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Evaluation of Hepatoprotective Activity of Ethanol Extract of *Ricinus communis* on Emamectin Benzoate-Induced Hepatotoxicity in Male Guinea Pigs

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Warda M. A. Kaidama*[1], Hussein A. Mahdi[2], Nabil A. AL-Yafrsi[2], Safwan A. AL-Baz[2], Mohammed A. Alshami[2], Shaaib Y. AL-Gumaei[2], Maged A. AL-Gumaei[2], Samam H. AL-Gumaei[2], Ali A.Badr[2]

[1]Biology Department, Science Collage, Ibb University, Ibb, Yemen-70270.

[2]Department of Pharmacy, Faculty of Allied Medical Sciences, Aljazeera University, Ibb, Yemen-70270.

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ABSTRACT

The objective of this study was to investigate the effect of emamectin benzoate (EB) an organophosphorus pesticide on biochemical parameters and enzyme activities in the liver of adult male guinea pigs as well as the possible role of *R. communis* extract in the attenuation of EB-induced change. The animals were divided randomly into seven groups of five guinea pigs in each group. Group I: served as the control group and were received oral administered distilled water, Group II: were orally administrated with EB (2mg/kg), Group III: were orally given EB (2mg/kg) + silymarin (100mg/kg), Group IV: were given the ethanol leaves extracts of *R. communis* (100 mg/kg) orally, Group V: was given the ethanol leaves extracts of *R. communis* (200 mg/kg) orally, Group VI: were orally given EB (2mg/kg) + ethanol leaves extracts of *R. communis* (100mg/kg) and Group VII: were orally given EB (2mg/kg) + ethanol leaves extracts of *R. communis* (200 mg/kg)daily for 10 days. On day 11, all animals were anesthetized with chloroform and blood was collected for biochemical analysis and they were dissected liver for histopathological examination, they were kept in fixative. EB increased the level of serum AST, ALT, ALP, and total bilirubin. EB also, caused many histopathological changes such as cytoplasmic vacuolization, dilated and congested blood vessels with hemorrhage, ballooning hepatocyte, infiltration with inflammatory cells, nuclear pyknosis, karyorrhexis and karyolysis, congestion of portal vein, hyperplasia of lining epithelium of bile duct indicated liver damage. While the ethanolic extract of *R. communis* (100 mg/kg and 200 mg/kg) protected the liver from intoxication induced by EB in guinea pigs through normalization of liver indicators and decreases the level of histopathological lesions.

INTRODUCTION

Folk medicine is the mother of all other systems of medicine and even modern medicine. The knowledge about certain herbs, which have curative and palliative effects on disease conditions, has been transmitted from one generation to another by experienced elders and also by tribal herbal specialists. Yemen with its uniquely variable climatic conditions possesses a huge wealth of flora, cultivated or wild. These found their way to folk medicine and are used widely and effectively for the treatment of various human and animal ailments, especially by natives in rural areas [1].

The disorders associated with the liver are numerous and varied because it is the key organ for metabolism. Many of these medicinal plants were extracted and used successfully in the treatment of jaundice and different liver disorders, but in most cases, their effectiveness has never been evaluated nor received any comprehensive scientific evaluation. Little information is documented concerning their other pharmacological effects [2]. Natural antioxidants from fruits and vegetables were reported to provide substantial protection that slows down the process of oxidative damage caused by reactive oxygen species (ROS) [1].

The *Ricinus communis* L plant is a species of the flowering plant. *Ricinus communis* has traditional medicinal use. The castor oil obtained from the seeds of the plant is still widely used traditionally and herbally as a medicine. The *R. communis* increase the regenerative and reparative capacity of the liver due to the presence of flavonoids and tannins [3].

