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Research Article

Hepatoprotective Activity of *Foeniculum vulgare* Against Paracetamol Induced Hepatotoxicity in Rabbit

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ABSTRACT

The hepatoprotective effects of a *Foeniculum vulgare* extract was determined in male rabbits *(Oryctolagus cuniculus).* The *Foeniculum vulgare* extract was used in different doses in Paracetamol induced toxicity in rabbits. *Foeniculum vulgare* seeds were obtained from market and identified by Punjab University. Extract was prepared by maceration method. *Foeniculum vulgare* seeds 100 gm were weighed after grinding then added in flask. 80% ethanol solution was added four times more than the weight of *Foeniculum vulgare* powder and then kept for 4 days on shaker and filtered through Whatman filter paper. Solvent extract was evaporated at temperature of 25°C. Extract powder was obtained. Powder extract 250 mg/kg/BW and 500 mg/kg/BW was filled in capsule of 2 & 0 size respectively. 16 rabbits, with an average age of 3-4 months, were taken. They were provided standard diet and water. Animals were kept at light/dark cycle (12/12 h) at a temperature (25 ± 2°C) and relative humidity (60 ± 5%). Animals were kept at Laboratory environment for one week. Study designed animal were divided randomly into 4 group, having 4 animal in each group. Group A served as positive control, given no medicine. Group B was intoxicated with a single dose of Paracetamol (Par-cm) 2 g/kg per oral (P.O.) using carboxymethyl cellulose (CMC) 1% as a vehicle. Group C received *Foeniculum vulgare* seeds hydroalcoholic extract (250 mg/Kg) P.O. daily for 9 days, followed by a single dose of Paracetamol 2 g/kg P.O. on 9th day using carboxymethyl cellulose 1% as a vehicle. Group D was pre-treated with *Foeniculum vulgare* seeds hydroalcoholic extract (500 mg /Kg) P.O. for 9 days, and then a single dose of Paracetamol 2 g/kg P.O. on 9th day using carboxymethyl cellulose 1% as a vehicle. Animals were slaughtered after 24 hours of the last treatment. Blood and liver sample were collected from all controlled and treated rabbits for Liver Function Tests and Histopathological studies. Serum Liver enzymes AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase), ALP (Alkaline Phosphatase) and bilirubin were used as indicators to monitor the status of the liver either healthy or damaged. Serum liver enzymes and bilirubin level increased in Group B as compared to Group A. The values of indicators were almost decreased in Group C and Group D. Histopathological studies were used for further confirmation of biochemical analysis. Histopathological study of Group A showed a normal hepatic cell structure. While Group B showed sinusoids congestion and ballooning degeneration. These parameters were found mild and moderate in Group C and Group D. These histopathological findings also supported the biochemical results. Thus the study concluded that the *Foeniculum vulgare* extract was effective against Paracetamol induced toxicity in rabbits.

Keywords: Natural product metabolomics; *Foeniculum vulgare*; Hepatoprotective; Paracetamol Par-Cm; Carboxymethyl Cellulose CMC.

INTRODUCTION

Liver is an important organ having a broad range of functions including metabolism, detoxification, excretion, synthesis of protein and construction of biochemicals required for indigestion. It participates on all biochemical pathways including growth, nutrient supply, reproduction, oppose to disease and provision of energy. Liver is a vital organ for regulation and homeostasis in the body. The liver is permanent susceptible to exogenous substances e.g. drugs, alcohol and environmental toxins, which can lead to liver disorders, such as hepatocellular, cholestatic (obstructive) and mixed type of the liver disorders. Almost all kinds of hepatic failure

