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Antihepatotoxic Effect of *Eruca Sativa* Mill. Extracts on Alcohol Induced Liver Injury in Rats

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Abstract

Objective: Food derived antioxidants have a special property for long term use as chemopreventive factor in disease states such as alcoholic liver diseases and hepatitis. This research decided to assess the property of several *Eruca sativa* Mill extract in ethanol induced liver injury in animals.

Material and Methods: 30 rats were divided into four groups included treatment groups with E. Sativa extracts (Water and methanolic), prophylactic and control. Oxidants/antioxidants profile, lipid profile, and serum liver functions tests were done.

Results: The finding revealed that extracts of *E. Sativa* decrease the alcoholic liver injury. In addition, the extracts of *E. sativa* may exert their prophylactic and cure role against ethanol oxidative stress by enhancing the antioxidant enzymes and antioxidant molecules levels.

Conclusion: Totally, this research proofed that the E. sativa leaves water extract is better than methanolic extract in decreasing alcoholic liver injury.

Introduction

The liver is one of the vital organs of the body that is involved in regulating many physiological activities. Any dysfunction of the liver causes a set of disorders that can cause irreparable damage to this organ [1]. Factors such as oxidative stress, free radicals, white alcohol, chemicals, viruses and drugs can damage liver tissue. The most sensitive and widely used diagnostic enzymes in the liver are

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aminotransferases which include: aspartate aminotransferase or AST and alanine aminotransferase or ALT [2]. These enzymes are normally produced by liver cells in certain amounts. When the liver is damaged, the liver cells increase the secretion of the above enzymes and cause their plasma levels to rise, which is a sign of liver damage in the blood. The functional role of aminotransferases is to catalyze chemical reactions in cells in which the amine group is transferred from a donor molecule to a receptor molecule [1]. This is why they are called aminotransferases. The AST enzyme is also called serum transaminase oxalavastate (SGOT) and ALT is also known as serum transaminase pyruvic glutamate (SGPT) [3]. Liver damage may involve a small or large part of the liver. In liver cell lesions, their contents leave the cell and enter the bloodstream. Therefore, enzymes such as AST, ALT and ALP are increased in the blood. Depending on the extent of the lesion, the surface of the enzymes also changes accordingly. Relative increases in serum ALT and AST can indicate the type of lesion [2]. In hepatocytes, AST is higher than ALT, and AST is present in the mitochondria and cytoplasm, whereas ALT is present only in the cell cytoplasm. In diseases such as viral hepatitis, in which the cell wall is damaged first, most of the enzymes in the cytoplasm enter the extracellular fluid and blood. As a result, ALT is higher than AST, but in diseases that involve the lesion of all parts of the cell, such as cirrhosis and hypoxia, the increase in AST is relatively higher than ALT [1]. Various chemical agents can cause liver damage and eventually cirrhosis are including alcohol, paracetamol, cadmium chloride, carbon tetrachloride, paracurat, paraxon, rifampin and Di-galactose amine. One of the most important of these substances is alcohol, which has been shown to cause severe liver damage in animal specimens (rats) due to the induction of toxicity by alcohol. The alcohol-induced fibrosis and cirrhosis are one of the oldest and most widely used experimental models based on this type of toxin [3]. Comprehensive experiments on biochemical and histological changes and changes associated with injury, inflammation and fibrosis have also been obtained using this model. Alcohol administration in mice over a shorter period of time leads to liver damage and cirrhosis, which is almost similar to cirrhosis in humans [2]. Researches have shown that antioxidants and phenolic compounds in herbs can prevent the toxic effects of drugs on the liver and reduce the release of liver enzymes into the bloodstream [2,3]. The body needs antioxidant compounds, because antioxidants are compounds that inhibit or eliminate the activity of free radicals and protect the body's cells from the harmful effects of these compounds. In fact, antioxidants are compounds that are used to prevent or slow down the damage caused by oxidative reactions in the body, and they act as neutralizers of free radicals and therefore prevent damage caused by these compounds in the body [2,3].