Castor plant has been cultivated as far back as 6000 years ago [4]. The botanical name of *R. communis* was coined by Swedish naturalist Carlos Linnaeus in the 18th century [5]. Some scholars believe its origin to be from Tropical Africa. In Saudi Arabia, it is commonly known as Kherwa [4]. It is an angiosperm shrub which is commonly known as castor oil plant. Castor bean is a fast-growing oilseed crop, suckering perennial herb belonging to (Euphorbiaceae) family [6]. Methanolic extract of *R. communis* leaves is supposed to have anti-microbial properties [7]. Water extract of root-bark has shown some analgesic activity in rats [8]. It also has anti-inflammatory and anti-histaminic properties [9]. Based on the above medicinal properties, the present study has been undertaken to investigate the hepatoprotective activity of ethanolic extract of leaves of *R. communis* against Emamectin benzoate(EB) pesticide-induced hepatic damage in male guinea pigs.

MATERIAL AND METHODS

Collection of plant

Fully matured dark green leaves of *Ricinus communis* were collected in February 2021. Plant species were identified by Dr. Essam aqlan, Assistant professor of Plant taxonomy and Flora, Department of biology, faculty of sciences, Ibb University.

Preparation of ethanol extract of *Ricinus communis* leaves

The plant leaves were thoroughly washed with tap water to avoid dust and other unwanted materials accumulated on the leaves from their natural environment. The dust-free leaves were allowed to dry at oven temperature 40 C° in the Pharmacy laboratory. The dried leaves were powdered by using an electric blender. Finally, fine powder was collected from the powdered leaves by sieving through the kitchen strainer and used for extraction. Twenty grams of powder plant material was kept in a 200ml conical flask then 100ml of ethanol 70% was added. The mouth of the conical flask was covered with aluminum foil and kept in a reciprocating shaker for 24h for continuous agitation at 150 rev/min for thorough mixing and also complete elucidation of active materials to dissolve in the respective solvent. Then, the extract was filtered by using Whatman no 1 filter paper. The solvent from the extract was removed by using an oven temperature of 40 C°. Finally, the residues were collected and used for the experiment.

Experimental design

A male guinea pigs weighing between 350-650g were gained from the animal house of Biology Department, Ibb University-Yemen conserved it for one week in environmentally controlled conditions (25±5C°, 55±5% humidity, and 12 h light-dark cycle) to accommodate with free access to food and water *ad libitum*. The experiment protocol was accepted by the Institutional Animal Ethics Committee of Ibb University-Yemen.

Male guinea pigs were randomized into seven groups of five guinea pigs in each group. The animal grouping was done in the following ways :

Group I: served as the control group and were received oral administered distilled water for 10 days. Groups II: were given oral administration of EB (2mg/kg) dissolved in distilled water daily for 10 days .Group III: were orally given EB (2mg/kg), then after 1 h. were treated with silymarin (100mg/kg) suspended in olive oil for 10 days.

Group IV: was given the ethanol extracts of *R. communis* (100 mg/kg) orally dissolved in distilled water daily for 10 days .Group V: was given the ethanol extracts of *R. communis* (200 mg/kg) orally dissolved in distilled water daily for 10 days .Group VI: were orally given EB (2mg/kg), then after 1 h. were treated by the ethanol extracts of *R. communis* (100 mg/kg) dissolved in distilled water for 10 days. Group VII: were orally given EB (2mg/kg), then after 1 h. were treated by the ethanol extracts of *R. communis* (200 mg/kg) dissolved in distilled water for 10 days. On day 11, all animals were anesthetized with chloroform and blood was collected to a tube does not contain anticoagulant to allow the blood clot at room temperature for 30 minutes and then centrifuged at 3000 rpm for 15 minutes to get the serum. The serum samples were taken into clean tubes and saved in the deep-freezer at -24C^{o} for biochemical analysis. They were dissected for liver, they were kept in Carnoy's fixative for 24h., then in 10% formalin for histological examinations.

Biochemical estimation

The serum was analyzed for various biochemical parameters like Aspartate transaminase (AST), Alanine transaminase[10] (ALT), Alkaline phosphatase[11](ALP), and bilirubin[12].

