animals (Deshmukh et al., 2010). External parasite infestation is one of the most prevalent and serious dermatological issues in rabbits (Darzi et al., 2007). It is the most stubborn, persistent, and zoonotically significant infectious disease (Kumar et al., 2002). Clinically, it is distinguished by pruritis, baldness, and prolonged illness and death as a result of cachexia (Roy et al., 2001). In the farms, ivermectin is the most commonly used antiparasitic drug across Egypt. Many kinds of research advised repeated administration of ivermectin dose as an effective curative protocol in rabbits affected with mange (Panigrahi and Gupta, 2013; Darzi et al., 2007; Mitra et al., 2014; Bharath et al., 2016) so a further investigation should be done to discover a hepato-renal protective agent to counter the adverse effect of repeated administration of ivermectin on liver and kidney.

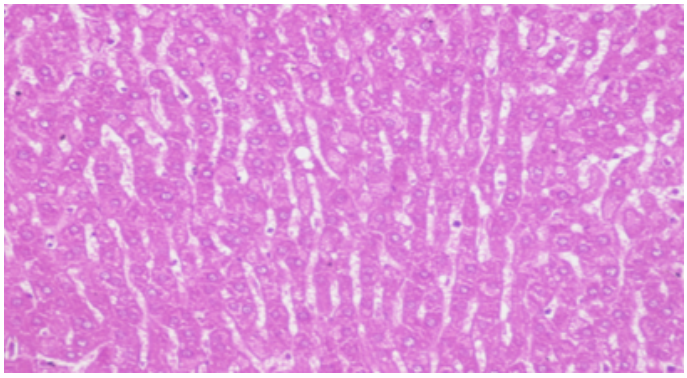

Figure 4: Hepatic parenchyma of group 2 revealed mild hydropic degeneration of hepatocytes associated with dilation of hepatic sinusoids (in some areas). H&E X 400.

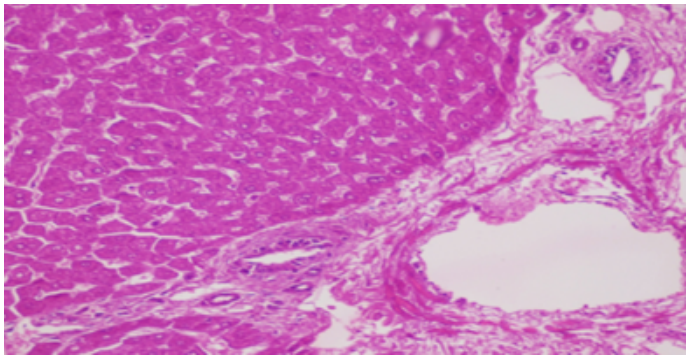

Figure 5: Hepatic parenchyma of group 3 revealed marked dilation of hepatic sinusoids associated with distortion of hepatic cords and fibrosis of portal area. H&E X 400.

The biochemical analysis

The serum biochemical markers are thought of as an internal mirror that shows the true impact of drugs such as the repeated dose of ivermectin and the herb marjoram as a counter on the liver and kidneys. The biochemical parameters that were measured were as follows: AST, ALT, ALP, GGT, total bilirubin, Cholesterol, Triglyceride, Albumin, Globulin, total protein, HDL-cholesterol, LDL- cholesterol, VLDL-cholesterol, creatinine, urea, sodium, potassium.

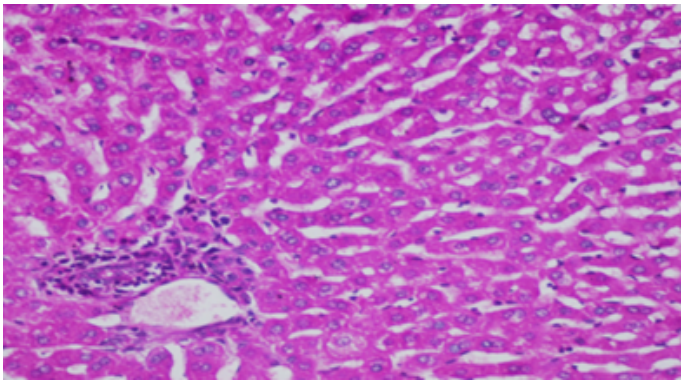

Figure 6: Hepatic parenchyma of group 4 revealed dilation of hepatic sinusoids associated with preservation of hepatic cords integrity. H&E X 400.