One of the medicinal plants that is used for the prevention and treatment of liver diseases in traditional medicine is *E. sativa* from *Cruciferae. E. sativa* is an annual herbaceous plant, belonging to the genus Shabboyan. The appearance of this plant was similar to horseradish and turnip [4]. This plant is rich in a variety of vitamins, especially vitamins A, C and K, and also contains minerals such as calcium, magnesium, potassium and phosphorus and is rich in antioxidants. Different parts of *E. sativa* are rich in vitamin K and therefore it is suitable for treating and improving blood thinning. This plant is rich in nutrients and vitamins and at the same time has a very low calorie content, so its consumption is recommended for slimming and weight loss [5]. Due to its minerals such as calcium, potassium, magnesium and phosphorus, it is beneficial for the health and strength of teeth and bones [4]. Another property of *E. sativa* in relation to maintaining bone health is reducing the risk of bone wear in old age. *E. sativa* is rich in antioxidants, so it strengthens the immune system and prevents cancer [5]. The use of this medicinal

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plant is very useful for preventing colds and has the same properties as turnips. Another property of *E. sativa* is to help maintain eye health, which is due to the abundance of vitamin A in this plant. This plant is effective in treating cataract eye disease. Due to having a variety of vitamins, it is effective on maintaining the health and vitality of the skin and hair (Bennett et al., 2006). This plant is a suitable and useful option to fight carcinogens due to its high content of sulforaphane. Incense of *E. sativa* is useful for relieving toothache, also one of the most interesting properties of *E. sativa* is to use this plant to help addicts to quit addiction. *E. sativa* improves shortness of breath, softens the chest and is prescribed for blood purification [5].

According to the above explanations, this research decided to assess the property of several *E. Sativa* extracts in ethanol induced liver injury in animals.

Materials and Methods

Experimental design

The animal experiments were conducted according to the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, 30 Adult male albino rats, weighing 150–200 g, fed with a standard pellet Diet and water were conditioned for 1 week before the start of the Experiment. All animals were kept under conditions that prevented them from experiencing unnecessary pain and discomfort according to guidelines approved by the ethical committee. Four groups of rats (sex rats/group/cage) were studied. The rats of group 4 (control) received the standard diet and water for 28 days.

Plant material

The plant *E. sativa* which used in this study was collected from Khartoum market in April, 2011. The plant part (leave) was air dried at room temperature then was weighted 100g for each extract.

Extraction method

By using soxhelt apparatus we begin extraction by using two reagents (Methanol and water). In extraction method we weighted 100g of the sample and put it in to the extract tube for methanol extraction then suitable amount of methanol were added after that was received in the flask and start the process, the process was continued 3hrs, after that the solvent extract was put in to the rotary evaporation to separate solvent from plant extract, finally the extract was put in to Petri dish to dry [6].. Then the sample was weighted and put in urine container (.On the other hand the rats were grouped in to four groups:

The first group was given pure alcohol.

The second group was given alcohol +methanol extract.

The third group was given alcohol +water extract.

The fourth group as control group.

The doses was started at 14/4/2011 .for each group 5mL of alcohol +0.5g of extract/kg body weight/day for group (2,3) and 5ml for group1. The doses were given during the day in form of 3ml in the morning and 2ml in the afternoon, the experimental period continued to 22days. after that the blood samples were collected from each group after surgery and put in plane container and we were isolated the serum from the hole blood and different parameter were estimated by Hitachi device including (AST-ALT –ALP-TG-CHOLESTROL-SERUM ALBUMIN and TOTAL PROTIEN)

Histopathological method

The rats' liver were collected and pre served in formalin 10% over night. In the second day 10% of formalin was prepared and then the preserved liver

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was placed in it. After that the livers dehydrated by using ethanol with different concentration (30-50-70-90-100-100%) 1hour for each concentration. Then the livers were placed in chloroform in step called clearing overnight.

In the second day the wax was prepared as liquid and then the livers were placed on wax blocks. Then by using microtome apparatus the slides were prepared and finally the slides were stained and dried and the effect was shown under microscope and the result was commented.

Serobiochemical methods

Blood samples were collected from the venous blood vessels of rat into plain containers, and serum were separated by centrifugation at 3000 rpm for 5 min and stored at -20°C until analyzed. The following methods for enzyme activity of control and test rats were performed according to the instructions in the manual of the Roche Diagnostic Hitachi 902 Analyzer.