Histological analysis

Animals were sacrificed and the kidney from each group was isolated and preserved in 10% formalin solution. After paraffin embedding, tissues were sectioned and stained with hematoxylin and eosin (H&E) for observing microscopic changes in the kidney.

Statistical Analysis

Data were expressed as the mean values ± standard deviation (S.D.) for each measurement. The data were also analyzed by one-way analysis of variance (one-way ANOVA) using SPSS (version 20) the test is significant at α 5%.

RESULTS

Effect of *R. communis* L extract on biochemical parameters in EB induced hepatotoxicity in guinea pigs

Aspartate Aminotransferase (AST) (U/L)

Administration of EB (2mg/kg) to male guinea pigs for 10 days significantly increased (P≤0.05) the serum activities of AST which was (87.67±1.32 U/L) compare with the control

group 57.50±0.91U/L. Silymarin (standard drug 100mg/kg) produced a significant decrease(P≤0.05) in AST which was 66.00±1.65 U/L when compared with the EB group. After 10 days of treatment with *R. communis* (100 mg/kg and 200 mg/kg) there was a significant decrease (P≤0.05) in AST which were 74.00±0.16 U/L and 73.50±0.19 U/L respectively when compared with EB only group (Tab: 1) (Figure: 1).

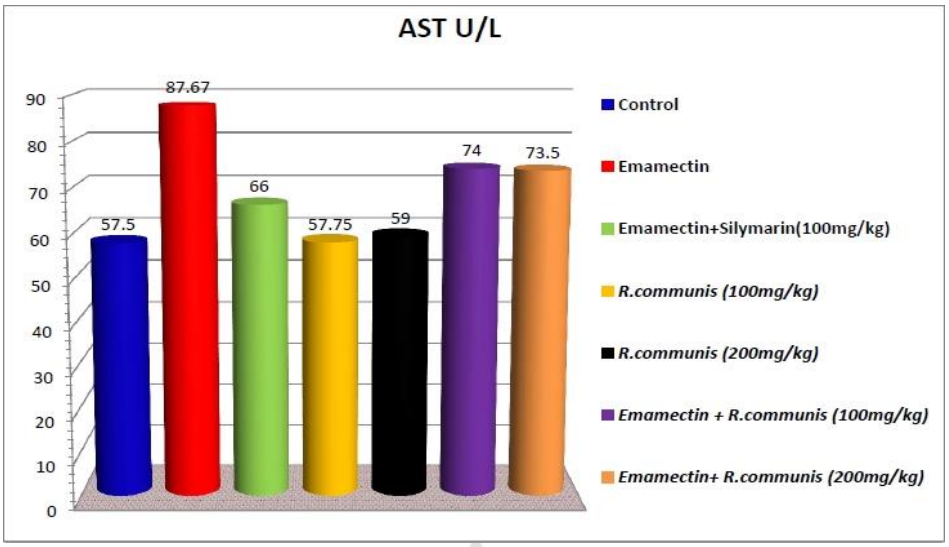

Figure No. 1: Effect of EB and *R. communis* extract on Aspartate Aminotransferase (AST) (U/L) in EB induced hepatotoxicity in guinea pigs

Alanine Aminotransferase (ALT) (U/L)

Table (1) showed that the average level of Alanine Aminotransferase (ALT) was 59.25±0.13U/L in the normal control group. Induction of hepatotoxicity by EB significantly increased (P≤0.05) in ALT level which was 98.25±1.95 U/L as compared with normal control (Figure: 2). The results showed that ALT level was significantly (P≤0.05) decreased in Silymarin 100mg/kg treated group which was 62.75±0.19 U/L. Also, there was found a significant decrease (P≤0.05) in *R. communis* (100 mg/kg and 200 mg/kg) treated groups which were 72.75±1.50 U/L and 70.00±1.96 U/L when compared with the EB group as present in (Table: 1).