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lead to death. Liver disorders are vast fatal diseases in all over the world. Many drugs are available but do not given satisfactory results for liver diseases because of adverse effects and high cost. Therefore there is need to search drugs for treatment of hepatic diseases that have more efficacy and safety [1]. The main function of the liver is secretion of bile and metabolism of fat, protein, carbohydrate, detoxification and storage of glucose and vitamins. Different types of disorders occurred in liver. Liver diseases e.g. jaundice, cirrhosis and fatty liver are very common in worldwide. After metabolism, several environmental toxins and carcinogens are converted into reactive compound resulting in tissue damage. Reactive oxygen and free radicals produce liver diseases and cirrhosis [2]. Pharmaceuticals and environmental chemicals are basic source to damage the liver. Many drugs cause liver injury and liver failure and reactive metabolites effect on cellular function and immune system [3]. The antibiotics were found to be causing liver damage more frequently. The hepatocellular pattern of damage was most common and directly correlated to the age [4]. Allopathic drugs used for liver diseases treatment are insufficient. So we required more drugs for liver treatment because available drugs have no satisfactory efficacy and safety. Almost 900 drugs are responsible for liver injury. Various medicinal plants and its products used for the liver diseases. Herbal drugs play a wonderful role in treatment of liver diseases and enhance the natural healing function of liver [5]. Demand of herbal drugs is increasing worldwide and Government of Pakistan has also made the laws to regulate the use of herbal products. According to one study there are about 600 commercially available herbal formulations which are being used worldwide for hepatoprotective effects. It is essential to evaluate the traditional herbal drugs by using standard experimental procedures to validate their claimed therapeutic uses [6]. The world market of natural products e.g. medicinal plants has been increased due to nutraceuticals and phytomedicines [7]. Plants play an important role in human food and medicine. Nutritional therapy concept increased in whole world in recent years. Use of natural plant's food and phytotherapy provide a new concept for improvement of health and prevention of diseases and its treatment. So study is required on nutraceuticals, phytonutrienta, nutritional therapy, phytotherapy, its related epidemiology and clinical studies [8]. All over the world, herbal treatment give a health benefits. According to WHO 80% population in developing countries rely on the use of herbal treatment. Demand of herbal products is increasing in developing and developed countries due to having minimum side effects and affordable prices [9]. Par-Cm is used as an analgesic and antipyretic which is metabolized in liver to form N-Acetyl-P-Benzoquinone Imine (NAPQI) that binds covalently with hepatic glutathione. Overdose of Par-Cm produces large quantity of NAPQI that causes liver injury [10]. In animals Par-Cm cause liver damage due to its high dose. At therapeutic dose, Par-Cm is converted to NAPQI that will be detoxified forming conjugates with glutathione. Cell death occurred if dose of Par-Cm is higher and hepatic glutathione is lower [11]. Hepatic necrosis occurred due to interaction of hepatocytes protein with reactive metabolites of Par-Cm. Liver injury occurred due to unintentional self-medication for fever and pain with dose more than 4 g/day of Par-Cm. Fasting and alcohol increased toxicity however it is managed by giving N-acetylcysteine (NAC). Morbidity and mortality occurred if overdosing is not managed in time or delay medical therapy for this toxicity [12]. At this time, we have need to increase interest about therapeutics benefits of herbal plants that have antioxidant ability to diminish the tissue injury that occurred by free radicals. Different kinds of therapeutic antioxidant products e.g. butylated hydroxyanisole, butylated hydroxytoluene and garlic acid esters are formulated for protection against oxidative activity but they are expensive and show bad effect on health such as boost up radio-sensitization, tumor and mutagenicity. So its use is decreased due to these side effects and plants have been searching and investigating for antioxidants. Liver disease is a big challenge therefore the world is shifting from synthetic to natural products [13]. Fennel *(Foeniculum vulgare)* is cultivated in different countries. This herb has history for being use as medicine [14]. Chromatographic analysis revealed that *Foeniculum vulgare*seeds extract contain fenchone, methylchavicol, trans-anethole, trans-anethole, limonene, fenchone, methylchavico, 3-carene, α-pinene, α-phellandrene and β-myrcene in different region of the world and being used as food and medicine as well as carminative, enhance libido, diuretic, stimulant properties, cough, galactogogue and indigestion. This fruit contain 1% to 3% of a volatile oil which consists of about 20% of d-fenchone and 50% to 85% of anethol. Anisic acid, d-apinene, dipentene, methyl chavicol and d-apinene are also present in this fruit [15]. Analgesic, antipyretic, diuretic and antioxidant abilities are present in this fruit. Essential oil gives a sweet aroma and taste [16]. *Foeniculum vulgare* reduce levels of Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Serum Aspartate Aminotransferase (AST) and bilirubin [17]. *Foeniculum vulgare* plant is also used for spleen disorders, suppressing appetite, improving digestive system and treatment of colic and irritable bowel. This plant has phytoconstituents to cure diseases in human beings and have antioxidant properties as well antimicrobial [18]. *Foeniculum vulgare* has been widely used as an estrogenic agent. This plant seeds extract have estrogen so it act on oviduct and mammary gland [19]. *Foeniculum vulgare* is also used in many other diseases like respiratory, endocrine, digestive and reproductive system diseases. In lactating mothers it is used as galactogogue agent, reduce stress and memory enhancing effects [14]. *Foeniculum vulgare* infusions are available for colic spasm in nursing babies. *Foeniculum vulgare* is also used as diuretic, eye lotion, secretolytic and antispasmodic whereas *Foeniculum vulgare's* powder is used topically for snake bites [20].