Aspartate aminotransferase (AST)

This enzyme is also known as aspartate transaminase, L-Aspartate: 2-oxoglutarate aminotransferase, glutamate oxaloacetate transaminase (GOT, E.C.2.6.1.1).

Serum AST activity was measured by a Hitachi 902 Analyzer using commercial kits (Biosystem Chemicals, Barcelona, Spain)

Test principle

The enzyme catalyzes the transfer of an amino group from aspartate to α-ketoglutarate (α-kg) in a reversible reaction. The end products formed in this reaction are oxaloacetate and glutamate. 2, 4, Dinitrophenyl hydrazine is added to form hydrazones of the keto acid present. These hydrazones sequentially react with sodium hydroxide to form a colour which can be read by the Analayzer and mean absorbance change per/min at 340 nm/min was utilized for enzyme activity.

Enzyme activity (i.u) = ΔA_{340}/min X 3333

Alanine aminotransferase (ALT)

Alanine transaminase, L-alanine: 2-oxoglutarate aminotransferase, glutamate pyruvate transaminase (GPT, E.C.2.6.1.2).

Serum ALT activity was measured by the Analyzer (Hitachi 902) using commercial kits (Biosystem Chemicals, Barcelona, Spain).

Test principle

The enzyme catalyzes the transfer of an amino group from alanine to α- ketoglutarate in a reversible reaction. The end products formed in the reaction are glutamate and pyruvate. The transaminase activity is proportional to the amount of pyruvic acid formed over a definite period of time and is measured by a reaction with 2, 4, dinitrophenyl hydrazine (DNPH) in alkaline solution (NaOH) and mean absorbance change per/min at 340 nm/min was utilized for calculating the enzyme activity as follows:

Enzyme activity (i.u) = ΔA_{340}/min X 3333

Alkaline phosphatase (ALP)

This enzyme is also known as Orthophosphoric monoester phosphohydrolase (ALP, E.C.3.1.3.1). The serum ALP activity was measured by the Analyzer (Hitachi 902) using commercial kits (Biosystem Chemicals, Barcelona, Spain)

Test principle

ALP catalyzes the hydrolysis of p-nitrophenyl phosphate liberating p-nitrophenol and inorganic phosphate. The rate of p-nitrophenol formation is proportional to ALP activity present in the serum.

P-nitrophenyl phosphate + H_2O → P-nitrophenol + inorganic phosphate.

Non-haemolysed serum was added to a chromogenic substrate and mixture was incubated at 37°C for 5 min. A colour developer was then

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added and mean absorbance change per min at 405nm /min was utilized for calculating the enzyme activity as follows:

Enzyme activity (i.u) = ΔA_{405} / min X 2764

Total protein

Total serum protein concentration was determined by the Hitachi 902 Analyzer using commercial kits (Biosystem Chemicals, Barcelona, Spain).

Test principle

The method is based on the biuret reaction in which a chelate is formed between Cu^{+2} ion and the peptide bonds of the protein in alkaline solution (NaOH) to form a violet coloured complex. The intensity of colour produced is proportional to the concentration of protein in the sample. The Rochelle salt (K-Na-tartarate) contained in the biuret reagent is utilized to keep the formed cupric hydroxide in solution which gives the blue colour . The absorbance of sample (A sample) and of standard (A standard) was read at 540 nm in the Hitachi Analyzer and serum total protein concentration (C) was calculated.

Albumin

Serum albumin concentration was determined by the Hitachi 902 Analyzer using commercial kits (Biosystem Chemicals, Barcelona, Spain).

Test principle

The measurement of serum albumin is based on the specific binding to the indicator, 3, 5, 5, 5, tetrabromocresol (Bromocresol green, BCG), an anionic dye, and the protein at acidic pH 4.2 with the formation of a coloured complex. The intensity of colour produced is proportional to the concentration of albumin in the sample.

Serum was mixed with a buffered BCG reagent and the mixture was incubated for 10 min at room temperature.

The absorbance of the sample (A sample) and of the standard (A standard) was measured against the reagent blank at 630 nm in the Hitachi 902 Analyzer. Serum albumin concentration (C) was calculated.