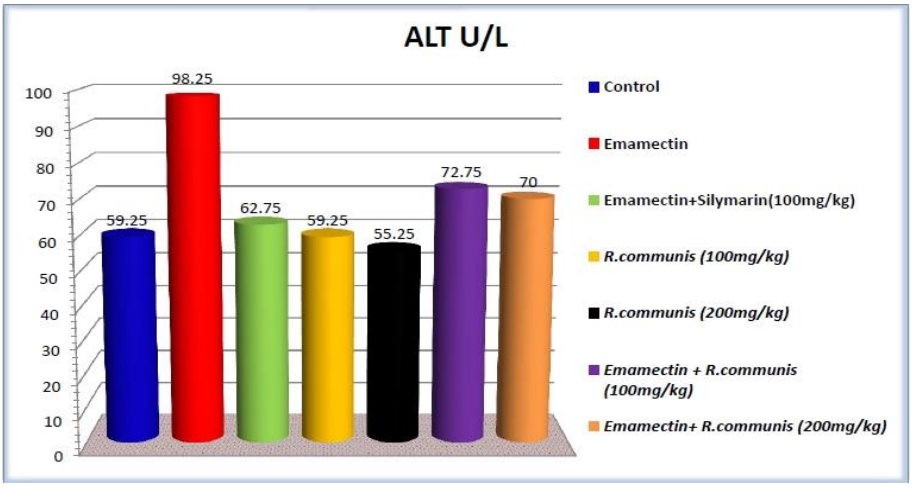

Figure No. 2: Effect of EB and *R. communis* extract on Alanine Aminotransferase (ALT) (U/L) in EB induced hepatotoxicity in guinea pigs

Alkaline Phosphatase (ALP) (U/L)

EB administration significantly increased the level of liver enzymes ALP which was 299.50±2.38 U/L compare with control 243.25±2.51 U/L. Silymarin 100 mg/kg produce a non-insignificant decrease in ALP concentration which was 276.00±1.45 U/L. Treatment with *R. communis* extract (100 mg/kg) and (200 mg/kg for 10 days) showed insignificantly reduced ALP enzymes which were 285.50±6.61 U/L and 282.00±6.60 U/L respectively compared to EB induced hepatotoxic pigs (Figure: 3).

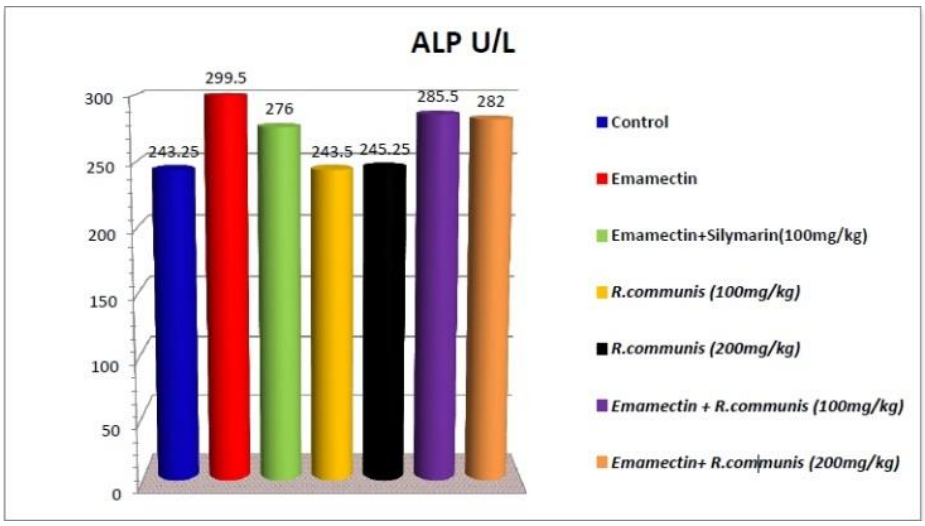

Figure No. 3: Effect of EB and *R. communis* extract on Alkaline Phosphatase (ALP) (U/L) in EB induced hepatotoxicity in guinea pigs

