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Full Length Research Paper

Carbon Tetrachloride (CCl_4) - induced hepatotoxicity in rats: Curative role of *Solanum nigrum*

Asif Mir[1]*, Farida Anjum[2], Naveeda Riaz[1], Hina Iqbal[1], Hussain Mustatab Wahedi[1], Jabar Zaman Khan Khattak[1], Muhammad Asrar Khan[3] and S. A. Malik[4]

[1]Department of Bioinformatics and Biotechnology, International Islamic University (IIU), Islamabad, Pakistan.
[2]Higher Education Commission (HEC), Islamabad, Pakistan.
[3]Department of Biosciences, COMSATS Institute of Information Technology, Islamabad, Pakistan.
[4]Department of Biochemistry, Faculty of Biology, Qauid-i-Azam University, Islamabad, Pakistan.

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Solanum nigrum **is used in hepatic disorders in folk medicines. The present study was aimed to evaluate the efficacy of *S. nigrum* on the liver functions in CCl_4 induced injuries. Enzymatic activities that are .AST, ALT and ALP, microscopic appearance of liver was used as parameter and hepatocurative studies were performed. In case of hepatocurative study that is post treatment of rats with aqueous extracts of plant (500mg/ orally, two doses with 24 h interval) prevented ($p < 0.001$) CCl_4 induced rise in activity of serum Transaminases (ALT and AST) and ALP and alcoholic extract did not prevent the rise of same enzymes compared to the sham control group in which liver was damaged by CCl_4 no treatment given. Histological examination of the liver of treated animals with aqueous extract of plant showed that fatty acids change was less in comparison to the sham control group. In treated group reduction in body weight was minimal and live enlargement was also less compared to the animals in sham control group. Treatment with aqueous extract of *S. nigrum* effectively attenuated the alteration within the parameter of present study and accredits the hepatocurative role of *S. nigrum.***

Key words: *Solanum nigrum,* hepatocurative, AST, ALT, ALP, histopathology, CCl_4 induced hepatotoxicity, aqueous extract.

INTRODUCTION

Plants have been used during the age for cure and treatment of diseases since the start of mankind. Phytotherapy is the use of plant, plant extract or pure chemicals isolated from natural products to treat diseases. Plants have been used to treat diseases such as diabetes, jaundices, cardiovascular diseases, heavy metal poisoning, congestion of abdominal and pelvic cavities and scarlet fever etc. (Said, 1996). It is estimated that out of 250,000 to 500, 000 species of plants only 1 to 2% of the terrestrial plants have been reasonably well investigated. Although today the synthetic drugs are larger in their number than the natural ones but still many synthetic drugs have their origin in the natural source and have been derived from plants and animals (Philipson, 1989).

Structurally different compound may be present in the plant which may have synergic effect. Complexity of these drugs and their biological variations make it necessary to evaluate their safety. Modern Science is now beginning to accept the use of standardized plant extracts. Therefore there should be attempts at the precise studies on such medicinal plants, which may be also used for liver diseases. Researchers have investigated several plants for their hepato-curative action and have demonstrated curative activity in their extract(s).

One of the plants *Solanum nigrum* is claimed effective in treating hepatic ailment by hakeems and traditional healer. *Solanum nigrum* belongs to Solanaceae (Potato family), which contains mostly vegetables and medicinal plants. The pharmacological constituents separated out from *Solanum nigrum* are reported to be steroidal glycoalkaloids (solamargine, solasonine and solanigrine), steroidal genin (titogenin), saponinus and vitamins

*Corresponding author. E-mail: mir77uspk@yahoo.com, asif.mir@iiu.edu.pk.

(riboflavin, carotene, nicotinic acid and ascorbic acid) (Varshney and Sharma 1965). *S. nigrum* has been reported to possess antiseptic, antispasmodic, immuno-modulating and anticonvulsant activities (Nadeem et al., 1991). The plant is also reported to be a valuable cardiac tonic, alterative, diuretic, sedative and diaphoretic.

Antioxidant activity is considered to be an important mechanism by which many of the medicinal plants used by traditional medical practitioners for the treatment of liver diseases. Investigations have been focused on extracting antioxidants with higher efficiencies and lower toxicities than currently available synthetics antioxidants. In this respect flavonoids and other polyphenolic compounds have received great attention (Constantino et al., 1992). Carotenoids are also reputed to be anti-oxidants (Klaui, 1982) and thus show anti-hepatotoxic activity (Oshima et al., 1984). Further, the hepato-protective potential of lignans was also reported (Faure et al., 1990).