Cholesterol

Serum cholesterol concentration was measured by the Hitachi 902 Analyzer using commercial kits (Biosystem Chemicals, Barcelona, Spain).

Test principle

Free cholesterol and cholesterol released from ester after enzymatic hydrolysis are oxidized enzymatically in the presence of O_2. Cholesterol in the sample is oxidized to cholestenone and H_2O_2, catalyzed by cholesterol oxidase. Quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Cholesterol ester+ H_2O → Cholesterol + Fatty

Cholesterol+O_2 → Cholestenone + H_2O_2

4-amino

antipyrine+phenol+H_2O_2 → Quinoneimine + H_2O

The intensity of the coloured quinoneimine forrmed is proportional to the amount of cholesterol present in the sample. The absorbance of sample (A sample) and of standard (A standard) was read against reagent blank at 550 nm in the Hitachi 902 Analyzer and serum cholesterol concentration (C) was calculated.

Triglyceride

A-Principle

Lipases catalyze the hydrolysis of triglyceride to yield glycerol and free fatty acid. The glycerol concentration is determined enzymetically with the trinder reaction using glycerol kinase (GK), glycerol-3-phsphate oxidase (GPO) and peroxidase (POD). The end product is a quinoneimine dye the

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concentration of which at 546 nm is directly proportional to the concentration of triglyceride in the sample.

Results

The results divided into four categories control, ethanol. (Methanol and water) extracts. And for each category we measured different parameters (cholesterol, TG, total protein, serum albumin and liver enzymes (ALT, AST, ALP). The results displayed as following:

Cholesterol

In the ethanol group there is a significant increase in the cholesterol concentration compare with the control group. In the methanol extract treated group there is a decrease in concentration compare with ethanol group. But there is slightly decrease in water extract treated group (Figure (1).