Total Bilirubin (mg/dl)

Guinea pigs from the EB alone group exhibited significantly increased (P≤0.05) levels of total bilirubin which was 0.9±0.20mg/dl compared to the control group 0.6±0.20 mg/dl. Silymarin (100 mg/kg body weight) also lowered insignificantly the total bilirubin which was 0.7±0.30 mg/dl compared to the EB alone group. In contrast, *R. communis* extract (100 mg/kg) and (200 mg/kg) treatment prevented the increase in these parameters resulting in nearly normal levels (figure: 4) which *R.communis* (200 mg/kg) extract noted a significant decrease P≤0.05 in total bilirubin level 0.6±0.10mg/dl while *R. communis* (100 mg/kg) extract noted insignificantly decrease in total bilirubin level 0.8±0.20 mg/dl compared to the EB alone group.

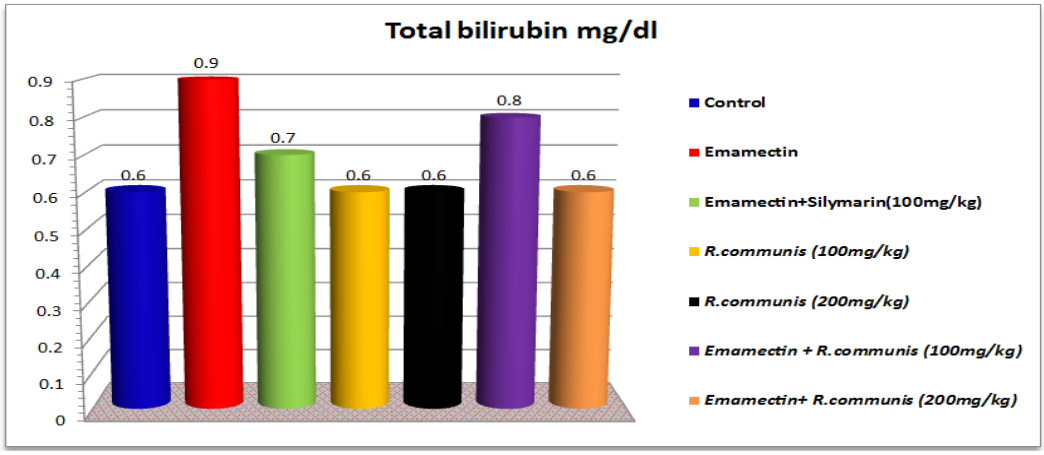

Figure No. 4: Effect of EB and *R. communis* extract on Total Bilirubin (mg/dl) in EB induced hepatotoxicity in guinea pigs

Table No. 1: Effect of EB and *R. communis* extract on biochemical parameters in EB induced hepatotoxicity in guinea pigs

	AST U/L	ALT U/L	ALP U/L	Total bilirubin mg/dl
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control	57.50±0.91	59.25±0.13	243.25±2.51	0.6±0.20
Emamectin	87.67±1.32$^{\#}$	98.25±1.95$^{\#}$	299.50±2.38$^{\#}$	0.9±0.20$^{\#}$
Emamectin+Silymarin(100mg/kg)	66.00±1.65*	62.75±0.19*	276.00±1.45	0.7±0.30
R. communis (100mg/kg)	57.75±0.62*	59.25±0.13*	243.50±2.59*	0.6±0.20*
R. communis (200mg/kg)	59.00±0.41$^{\#*}$	55.25±0.19*	245.25±2.51*	0.6±0.20*
Emamectin + *R. communis* (100mg/kg)	74.00±0.16$^{\#*}$	72.75±1.50$^{\#*}$	285.50±6.61$^{\#}$	0.8±0.20
Emamectin+ *R. communis* (200mg/kg)	73.50±0.19$^{\#*}$	70.00±1.96$^{\#*}$	282.00±6.60$^{\#}$	0.6±0.10*

All value represents the mean± SD of 5 animals. # P≤0.05 compared with the normal control value. * P≤0.05 compared with EB group alone values.