The Greek for liver is “Hepar”, a word used in combed form as hepato-, hepatic and hepatitis (Wynaber et al., 1995). Liver is usually large, compound tubular gland. The structural and functional unit of liver is lobule that contain the central vein running longitudinally through these lobules. There are three concentric zones of classical lobule, Zone I, an ellipsoidal area, Zone II, intermediate and Zone III near the end of the lobule. Within the lobules, liver cells or hepatocytes are arranged in one-cell-thick plate like layers. These hepatocystes make 80% of the cell population of liver. Histologist using light microscope noted minor differences in the appearance of hepatic cells in three concentric zones within the classical lobule. Liver can be damaged by drugs like tetracyclines, sulphonamides and antihypertensive drug methyldopa. One of the best models of injury produced in liver is by CCl_4. CCl_4 is used as hepatotoxic agent in animals research work to study the hepato-curative action of plants and other compounds (Aliyu et al., 1995; Bishayee et al., 1995).

The present work has been carried out to evaluate the action of the aqueous and alcoholic extracts of *S. nigrum* against the CCl_4 induced hepatotoxicity in the albino rates of Sprague Dowley strain.

MATERIALS AND METHODS

S. nigrum whole plants were collected from the fields at NARC, Islamabad and put to dry for two weeks in the sunlight. Dried whole plant and its fruit were made clear of dust and other impurities. The plants and its dry fruit were identified and authenticated as plants of *S. nigrum* by the taxonomy section of the Department of Biological Sciences, Quaid-i-Azam University, Islamabad.

Preparation of powder

The whole dried plants and its fruit were separately powdered mechanically with a china herb grinder. The powder of whole plant and fruit were mixed in equal ratio manually. The prepared powder was kept in dry, clean, airtight glass jar and stored at 4°C until used.

Preparation of aqueous extract

The prepared powder weighing 100 g was macerated in 500 ml of distilled water for 24 h. It was then filtered and filtrate was dried in Petri dishes and concentrated to dark green residue by heating at 40°C, till complete evaporation of water was achieved.

Preparation of Alcoholic Extract

The prepared powdered weighing 100 g was mixed with 250 ml of absolute ethanol (Merck) for 24 h. The mixture was filtered and the filtrate was dried in Petri dishes in an oven at 40°C. The dried ethanol extract so obtained was stored in a refrigerator until used. It was dissolved in 10 ml distilled water just before treatment to their respective groups of rats (Alcoholic groups).

Animals

Male Sprague Dowley, Albino rats weighing between 150 to 350 g were obtained from the animal house of National Institute of Health (NIH), Islamabad. Rats were kept in wire cages at animal house of Quaid-I-Azam University, Islamabad under standard conditions. Food and water was available to the rats.

DOSES AND ROUTE OF ADMINISTRATION

In the study animals, hepatic injury in all groups except standard control was induced by single oral administration of CCl_4 mixed with olive oil as vehicle in 1:1 ratio (3 ml/kg of rat body weight). Animals with hepatic injury were post treated with extracts of *S. nigrum*. Extract was given in doses of 500 mg/kg orally with the help of gastric tube.

EXPERIMENTAL DESIGN

Animals were randomly divided into four groups, each of ten animals. Each group was named according to extract used in two different. 1. Standard control group (received only vehicle i.e. olive oil (1.5 ml/kg of rat body weight) orally, instead of plant extract and CCl_4) 2. Sham control group (CCl_4 was administrated to induce hepatic injury. Four oral doses (500 mg/kg of rat body weight) with 24 h interval of an inert plant extract). 3. Aqueous extract group. (All the rats in this group were given four oral doses (500 mg/kg of rat body weight) of aqueous extract with 24 h interval after the hepatic injury was induced by CCl_4 along with vehicle before four hour of first dose). 4. Alcohol extract group (Animals in this group were given two oral doses (500 mg/kg of the rat body weight) of alcoholic extract after the hepatic injury was induced)

BLOOD COLLECTION

Each animal was anaesthetized with diethyl ether. Heart puncture was done with a 5 ml disposable syringe and 2 ml blood was drawn very gently and slowly. The blood collected was shifted immediately to clean dried centrifugation tubes, allowed to clot and serum was separated by centrifugation at 3000 rpm for 15 min. Serum was separated and then preserved in the cuvettes at -20°C in the freezer until analysis. Biochemical estimations were made the following day.