OBJECTIVE

To determine the hepatoprotective effect of hydroalcoholic extract of *Foeniculum vulgare* seeds at different doses in Paracetamol induced hepatotoxicity in Rabbit.

MATERIALS AND METHODS

Chemicals and drugs

Paracetamol (Par-Cm) micronized powder was obtained from Munawar Pharma (Pvt) Ltd. Lahore and Carboxymethyl Cellulose (CMC) was obtained from Cure Inn Phytoceuticals.

Extraction

Foeniculum vulgare seeds were obtained from market and identified by Punjab University. Extract was prepared by maceration method. *Foeniculum vulgare* seeds 100 gm were weighed after grinding then added in flask. About 80% ethanol solutions was added four times more than the weight of *Foeniculum vulgare* powder and kept for 4

days on shaker at temperature of 25°C. Solvent extract was filtered through Whatman filter paper and powder extract was obtained. Powder extract 250 mg/kg/BW and 500 mg/kg/BW was filled in 2 & 0 size capsule respectively [21].

Experimental animals

16 male Rabbits *(Oryctolagus cuniculus)* with weight (550-1000 gm) and average age of 3-4 months were obtained from market. Animals were kept at light/dark cycle (12/12 hr) at a temperature (25 ± 2°C) and relative humidity (60 ± 5%). Animals were kept at laboratory temperature. They were provided standard diet with water. Animals were acclimatized to the laboratory atmosphere for one week. Animals study protocol was approved by Research & Ethics Committee, Hajvery University Lahore.

Grouping of animals and treatment protocols

Test animals were divided into 4 groups, each group having 4 animals,

- Group A was kept as control (normal saline).
- Group B received a single dose of Par-Cm[2] gm/kg P.O. on 9th day by using CMC 1% as a vehicle.
- Group C received *Foeniculum vulgare* hydroalcoholic extract (250 mg/Kg P.O.) daily for 9 days, followed by a single dose of Par-Cm 2 gm/kg P.O. on 9th day using CMC 1% as a vehicle.
- Group D was administered *Foeniculum vulgare* hydroalcoholic extract (500 mg /Kg P.O.) for 9 days and then a single dose of Par-Cm 2 gm/kg P.O. on 9th day using CMC 1% as a vehicle

Animals were slaughtered after 24 hours of last treatment. Blood was collected from all treated and controlled rabbits for Liver Function Tests. Liver was obtained, washed with distilled water, weighed and then stored by using 10% formalin solution. Tissue section of 4-6 µm thickness was obtained. Hemoxyline and Eosin staining was performed for histopathological assessment.

Biochemical analysis of total bilirubin and liver enzymes diagnostic kits

Micro Lab 300 (Semi-automated clinical chemistry analyzer) was used for biochemical test, ALP, ALT and AST. Analysis Merck, France Diagnostic kits was used and Diasys Diagnostic Systems and Germany kit were used to determine total bilirubin.