Effect of *R. communis* L extract on liver histopathology

Histopathological examination of liver sections of control group 1 and group 4, 5 showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein (Figure: 5 a, 5b, 5c). After 10 days of EB administration, many histopathological changes were observed in the liver sections. These changes were cytoplasmic vacuolization, dilated and congested blood vessels with hemorrhage, ballooning hepatocyte, infiltration with inflammatory cells, nuclear pyknosis, karyorrhexis, and karyolysis indicated liver damage, congestion of portal vein, hyperplasia of lining epithelium of bile duct(Figure: 5d, 5e). The examination of liver sections obtained from guinea pigs administrated with EB and silymarin treated group for 10 days showed normal view with just little vacuolization and nuclear pyknosis compared to EB only group(Figure: 5f).

The liver section of the guinea pigs treated with 100mg/kg and 200mg/kg ethanol leave extract of *R. communis* administrated with EB for 10 days showed little histological changes

when compared to animals of EB only group such as mild congestion of central vein, vacuolization and nuclear pyknosis (Figure: 5 g, h).

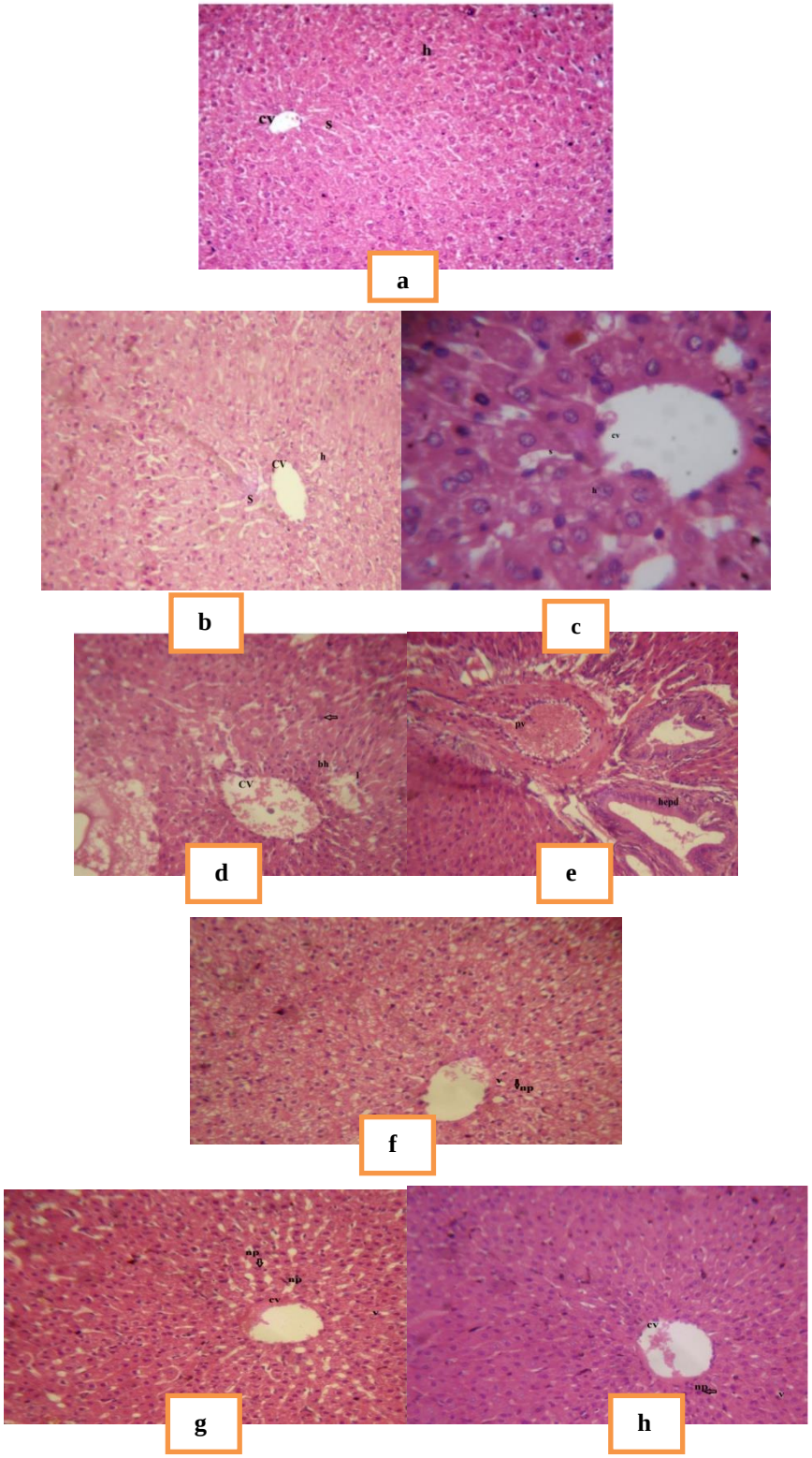

Figure No. 5: Histopathological section of liver tissue of guinea pig

(**a**) Section of liver from control group guinea pigs (2ml/kg)distilled water for ten days showed normal cellular architecture with distinct hepatic cells(h), sinusoidal spaces(s) and central vein(cv) (H&E×100); (**b, c**) sections of the guinea pigs administrated with *R. communis* (100 and 200 mg/kg) leave extract only showed normal liver architecture, the hepatocytes (h)appeared as polygonal cells with rounded nuclei and there is a few spaced hepatic sinusoids (s) with the fine arrangement of Kupffer cells (H&E×200); **(d,e)** Liver of guinea pig administrated with EB only showed vacuolization(v), dilated and congested blood vessels (cv) with hemorrhage, ballooning hepatocyte (bh), infiltration with inflammatory cells(i), nuclear pyknosis (np), karyorrhexis and karyolysis (k) indicated liver damage, congestion of portal vein (pv) hyperplasia of lining epithelium of bile duct (hepd) (H&E×100, 200);(**f**) Silymarin (standard drug) treated group showed little vacuolization (v) and nuclear pyknosis compared to EB only group (H&E×100); (**g, h**) sections of guinea pigs treated with 100mg/kg and 200mg/kg ethanol leave extract of *R. communis* showed little histological changes such as mild congestion of central vein (cv), vacuolization (v) and nuclear pyknosis (np) (H&E×100).

DISCUSSION

The liver is often the primary target for the toxic effects of xenobiotics. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver [3]. Therefore, the liver can be used as an index for the toxicity of xenobiotics. Hence, the activities of some enzymes and levels of certain biochemical parameters represent the liver function. Alanine aminotransferase (ALT) is an enzyme that helps metabolize protein. When the liver is damaged, ALT is increased in the liver and released into the bloodstream, so high level of this enzyme is observed. The estimation of this enzyme is a more specific test for detecting liver abnormalities since it is primarily found in the liver, also this enzyme showed elevated levels during hepatocellular necrosis. Aspartate aminotransferase (AST) is another liver enzyme that aids in producing proteins. It catalyzes the reductive transfer of an amino group from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate. Aspartate amino-transferase is the mitochondrial enzyme, predominantly found in the liver, skeletal muscles, and kidneys. Injury to any of these tissues can cause an elevated blood level. It also helps in detecting hepatocellular necrosis. The ratio of serum AST to ALT can be used to differentiate liver damage from other organ damage[13-14].

In the present study ALT, ALP, and AST values were elevated in pigs administrated with EB.