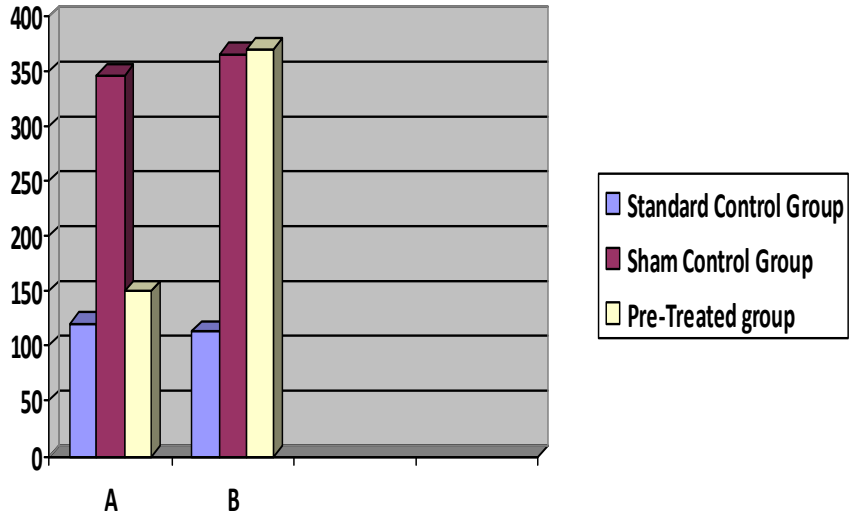

Figure 1. Activity of AST (U/L) in serum of various hepatocurative groups of rats. The activity of AST is shown as mean ± SD of 10 observations.

BIOCHEMICAL ANALYSIS OF SERUM SAMPLES

Serum samples collected from different groups were analysed for Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphate (ALP) using procedure and packed kits made by Bicone, Germany. The absorption was recorded using a 4010 Spectrophotometer.

LIVER HISTOPATHOLOGICAL ASSESSMENT

Liver sections taken immediately after dissection from the liver, fixed in 10% buffered formalin (Lin et al., 1997), dehydrated in gradual ethanol (50 to 100%), cleared in xylene, and embedded in parafine. Sections (4 to 5 um thick) were prepared and then stained with Haematoxylin and Eosin (H and E) dye for photomircoscopic observations like cell necrosis, fatty change, hyaline degeneration, ballooning degeneration, infiltration of kupffer cells and lyphocytes under power 40X.

Statistical analysis

All statistical analysis was made by means of student's t-test using computer programme Statistica (1994). A "p" value of 0.01 was taken as a level of significance.

DNA ladder assay

DNA ladder was carried out according to the procedure of Wu et al. (2005). 5 µg of DNA of rats separately was loaded in 1.5% agarose gel containing 1.0 µg/ml ethidium bromide including DNA standards (0.5µg per well). Electrophoresis was performed for 45 min at 100V. After electrophoresis gel was studied under gel doc system and was photographed.

RESULTS

Serum levels of alkaline phosphate (ALP), Aspartate Transaminase (AST) and Alanine Transaminase (ALT) activities, loss/gain in body weight (data not shown), gross and microscopic appearance of liver were studied and results are presented in the following paragraphs.

Aspartate transaminase (AST)

Figure 1 shows the mean activity of AST in standard control groups in each experiment were 120.6 ± 15.13 and 113.2 ± 22.83 respectively for aqueous and alcoholic extract experiment while in sham control groups these were 346.8 ± 27.98 and 365.4 ± 17.68.

The activity of AST in alcoholic extract group was 370.8 ± 33.75 while in aqueous extract groups were 150.2 ± 22.98. Figure 1 shows a comparison of aqueous and alcoholic extracts with their standard control group and sham control group. It is clear from the figure that the activity of AST in aqueous extract group was close to its standard control group ($P < 0.001$) and lower than the sham control group ($P < 0.001$) which is suggestive of effectiveness of the aqueous extract of the plant. The same in case of alcoholic extract group was much higher compared to their respective standard control group ($P < 0.001$) and very close to their respective sham control group ($P < 0.005$), which suggests that alcoholic extract of the plant is ineffective.