Histopathological studies

After slaughtering the rabbits, the liver of rabbit was taken, all the adhered extra connective tissues from the liver were removed. Distilled water was used for liver washing; gross examination of liver was completed and recorded. Liver was sliced in small pieces then immediately placed in 10% Neutral Buffered Formalin (10% NBF). For proper fixation achievement, the liver pieces were properly dipped in the (10% NBF).

Statistical analysis

One-way ANOVA followed by Tukey HSD was used to determine the statistical differences between the means of study groups using the SPSS computer software (Version 23).

RESULTS

Liver function tests

These tests include ALT, total bilirubin, ALP and AST. It was found that *Foeniculum vulgare* 500 mg/kg (0.570+0.010) mg/dl slightly reduced the bilirubin level in comparison with *Foeniculum vulgare* 250 mg/kg (0.590+ 0.010) mg/dl with a p=1.000. However, *Foeniculum vulgare* 500 mg/kg (0.570+0.010) mg/dl significantly controlled bilirubin level in comparison with Par-Cm (17.000+0.577) mg/dl alone with p=0.000 as shown in Figure 1. *Foeniculum vulgare* 500 mg/kg (123.00+1.000) IU/L considerably decreased the ALT levels as compared to *Foeniculum vulgare* 250 mg/kg (165.00+1.000) IU/L and Par-Cm alone (309.00+1.000) IU/L groups with a relatively high level of significance p=0.0001 indicated in Figure 2. Similar is the case with AST, *Foeniculum vulgare* 500 mg/kg (28.000+1.000) IU/L consistently reduced the AST level than *Foeniculum vulgare* 250 mg/kg (70.000+1.000) IU/L, Par-Cm alone (108.000+1.000) and Control (64.000+ 1.000) IU/L groups with p=0.000 as demonstrated in Figure 3. Alkaline phosphatase level was much reduced by *Foeniculum vulgare* 500 mg/kg (21.000+1.000) IU/L as compared to *Foeniculum vulgare* 250 mg/kg (27.000+1.000) IU/L group indicated in Figure 4. In short, it is found that liver function enzymes were significantly reduced by *Foeniculum vulgare* 500 mg/kg group among all treatments. Therefore, this study showed that *Foeniculum vulgare* 500 mg/kg is found most hepatoprotective in all defined treatments.

Histopathology

Animals were slaughtered after 24 hours of the last treatment. Blood were collected from all treated and controlled rabbits

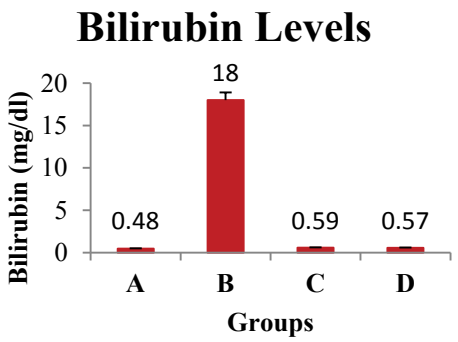

Figure 1: Shows slight decrease in bilirubin level in group D as compared (Group C).

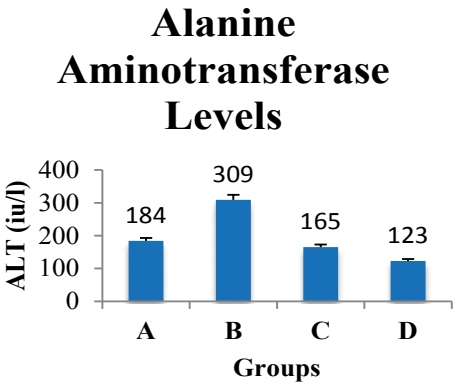

Figure 2: Shows that *Foeniculum vulgare* 500 mg/kg concentration significantly decreased ALT among all groups.