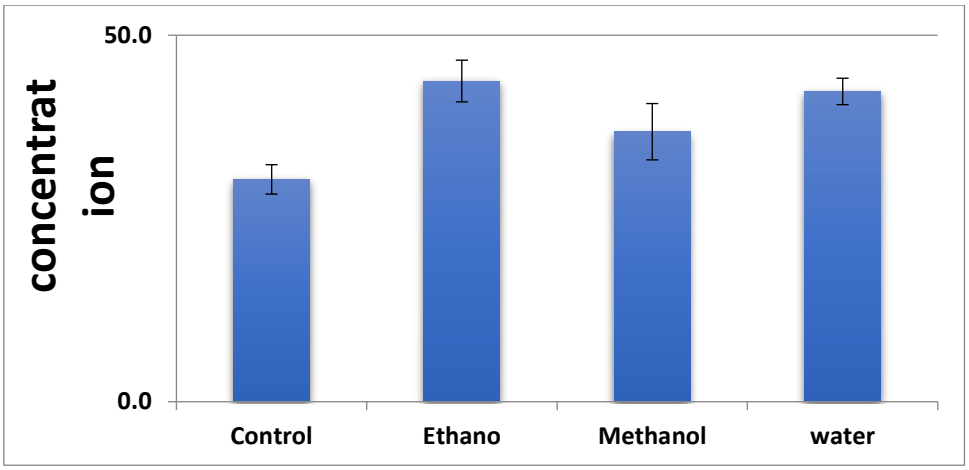

Figure 1. *Concentration of cholesterol in different samples***3.2.**

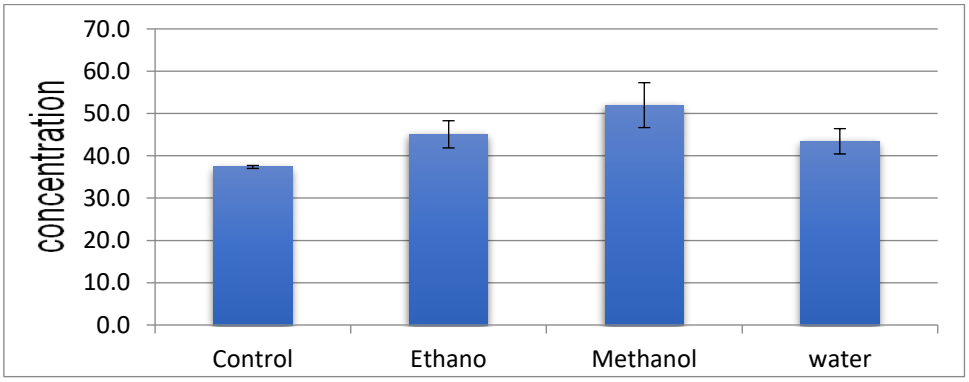

Figure 2. *Concentration of triglyceride in different samples*

Triglyceride

In the ethanol group thier is an increase in TG concentration compare with the control group. In the methanolic extract treated group there is an increase in concentration compare with ethanol group. But in the water extract treated group there is a decrease in concentration Figure (2).

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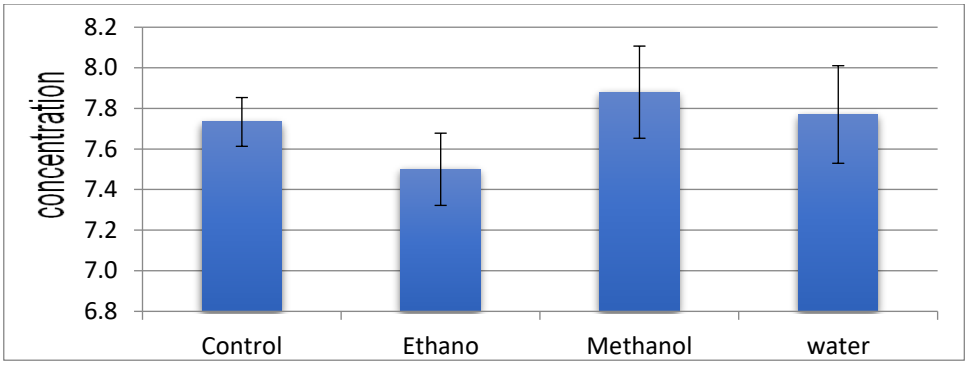

Figure 3. Concentration of T. protien in different samples

Total protein

In the ethanol group there a decrease in total protein concentration compare with the control group. But in the treated groups (water and methanolic extract) there are an increase in concentration (Figure 3).

Serum albumin

In the ethanol group there is an increase in serum albumin concentration compare with the control group. But in the treated groups (Water and methanolic extract) there are an increase in concentration (Figure 4)

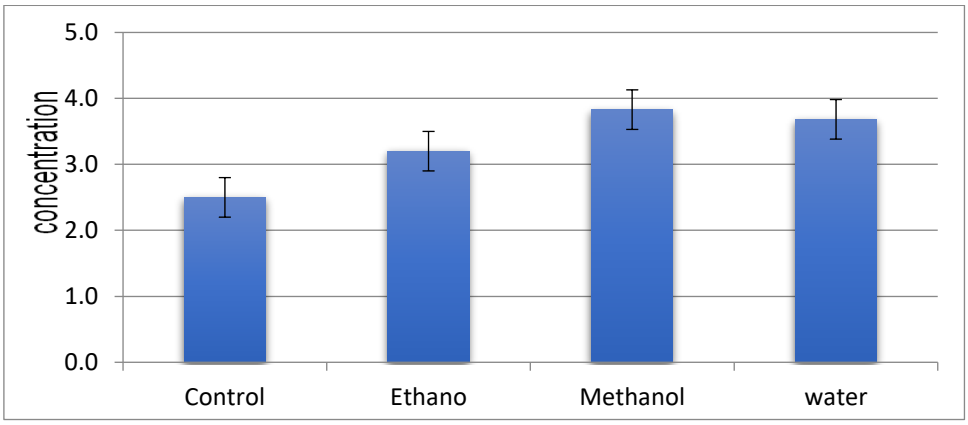

Figure 4. Concentration of serum albumin in different sample

Alkaline phosphatise

In the ethanol group thier is an increase in ALP concentration compare with the control group. But in the treated groups (Water and methanolic extract) there are a decrease in concentration (Figure 5).