Alanine transaminase (ALT)

Figure 2 shows the mean activity of ALT in standard control groups in each experiment were 36. 6 ± 9.97 and 24.4 ±13.85 respectively for aqueous and alcoholic extract while in sham control groups these were 246.8 ± 39.00 and 343.8 ± 25.49 respectively.

The activity of ALT in alcoholic extract group was 307.6

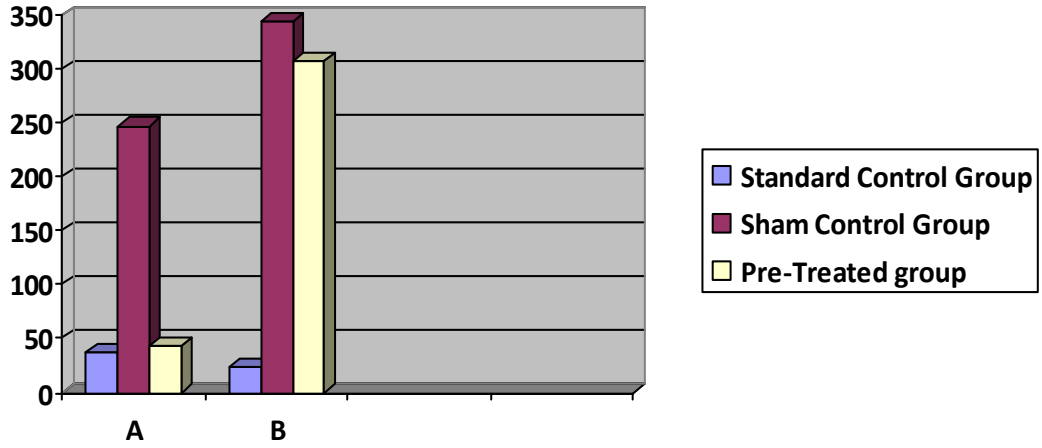

Figure 2. Activity of ALT (U/L) in serum of various hepatocurative groups of rats. The activity of ALT is shown as mean ± SD of 10 observations.

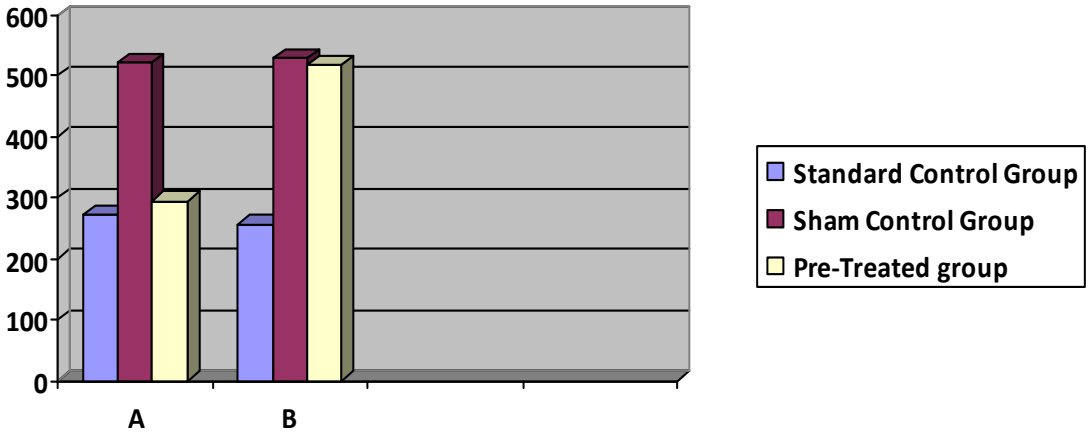

Figure 3. Activity of ALP (U/L) in serum of various hepatocurative groups of rats. The activity of ALP is shown as mean ± SD of 10 observations.