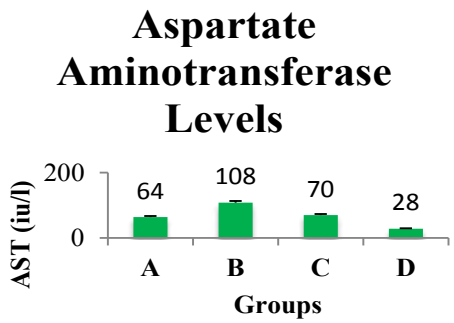

Figure 3: Shows that *Foeniculum vulgare* 500 mg/kg concentration significantly reduced AST levels among all groups.

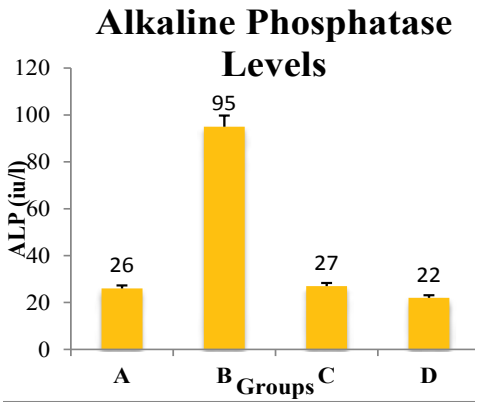

Figure 4: Figure indicates that ALP is significantly reduced by *Foeniculum vulgare* 500 mg/kg concentration in all groups.

for Liver Function Tests. Liver was washed with distilled water, weighed and stored in 10% formalin solution. Tissue sections of 4-6 µm thickness were obtained and Hemoxyline and Eosin staining was performed for histopathological assessments. For group A rabbits section revealed liver parenchyma composed of unremarkable hepatocytes. The sinusoids showed normal pattern. The hepatocytes have trabecular pattern of arrangement around portal tract and central vein. No evidence of fibrosis, hepatitis or staetosis was seen Figure 5. In subjects of group B, section revealed liver parenchyma showing neutrophils within the sinusoids, the sinusoids appear congested. The hepatocytes showed ballooning degeneration, focal vacuolisation and disorganisation. At places, steatosis of microvesicular type was seen. The central vein was slightly disturbed. No evidence of fibrosis or hepatitis was seen Figure 6. In group C animals, section revealed liver parenchyma composed of unremarkable hepatocytes showing trabecular arrangement around portal tract and central vein. Few congested blood vessels were identified. Some of the hepatocytes showed acidophilic and granular change. Sinusoids were unremarkable. No evidence of steatosis, fibrosis or hepatitis was seen Figure 7. In experimental group D, section revealed liver parenchyma composed of unremarkable hepatocytes showing trabecular arrangement around central vein. The portal tracts and sinusoids were unremarkable. No evidence of fibrosis or hepatitis was seen Figure 8.

DISCUSSION

Paracetamol (Par-Cm) induced hepatotoxicity is a genuine method for observing the herbal plants hepatoprotective effects Jaeschk et al. reported Par-Cm is responsible for oxidative damage by enhancing hepatic cells membrane peroxidation by free radicals formation [22,23]. Jaeschk et al. explained that free radicals disturb hepatocytes membrane. Liver cell disturbances were measured from value of ALP, ALT and AST enzymes in serum. These values showed the liver physiological status when Par-Cm toxic dose was given. Cellular insult occurred and biochemical indicators (bilirubin and alkaline phosphatase aminotransferases) present in liver cells entered in blood stream [24]. Glutathione is a major antioxidant and it protect the liver from the toxicity of Paracetamol but high dose of Paracetamol caused increased level of bio-indicators. Hussain et al. and Akhtar et al. reported that the extent of liver damage is determined by released cytolasmic enzymes ALP, AST and ALT [25,26]. When liver cells disturbed then

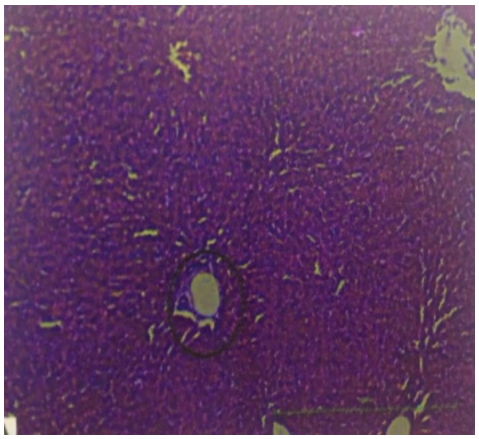

Figure 5: Shows no evidence of fibrosis, hepatitis or staetosis was seen (Group A).