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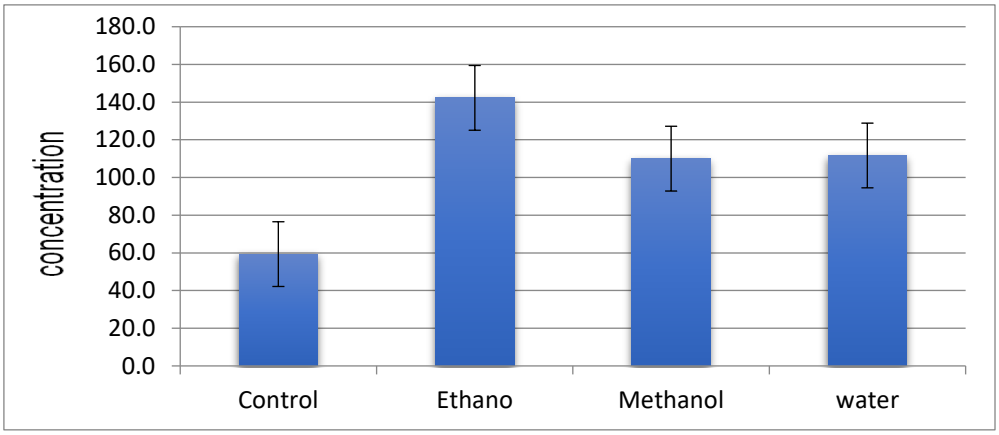

Figure 5. Concentration of ALP in different samples

Aspartate transferase

In the ethanol group thier is an increase in AST concentration compare with the control group. But in the treated groups (water and methanolic extract) there are an decrease in concentration (Figure 6).

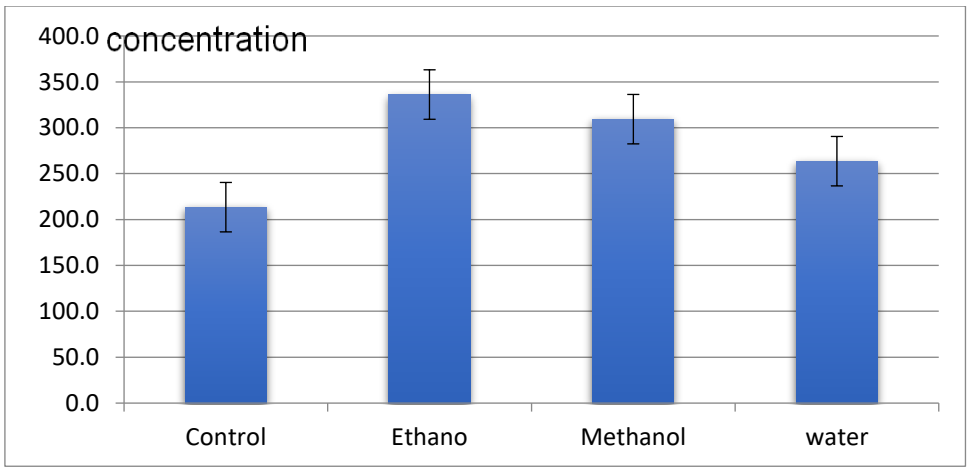

Figure 6. Concentration of AST in different samples

Alanine transaminase

In the ethanol group thier is an increase in ALT concentration compare with the control group. In the methanolic extract treated group there is an increase in concentration. But in the water extract treated group there is a decrease in concentration (Figure 7).