± 45.07 while in aqueous extract group was 43.4 ± 12.12. Figure 2 shows the comparison of group with aqueous and alcoholic extracts with their standard control group and sham control group in each experiment. It is clear from the figure that the activity of ALT in aqueous extract group was close to its standard control group ($P < 0.001$) and lower than its sham control group ($P < 0.001$) The same in case of alcohol extract group was much higher compared to their respective standard control group ($P < 0.001$) and very close to their respective sham control group ($P < 0.05$).

Alkaine phosphatase (ALP)

(Figure 3) shows the mean activity of ALP in standard control groups in each experiment were 271 ± 21.08 and 256.2 ± 36.46 respectively for aqueous and alcoholic extract experiments while in sham control groups these were 522 ± 66.45 and 528.6 ± 13.96 respectively.

The activity of ALP in alcoholic extract group was 518.6 ± 29.10 while in aqueous extract group was 295 ± 30.14. (Figure 3) shows a comparison of group with aqueous and alcoholic extracts with their standard control group and sham control group in both experiments. It is clear from the figure that the activity of ALP in aqueous extract group was close to its standard control group ($P < 0.001$) and lower than the sham control group ($P > 0.05$).The same in case of in alcoholic extract group, it was much high compared to their respective standard control group ($P > 0.05$) and very close to their respective sham control group ($P < 0.05$).

Histopathology of rat liver

The results of histological examination are presented in (Figures 4, 5, 6, and 7). Figure 4 is the section of the