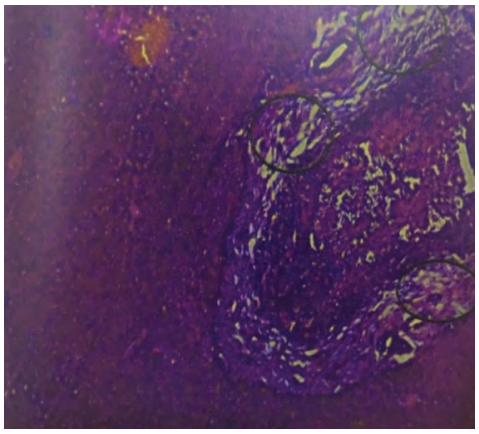

Figure 6: No evidence of fibrosis or hepatitis was seen (Group B)

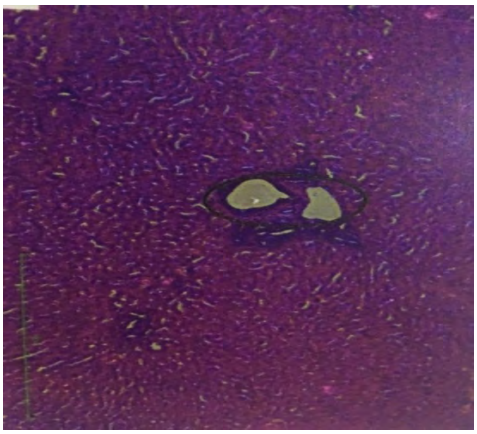

Figure 7: No evidence of steatosis, fibrosis or hepatitis was seen (Group C).

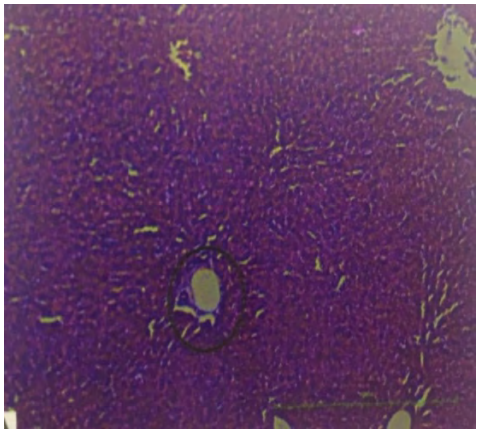

Figure 8: No evidence of fibrosis or hepatitis was seen (Group D).