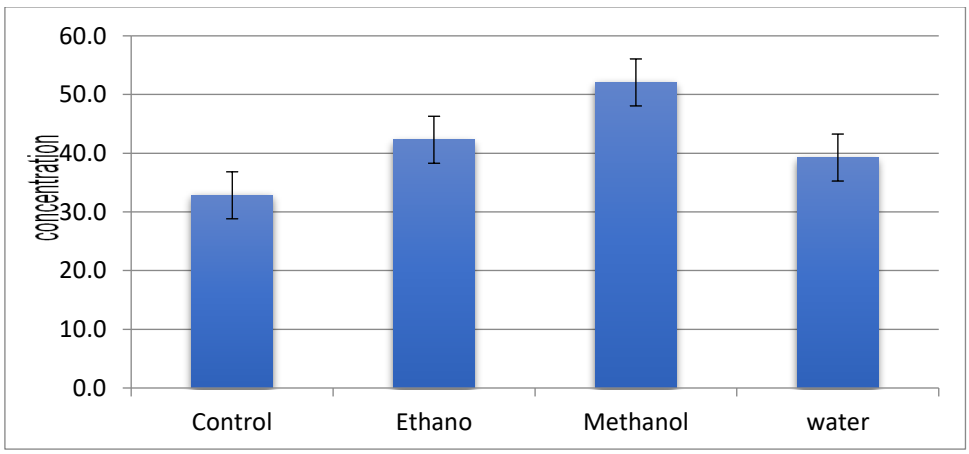

Figure 7. Concentration of ALT in different samples

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Histopathological results

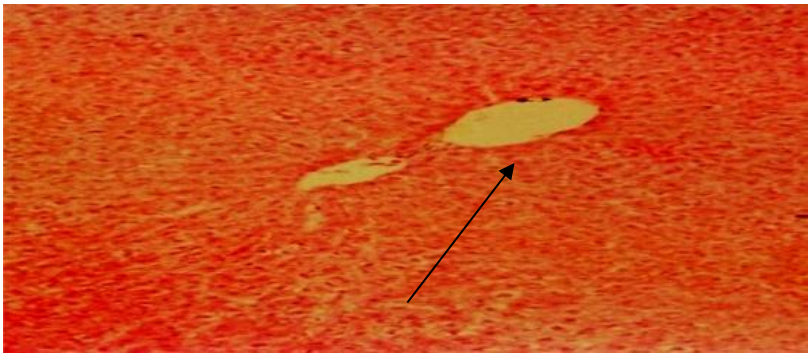

Figure 8. *Liver of a rat receiving daily constant diet showing normal hepatocyte H& E X100*

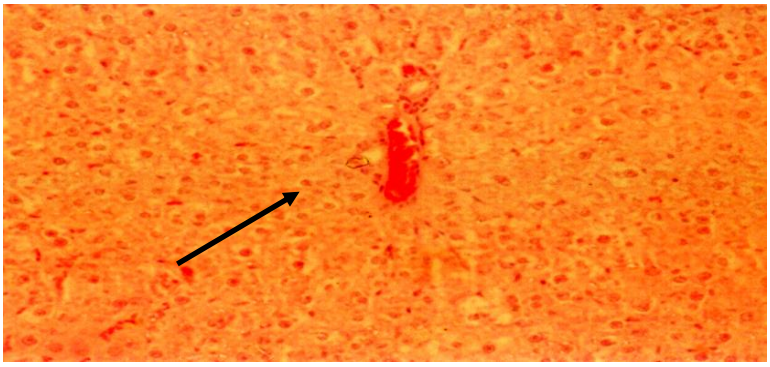

Figure 9. *Liver of a rat receiving daily oral doses of ethanol at 5ml for four weeks showing fatty cytoplasmic vacuolation of the centrilobular hepatocyte and lymphocytic infilteration and heamorhage H&E X100.*

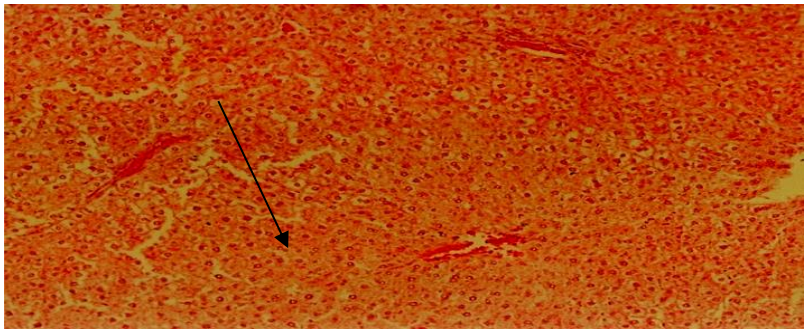

Figure 10. *Liver of a rat receiving daily oral doses of Eruca Sativa leaves methanol extract at 0.5 g\Kg for four weaks showing faty cytoplasmic vacuolation of the centrilobular hepatocyte andlymphocytic infilteration and heamorhage H&E X100.*