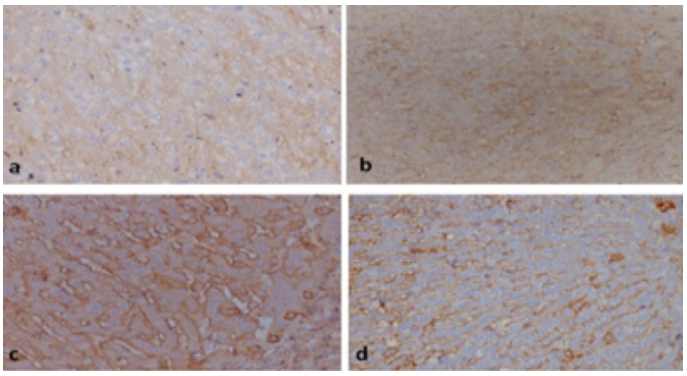

Figure 7: PECAM-1 expression showing; absence of expression in control negative group (a), minute expression of some dilated hepatic sinusoids, in group 2 (b), intense expression in Invermectin group (c), and weak expression at the margin of dilated hepatic sinusoids in Ivermectin-O.M. treated group (d). HRP/ PECAM-1 X 400.

The present work showed no significant differences in all aforementioned biochemical parameters between group two (positive control group) and group one which consequently indicated no adverse effect of the O.M. when being used in the rabbits by a daily dose 200mg/kg b.wt. for 60 consecutive days, similar results were demonstrated by (Soliman et al, 2019) who used rats which have been received orally aqueous extract of organium (200 mg/kg), once daily for 30 consecutive days, (Nashwa and Faten, 2014) who used a daily dose of marjoram oil in rats for 2 months and (El-Shafeey and Taha, 2013) who used a daily dose of oregano water extract in female rats for 14 days.
ALT and AST are sensitive biomarkers that are directly engaged in liver damage and toxicity (El-demerdash, 2004). The increase in serum ALT and AST levels could be related to hepatotoxicity caused by increased hepatocyte cells membrane permeability or even rapture, resulting in enzyme leakage into the bloodstream (Rabab et al., 2015).

In the current research (Table 1) biochemical analysis indicated significant elevation in ALT, AST, and ALP in group three in comparison with group one. Such results demonstrated the adverse effect of ivermectin on the liver tissue agrees with those are mentioned by other researchers about the microcyclic lactones reverse effect on rabbits (El Far, 2013; Seddiek et al., 2013; Ismaiel et al., 2017) and rats (Arise and Malomo, 2009; Shoeb, 2013; Rabab et al., 2015).

Ivermectin is a relatively safe anti-parasitic treatment for animals, but, it may influence the balance of oxidants and antioxidants (El-Shenawy, 2010). Ivermectin can generate free radicals, which can have a deleterious effect on the parasite. Nitric oxide is engaged in a variety of physiological activities along the journey. Through its cytotoxic impact, it works as a free radical and as a host defense mechanism (Tamarozzi et al., 2011). Ivermectin was reported to counteract against scabies agents by inducing free radicals associated damage and by decreasing antioxidant enzyme activity (Behera et al., 2011).

The nearly normal levels in the serum ALT, AST, and ALP in group four (Table 1) suggest a reasonable hepatoprotective effect of this treatment, reduce the damage of plasma membrane of these tissues at a cellular level leading to decrease efflux of these enzymes into the extracellular fluid and bloodstream.

Such result agrees with those are mentioned by other researchers which demonstrated the hepatoprotective effect of O.M. on the liver tissue against other hepatotoxic substances in rats such as sodium nitrite (Nashwa and Faten, 2014), CCL_4 (El-Shafeey and Taha, 2013), and paracetamol (Soliman et al., 2019), this result may be attributed to the presence of a high concentration of phenol in O.M and that is the reason which gives it the unique antiradical and antioxidant activities (Soliman et al., 2019; Chishti et al., 2013).

The present study showed significant increases in GGT in group three as compared to group one (control group), this increase may be attributed to degenerative and necrotic changes in the epithelial lining of the bile duct (Allam et al., 2020), also the maintaining of GGT serum level in group four near to normal level (which was demonstrated in group one) was observed and illustrated a reasonable protective effect of O.M against the adverse degenerative effect of repeated s/c ivermectin injection.