transport mechanism of liver enzymes and membrane permeability changed these liver enzymes entered in serum from the cells so level of biochemical's parameters (AST, ALP, ALT and Bilirubin) decreased in liver cells and increased level in serum [27,28]. The AST enzyme is present in hepatocytes mitochondria whereas bilirubins, ALP, ALT are also found in liver cells, so liver damage is measured by these parameters [6]. In this study hepatic damage occurred due to increased level of AST, ALP, ALT & Bilirubin. ALP, AST, ALT & total bilirubin measured in Par-Cm intoxicated group were 108.000 ± 1.000 IU/L, 95.000 ± 1.000 IU/L, 309 ± 1.000 IU/L & 17.000 ± 0.5777 mg/dl respectively, which were higher than group A (controlled group). The values of group A 64.000 ± 1.000 IU/L, 26.000 ± 0.0001 IU/L, 184 ± 0.000 IU/L & 0.48 ± 0.000 mg/dl. Par-Cm caused hepatic cell membrane damage that's why enzyme entered in blood stream from liver cells. This study results comply with Ahmad et al., Rehman et al., Rehman et al. and Mumtaz et al. showing same hepatotoxicty with Par-Cm [29-32]. *Foeniculum vulgare* extract 250 mg dose showed the hepatoprotective effects and decreased the level of AST, ALP, ALT and bilirubin 70.000 ± 1.000 IU/L, 27.000 ± 1.000 IU/L, 165.000 ± 1.000 IU/L & 0.590 ± 0.010 mg/dl respectively in serum as compared to the group B (diseased group). So this indicates that *Foeniculum vulgare* extract exerts hepatoprotective effect on liver tissue which was damaged by using the Par-Cm. When dose of *Foeniculum vulgare* extract increased 500 mg it showed more hepatoprotective effects. The values of AST, ALP, ALT and Bilirubin 28.000 ± 1.000 IU/L, 21.000 ± 1.000 IU/L, 123.000 ± 1.000 IU/L & 0.570 ± 0.010 mg/dl decreased respectively as compared to group B and C. Damaging signs were seen with Par-Cm administration in histopathology of liver. In histology of liver *Foeniculum vulgare* showed positive effects. Group B received Par-Cm toxic dose showed many negative changes and disturbances in tissues of liver like sinusoids and central vein disturbed and ballooning degeneration. These same histopathological changes and signs were observed with toxic dose of Par-Cm by Rehman et al. and Naveed et al. [30,33]. Liver cell congestion occurred in this study was same as the result of Ruepp et al. [34]. Reeves et al. reported that formation of large collagen, connective tissue and hepatic fibrosis deposition results in liver injury [35]. Jaeschke et al. reported that NAPQI formation occurred due to large dose of Par-Cm and free radicals generated leads to hepatic cell necrosis [23]. *Foeniculum vulgare* protected damaging effects of liver due to Par-Cm. Par-Cm caused sinusoids congestion and microscopic examination of liver exhibited ballooning degeneration in Group B. In group C *Foeniculum vulgare* reduced the damaging effects of liver and intensity of destructive changes in liver histology. Lesser congested blood vessels were identified. In group D when dose of *Foeniculum vulgare* increased then microscopic examination of liver cells showed increased hepatoprotective effects and no phenomenon of fibrosis were seen. Thus, results suggested that *Foeniculum vulgare* is effective against liver toxicity caused by Par-Cm. *Foeniculum vulgare* reduces the liver damaging effects due to Par-Cm. *Foeniculum vulgare* contain fenchone, camphene, pinene, ß myrcene, ß-pinene, phellandrene, limonene, 3-carene, camphor, methylchavicol, trans-anethole, and cisanethole. D-limonene increased concentration of reduced Glutathione (GSH) in the liver. So that glutathione covalently bind with NAPQI (N-acetyl-p-benzoquinone imine) and decreased toxicity of Par-Cm and Al-Amoudi et al. study showing decreased the toxicity of liver due to present d-limonene in *Foeniculum vulgare* [18]. The herbal product showed a remarkable protection of hepatic cells which injured due to toxicated dose of Par-Cm and also lowered the level of enzymes which increased by Par-Cm intoxication. When Par-Cm was administered in rabbit model then all bio-marker enzymes value was increased. The study results showed a positive response by herbal remedy *(Foeniculum vulgare)* and if herbal remedy dose is increased to 500 mg then it showed more positive response as compare to lower dose 250 mg. Histopathological findings also showed that the herbal drug exhibit magical healing process on Par-Cm toxicated hepatic cells. *Foeniculum vulgare* is a powerful antioxidant and beneficial for hepatoprotectivity Figures 7 and 8.

CONCLUSION

Foeniculum vulgare at different doses is a beneficial for its hepatoprotective effect in animals with large doses of Paracetamol (Par-Cm) induced hepatotoxicity. Further studies are warranted with large number of animal and other animal species. Histopathological studies were used for further confirmation of biochemical analysis. Histopathological study of Group A showed a normal hepatic cell structure. While Group B showed sinusoids congestion and ballooning degeneration. These parameters were found mild and moderate in Group C and Group D. These histopathological findings also supported the biochemical results. Thus the study concluded that the *Foeniculum vulgare* extract was effective against Paracetamol induced toxicity in rabbits.

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