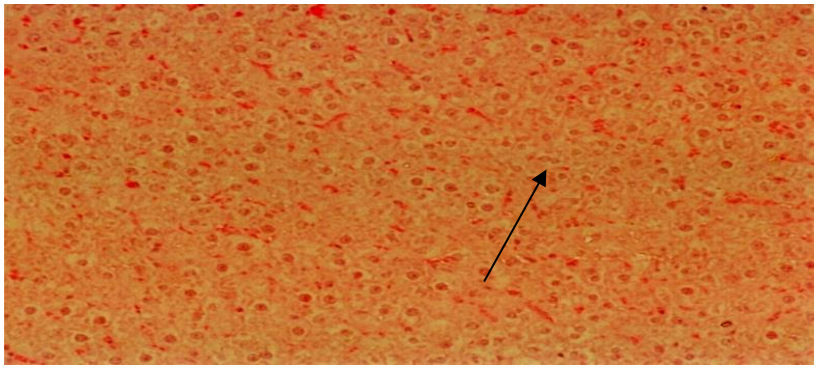

Figure 11. *Liver of a rat receiving dialy oral doses of Eruca Sativa leaves water extract at 0.5 g\Kg for four weeks showing faty cytoplasmic vacuolation of the centrilobular hepatocyte H&E X100.*

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Discussion

The liver is the largest gland in the body, located on the right side of the stomach and on the gallbladder. This important organ is responsible for about 500 different functions in the body. One of its most important tasks is its key role in the metabolism of proteins and lipids, including breaking down fats and trapping toxins and refining them by turning them into harmless substances for the body [7]. The liver is able to metabolize only one unit per hour, ie 7 to 8 grams or 10 ml of pure alcohol (hepatocytes) have difficulty in detoxification function. Alcohol-induced liver disease is the name of a group of liver diseases that are associated with excessive alcohol consumption [8]. These diseases are the most common physical disorders of alcohol abuse. They are divided into three general categories: alcoholic fatty liver-liver cirrhosis and alcoholic hepatitis. Alcoholic fatty liver can develop after a short period of high ethanol intake. In this complication, liver cells that are damaged by high levels of alcohol are unable to break down fats, and as a result, fat vacuoles accumulate in these cells [7]. It should be noted that the presence of fat in the liver is normal, but if the amount reaches more than 5 to 10% of the weight of the liver itself, the person will develop fatty liver disease. If a person who consumes high amounts of alcohol is overweight and has a stressful life, he or she is more likely to develop alcoholic fatty liver [8].

In the recent research, the alcohol was used for liver injury and enhancing the liver enzymes. The findings of this study were according to the results of the Rajakrishnan and Menon (2001) study [7]. The previous study indicated the consumption of alcohol increased the liver enzymes leakage and their circulation to the blood. In another study, Das et al., (2005) revealed the similar results with our and Rajakrishnan and Menon (2001) studies [7, 8]. Treated by *Eruca sativa* aqueous leaves reduced the AST, ALT and ALP levels similar to the El-Nattat and El-Kady (2007) [9] and Ahmed et al (2002) studies [10]. But there increase in serum albumin and this result are disagreement with Ahmed et al (2002) [10].

In the recent research, serum albumin and total proteins were raised in the treated group of extracts of *Eruca sativa* compared to untreated group. The recent results were seen at the study of El-Missiry and El-Gindy (2000) [11, 12]. Siler et al., 1999 indicated the alcohol removed the whole–body lipid balance [13]. Our results confirm the previous study, because the serum cholesterol and TG levels at the present of alcohol. Kumar et al. (2002) also confirmed our results about the fat profiles. In the examined groups, *Eruca sativa* reduced the cholesterol in serum [14]. These findings were in according to El-Gengaihi et al., (2004) who revealed that extract of *Eruca sativa* induced a significant reduce in various lipid parameters values [12]. But serum triglyceride decreased with increase in methanolic extract due to error result.

The histopathological test show that the administration of ethanol caused a haemorrhage and slightly necrosis. And in the treated figures the water extract reduce the effect of ethanol (haemorrhage and necrosis). But the methanolic extract didn't effect on the liver injury.

Conflicts of Interest

The authors hereby declare no conflicts of interest.

Authors` Contribution

All authors contributed in the experiments, analysis and preparation of this manuscript.

Funding/Support

Not Applicable

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