In vertebrates, bilirubin is a yellow molecule that arises in the normal catabolic process that breaks down heme. This catabolism is a crucial process in the body's elimination of waste materials produced by the destruction of aging or dysfunctional red blood cells (Braunstein, 2019). According to our findings, liver cell death inhibits I bilirubin hepatic absorption, decreases conjugation, and prevents d-bilirubin excretion. Darwish and Eldakroury (2020) which caused a

significant elevation of serum total bilirubin concentration in group three as compared to group one, the protective effect of O.M reduce liver cell destruction and succeeded to restore serum total bilirubin level near its normal level (control group).

In the present investigation, Injection of ivermectin into rabbits at a rate of 200 micrograms per kilogram of body weight monthly for two months resulted in a considerable increase in blood urea and creatinine levels; a similar increase in urea and creatinine levels following ivermectin injection has been documented by other researchers such as (Arise and Malomo, 2009). These elevations may be attributable to the direct influence of ivermectin or its metabolites on renal tissue. The increase in creatinine and urea concentrations in ivermectin-treated animals may be related to a decrease in glomerular renal filtration or renal tubule malfunction (Walmsley and White, 1994).

Ivermectin, which was discovered to significantly reduce the total body's antioxidant capacity and to increase the production of nitric oxide (Atakisi et al., 2009), which can harm the renal tissue, leading to kidney malfunction. Failure in the glomerular filtration results in the retention of substances such as urea and creatinine, which may be responsible for their high serum levels in group three in comparison with group one (Selvakumar et al., 2013).

O.M. genus is a plant that has shown to possess several types of biological activities such as, antiradical activity and it is a rich source of polyphenols which are known natural antioxidants (Chishti et al., 2013) which can tolerate increment in the production of nitric oxide and compensate the reduction of total body's antioxidant capacity caused by ivermectin which may be the cause of O.M. renal protective effect and can be observed as a maintaining in serum urea and creatinine levels in group four near-normal levels which have been observed in group one.

As is well known, the functional capacity of the kidney can be assessed by measuring sodium and potassium levels in the serum and excretory constituent concentrations (Whelton et al., 1994). Sodium and potassium are abundant in the body's extracellular and intracellular fluids. They are the most essential components in the transport of electrolytes between the extracellular and intracellular compartments due to their propensity to rapidly break down into their constituent ions or radicals. The significant increase in serum Na+ concentration after ivermectin administration once a month for two months could be attributed to increased production of aldosterone (which has been shown to stimulate membrane aldosterone receptors) and other mineralocorticoids that increase tubular reabsorption of Na+ (Tietz et al., 1994).

As well as the considerable increase in serum K+ concentration in the ivermectin-treated group could be attributed to enhanced sensitivity of the nephron to aldosterone and other mineralocorticoids, which are responsible for electrolyte reabsorption and retention, respectively. this result agrees with what has been mentioned before by (Arise and Malomo, 2009) who used male albino rats which received a therapeutic dose of 0.4 milligram per kilogram of body weight ivermectin daily for 15 days, in our study the administration of O.M. with ivermectin succeeded to keep the levels of Na and K near normal levels which have been recorded in group one and this may be attributed to the ameliorative effect of the plant extract on kidneys.

Liver insufficiency in combination with ivermectin as a Farnesoid X receptor (FXR) ligand may be the cause of the non-significant decrease of cholesterol and triglyceride serum concentration levels in group three in comparison with group one, this result agreed with (Chahrazed et al., 2020) who demonstrated that ivermectin administration provoked a decrease in cholesterol and triglyceride in Healthy male young Algerian rabbits, (Jin et al., 2013) revealed that ivermectin reduced serum cholesterol in mice, which is attributed to the fact that the antiparasitic drug, ivermectin, is a Farnesoid X receptor (FXR) ligand that maintains bile acid and cholesterol homeostasis and can effectively improve hyperglycemia and hyperlipidemia in diabetic mice models by regulating gene expression.

The usage of O.M in combination with ivermectin (group 4) slightly decrease the serum cholesterol and triglycerides levels than levels in group 3 and this may be attributed to the combination between ivermectin as a Farnesoid X receptor (FXR) ligand and the anti-hyperlipidemic effect of O.M., such result agreed with what have mentioned by (Pimple et al., 2012) who mentioned that the oral administration of aqueous extract of O.M. in a dose of 200 milligrams per kilogram of body weight succeeded to restore the lipid values to normal in non-insulin dependent diabetes mellitus rats even more significantly than the hydro distilled volatile oil, the petroleum ether extract and the methanolic extracts which have been used in concentrations of 100, 200, 400 milligrams per kilogram of body weight.