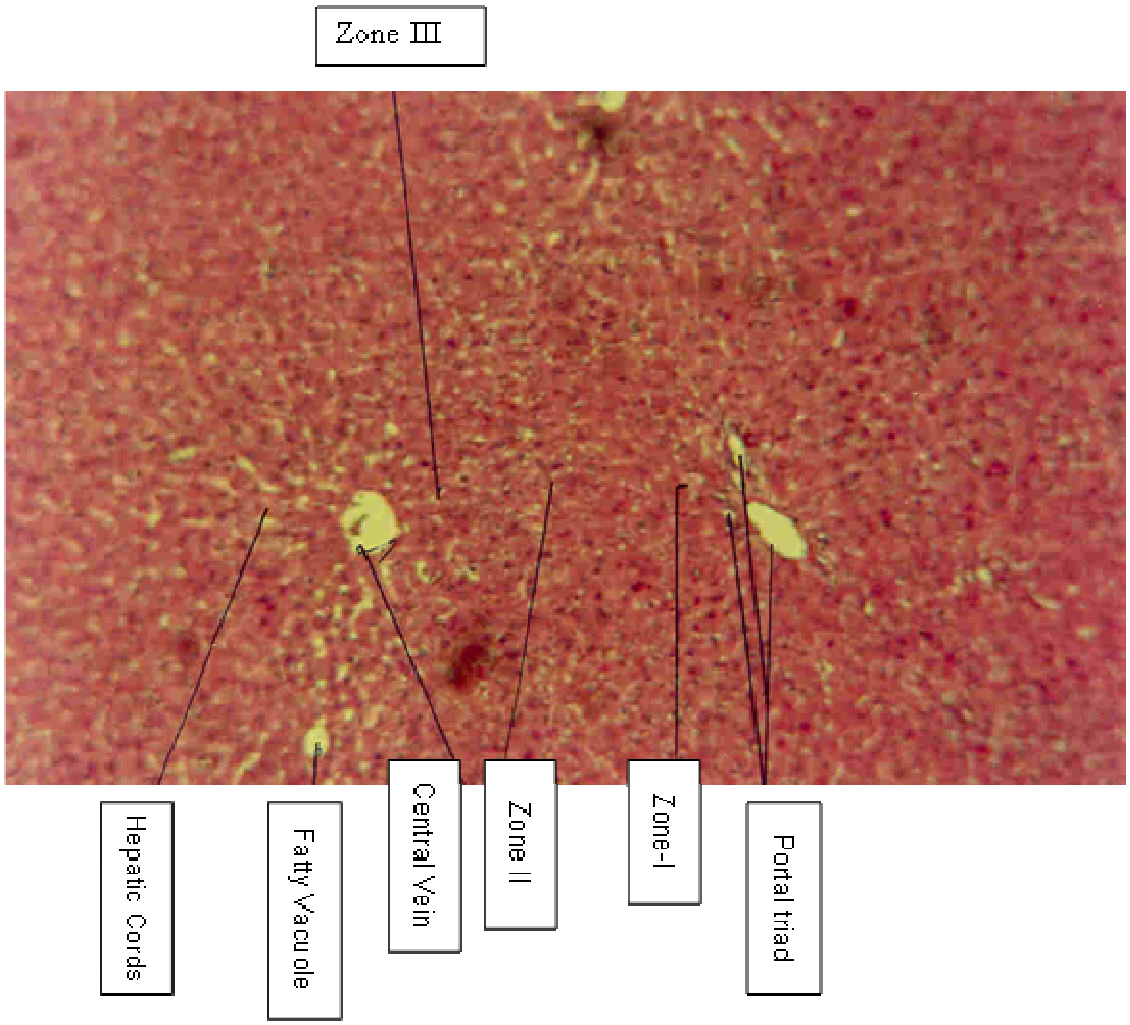

Figure 4. Section of the male rat liver (Standard Control) showing hepatic lobule with central vein and radiating cords of hepatocytes. Haematoxyline and Eosin stained (H and E) 5 micron thick section. Photomicrograph 40x.

male rat liver of standard control group, revealed that the architecture of the lobule was intact, with hepatocytes arranged as radiating plates around the central vein, the hepatic lobule was divided into three zones, Zone I comprised of area at the periphery of the hepatic lobule close to the portal triad. Area around the central vein was labeled as Zone III of the hepatic lobule. Area of the lobule in between Zone I and Zone III was labeled as Zone II. Parenchyma showed occasional fat containing cells. These were visible clearly inside the hepatocytes. (Figure 5) is the section of male rat liver of sham control group animals, revealed the dilated and congested blood cells and fatty acid changes. Hepatic cords were slightly distorted due to focal necrosis. Fatty changes were present moderately in the centribular area visible as small round clear yellowish areas. One gross examinations liver was enlarged in all animals. It was pale and there were tiny red spots on the surface of liver. (Figure 6) shows the section of male rat liver, pretreated with

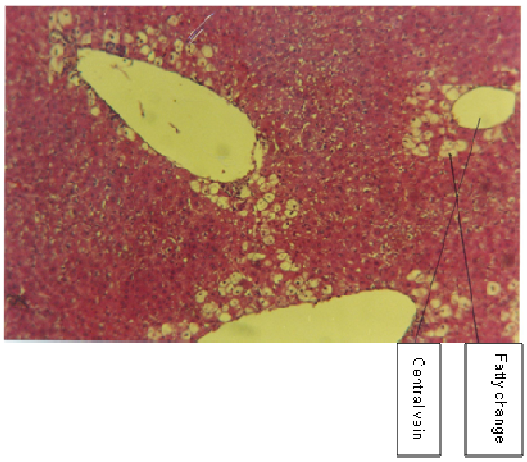

Figure 5. Section of the male rat liver of Sham Control group showing dilated and congested central vein and fatty changes are seen. H and E stained 5 micron thick paraffin section. Photomicrograph 40x.

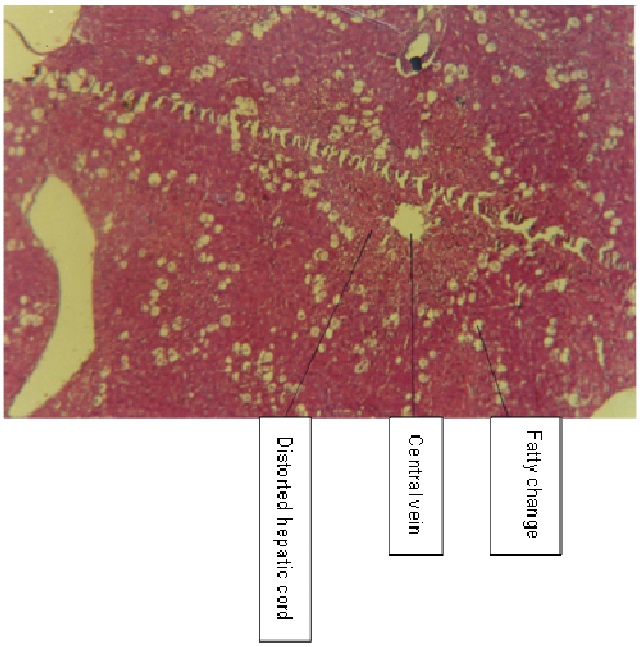

Figure 6. Section of the male rat liver post-treated alcoholic extract group showing dilated and congested central vein and fatty changes are seen. H and E stained 5 micron thick paraffin section. Photomicrograph 40x.