The elevation of AST enzyme of the hepatocytes reflects the increase of plasma membrane permeability resulting from the damage of hepatocytes and this is a parameter of liver damage[15]. The alteration in serum levels of alanine aminotransferase (ALT) may be indicating internal organs damage especially in the liver[16]. In addition, the total bilirubin concentration increases due to the effect of the emamectin benzoate, which lead to the rupture of the cell membrane and the leakage of these substances into the bloodstream at high rates. The obtained results are in agreement with the results obtained by the [17] that was conducted on female mice treated with both the pesticides emamectin benzoate and thiamethoxam, where a significant increase in the level of hepatic enzymes treated with emamectin benzoate at a concentration of 5 mg/ kg and thiamethoxam at a concentration of 100 mg/kg was observed, as well as changes occurring severe to the liver.

Our results provide the first experimental evidence that *R. communis* extract improves EB-induced liver damage in guinea pigs, as shown by normalization and restoring of these liver enzymes to normal levels. The present findings complemented the other studies on this plant extract as a study with[18] which studied the extract of *R. communis* leaves mediated alterations in liver and kidney functions against a single dose of CCl_4 induced liver necrosis in albino rats, the results indicated that both the doses (0.5 and 1.0 ml/kg body weight single dose) of extract of *R. communis* leaves lowered the activities of AST, ALT, ALP and contents of bilirubin's, urea and creatinine, indicating the protection of liver and some stress on kidney functions. And our results were in agreement with [19] they studied the protective role of *Carissa edulis* ethanolic extract against dimethoate-induced hepatotoxicity in guinea pigs. The DM caused a significant increase in the serum level of liver enzymes (AST, ALT, ALP) when compared with control animals, whereas CE extract and Liv-52 pre-treatment to the DM-intoxicated animals resulted in a significant normalization of the activities of liver enzymes.

Hepatotoxicity is common pathological toxicity that may result from the long-term accumulation of toxic chemicals, as it showed severe effects represented by severe tissue changes. Moreover, guinea pigs administrated with the pesticide emamectin benzoate at a concentration of 2 mg/kg of body weight showed severe tissue changes. At the same time, a study conducted by [20] demonstrated the presence of lymphocyte infiltration and thrombotic necrosis in hepatocytes in mice treated with emamectin benzoate. Also, liver tissues rich in their content of ALP enzymes, ALT, AST, and Total bilirubin suffer significantly from loss through many pathological and thus the biochemical examination data obtained from this

experiment support the imbalances in which liver enzymes appeared conditions [20]. Moreover, light microscopic analyses revealed that the EB-treated animals which received *R. communis* extract and silymarin exhibit little changes compared with that seen in the liver of the EB-administrated group. Thus, *R.communis* could ameliorate and alleviate the liver damage induced by EB intoxication. There are several reports supported the role of antioxidants in attenuating the toxicity of some pesticides and toxins in experimental animals. For example, a synthetic antioxidant acetyl gallate derivate showed protective capacity against hepatic oxidative stress and brain DNA damage induced by EB in male rat [21]. Furthermore, the wide range of biological activities of many medicinal plants extract may be due to the presence of different biologically active components. The *R. communis* extract with the potentiality to scavenge the free radicals contains flavonoids and tannins. Flavonoids and tannins have been reported to have anti-peroxidative effects, thus, this finding suggests that the *R. communis* extract was effective in bringing about functional improvement of hepatocytes [22].

Moreover, the groups that received only ethanol extract of the *R. communis* without the administration of EB (groups 4 and 5) did not show any significant differences to the control group based on biochemical parameters (AST, ALT, ALP, and total bilirubin) and the histopathological findings confirmed normal hepatic architecture and no signs of liver damage.

CONCLUSION

Our study suggested a significant protective effect of *R. communis* plant extract against Emamectin benzoate pesticides-induced hepatotoxicity. *R. communis* extract exerts this protection through amelioration of lipid peroxidation by its scavenging activity of free radicals and enhancement of the antioxidant defense system.

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