There was a non-significant increase in HDL-Cholesterol serum level in group three in comparison to group one, this result agrees with (Chahrazed et al., 2020) who demonstrated that ivermectin treatment decreased all lipid parameters except HDL-C, which increased at 14 days. this level has been maintained near to normal level in the fourth group, such result agrees with what was mentioned by (Soliman et al., 2019) who mentioned that O.M. when used with paracetamol toxicity, succeeded to maintain

HDL-Cholestrol serum level which has been recorded in paracetamol treated group of rats near to normal level.

Liver disorders and malfunction is also a big factor because albumin and globulin are made in the liver, so any disorder that affects the liver may reduce albumin and globulin production (Spinella et al., 2016). Other health condition like kidney disorders may also results in reduction of albumin and globulin production (Levitt and Levitt, 2017).

In our study, there was a significant decrease in albumin, total protein and subsequently decrease in the level of calculated globulins and A/G ratio in group three in comparison with group one which may be attributed to liver and kidney affections.

this decrease has been maintained near to normal level by using O.M in group four, such results agreed with (El-Speiy et al., 2016) who treated rabbits with ivermectin injection by double therapeutic dose 1 mg kg-1 of ivermectin via subcutaneous injection.

Histopathological study

This study aimed to detect the possible hazardous effect of ivermectin on rabbit kidneys and liver criteria and investigate the ability of O.M. to restore renal and hepatic histological criteria with concern for vascular integrity.

Despite of the known safety profile of ivermectin, within the recommended dose, for cheyletiellosis in rabbits (Mellgren and Bergvall, 2008); but ivermectin-related serious adverse drug reactions (sADRs) including toxidermia, renal and hepatic disorders had been recorded (Campillo et al., 2021). Ivermectin is extensively metabolized by human liver microsomes via cytochrome. So, ivermectin and its metabolites were excreted mainly in feces (Gonzalez et al., 2008) and less than 1% in urine (Mathachan et al., 2021).

Regarding to our histopathological investigation, there was dilation of periglomerular space indicate to increase in glomerular tuft vascular permeability ; this was detected in group 3 and restored in group 4; with suggestion to pathophysiological effect mediated by glomerular vascularity dysfunction.

PECAM-1 is a marker for blood vessels permeability modification with indication of transfer from fenestration to capillarization and hence loss of physiological function. The angiogenic effect of PECAM-1 is tissue specific and the endothelial proliferation depend on the level of PECAM-1 (Tsuneki and Madri, 2014).

PECAM-1 had a principal role in normal kidneys' endothelial cells adhesion and migration, hence establishment of kidneys' vasculature physiology require vasculogenesis and angiogenesis (Kondo et al., 2007). In normal kidneys, PECAM-1 is expressed in glomerular tufts and interstitial peritubular endothelial cells, this expression gets lost in case of sclerotic and /or fibrotic conditions (Sivridis et al., 2003).

In the present investigation PECAM-1 expression showed; marked loss in group 3 and re-expression was noticed in group 4.

Loss of PECAM-1 expression in some glomerular tufts of treated group (4) could be attributed to vasculopathy of some glomeruli. Meanwhile expression of PECAM-1 by tubular epithelium could be an indicator for release of PECAM-1 from fragmented glomerular endothelial cells after glomerular damage and subsequent uptake by tubular epithelia (Sivridis et al., 2003).

Permeability leakage appeared only following endothelial contraction and the restoring of microvascular integrity is dependent on PECAM-1– induced glycolytic response that reestablish cytoskeletal remodeling and junctional repair. High extracellular glucose level with further increase in ROS; lead to podocytes apoptosis and hence permeability impairment (Susztak et al., 2006).

Other factor that could affect the blood vessels function is oxidative stress which, leads to lipid peroxidation and impairment of cell membrane functions. Origanum (*O. vulgare*) possess nephron-protective effect through its anti-inflammatory and anti-oxidant activities mediated by increase Nrf2 and subsequent increase the intra-cellular level of SOD and CAT (Lui et al., 2017). On contrary; high dose of Orignum (800mg/kg) showed nephropathy effect with high urea and creatinine levels (Sharifi-Rigi et al., 2019).

Glomerular PECAM-1 expression is decreased with disordered glomerular architectures, and increase during recovery phase (Khan et al., 2011), that in accordance with our result as PECAM-1 expression reduced in group 3 and restored in group treated with O.M. (group 4).

Hepatic sinusoids are fenestrated blood vessels, under some pathological stats they undergo capillarization with loss of fenestration as an early stage of fibrosis associated by angiogenesis and finally capillarization (Baiocchini et al., 2019). Capillarization of hepatic sinusoids could explain their dilation and subsequent disorganization of hepatic cord; this feature was noticeable in group 3 (ivermectin).

Hepatic sinusoidal expression of PECAM-1 indicates to sinusoidal capillarization, reduction of cellular fenestration with deposition of basement membrane (Coulevard et

al., 1993) and abnormal differentiation of pre-existing sinusoids (Schaffner and Popper, 1963); this expression was detected in group 2 and 3 at mild to severe degree respectively and almost lost in group 4 (O.M. + ivermectin) indicating to re-establishment of sinusoidal physiology with loss of PECAM-1 expression but persistence of abnormally proliferated/ dilated sinusoids as previously mentioned by Schaffner and Popper (1963) and indicated as irreversible changes by Wikinson et al. (2020).

Our study detected marked hazardous effect of ivermectin on hepatic sinusoids, this vasculopathology had an effect on the small blood vessels and hence the tissues supplied by them (Wani et al., 2017). This effect was also demonstrated in group 3, while restoration of hepatic sinusoids integrity was evident in groups 4.

Vascular property changes could also be attributed to the effect of nitric oxide. Nitric oxide (NO) molecules participate in regulation of vascular tone, remolding, endothelial permeability and angiogenesis through its regulation of endothelial cells survival, proliferation and migration. Endothelial NOS (eNOS) is the NO synthase (NOS) in endothelial cells (Gover et al., 2002).

Other possible role of Origanum Majoranum Extract (OME) is the ability to restore renal and hepatic pathy through up - regulation of lipid and glucose metabolic responsible genes mainly of adiponectin and GLUT2. Badran et al. (2019) report that OME induce, in dose dependent, relaxation of endothelium intact rings via enhancing the conversion of NO production by endothelial NO synthase (eNOS) with end result of vasodilator effect, quenching of free radicals with further anti-hypertensive effect (Peixoto-Neves et al., 2010). The vasodilator effect of adiponectin is mediated by the biosynthesis of eNO from NO that induced by high glucose concentration and is associated with reduction in PECAM-1 expression (Lee et al., 2015).

PECAM-1 affect vasodilation is mediated by NO. PECAM-1 is participated in intra-cellular eNOS expression and activities, so it is an essential agent in angiogenesis, vascular development and integrity, PECAM-1 expression may contribute to appropriate activation of eNOS (Park et al., 2015). But the effect of PECAM-1 deficiency on eNOS expression is tissue dependent (Dimaio et al., 2008).

Moreover, carvacrol and thymol, the main polar phenols of OME, possess antagonistic effects, anti-oxidative and irritant effects, respectively; so they suggested to restore hepato-pathic effect mainly through the anti-oxidative effect (Rašković et al., 2015). Both act as an initiator of cell proliferation and even liver regeneration (Abdel-Aziem et al., 2014). Capillarization of Liver sinusoidal endothelial cells (LSEC) associated with increase in expression of PECAM-1 and characterized by loss of sinusoidal endothelial cells fenestration and basement membrane synthesis and hence adopt capillary like phenotype which is irreversible (Wikinson et al., 2020).

CONCLUSIONS AND RECOMMENDATIONS

In the current study, it was discovered that O.M. extract restores all of the ivermectin affected serum biochemical parameters to near-normal levels, and also that the O.M protective effect is mediated by vascular integrity with variable mediators and pathways. Thus, its aqueous extract exhibits a protective effect that reduces the hepato-renal adverse effects generated by the repeated use of ivermectin, implying its usage as a strong hepatoprotective and nephroprotective agent.

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NOVELTY STATEMENT

This research paper is considered one of the first studies which demonstrated the hepato-renal protective effect of O.M. which in turn could provide more information about its ability to counter the adverse effects of ivermectin which are generated by its repeated use in animals treatment.

AUTHOR'S CONTRIBUTION

Both authors participated in design, experimental procedure, writing, revising , and reviewing of the manuscript

Conflict of interest

The authors have declared no conflict of interest.

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