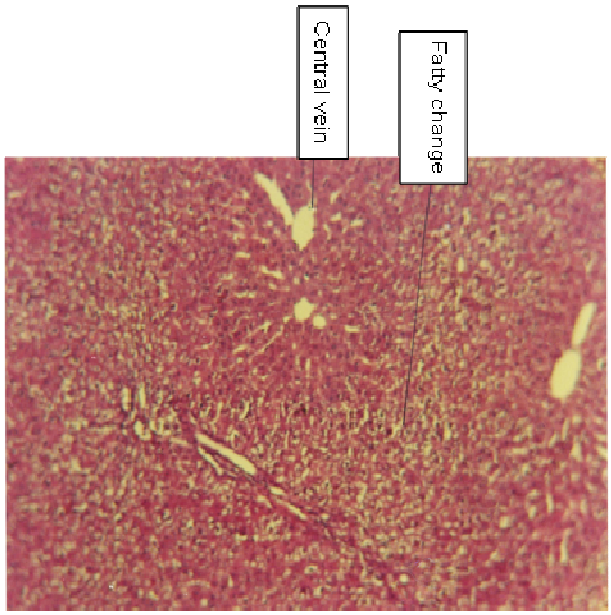

Figure 7. Section of the male rat liver post-treated aqueous extract group showing mild to moderate improvement. Central veins slightly dilated and congested with mild fatty change. H and E stained 5 micron thick paraffin section. Photomicrograph 40x.

alcoholic extract and then diseased with CCl_4. It revealed dilated and congested central vein. Hepatic architecture was slightly disturbed due to distortion of the hepatic cords. On examinations, liver was enlarged, pale and surface was smooth and tiny red spots were seen on its surface. (Figure 7) is cross section of male rat liver post-treated with aqueous extract, shows mild to moderate improvement. Hepatic cords were slightly distorted. Central vein slightly dilated and congested with mild fatty change visible as small rounded yellowish areas.

DNA ladder assay and DNA fragmentation

Treatment of rats with CCl_4 increased the percentage of DNA fragmentation (61.8 + 2.0) significantly as compared to control group (38.6 ± 3.0). By contrast, the group of rats showed significant lower percentage of DNA fragmentation (54.6 ± 2.4) in the aqueous-treated group. A peculiar type of continuous DNA fragmentation pattern was observed in electrophoresis (Figure 8). DNA fragmentation percentage and DNA ladder assay were intimately correlated with each other. Treatment of aqueous extract showed marked repairing potential against CCl_4 and alcoholic extract.

DISCUSSION

Hepato-curative study was performed to evaluate the curative effects of the *S. nigrum* extract on the damaged liver. In this study single dose of CCl_4 was given first and separate groups were administered with Aqueous and alcoholic extracts of the plant.

The parameters used to confirm the effects of the herbal medicinal plants in this study were biochemical and histological. Arise in plasma Transaminases activities is a sensitive indicator of damage to cytoplasm and/or mitochondrial membranes even if there is no detectable impairment of function.

Animals of standard control group were active and response too stimulus was quick as compared to sham control group which had sluggish behaviour .This may be due to the fact that CCl_4 is anaesthetic agent which may be causing slowness in all reflexes of the animals and other general behaviours (Klaassen, 1985; Sherlock and Dooley, 1989).

In Alcoholic extract experiments, standard control group showed the serum AST, ALT and ALP levels of 113.2 ± 22.83, 24.4 ± 13.85 and 256.2 ± 36.46 respectively where as sham control group showed the same values as 365.4 ± 17.68, 343.8 ± 25.49 and 528.6 ± 13.96 respectively. These results show that CCl_4 hepatotoxicity was effectively produced in sham control group. With CCL_4 of ALP was slighty elevated whereas the activities of the AST and GPT were markedly raised. This finding is in agreement with the work already reported (Gilani and Janbaz, 1995; Janbaz et al., 1998). Animals in this group appeared ill looking, less hungry as compared to standard control group and lost body weight.

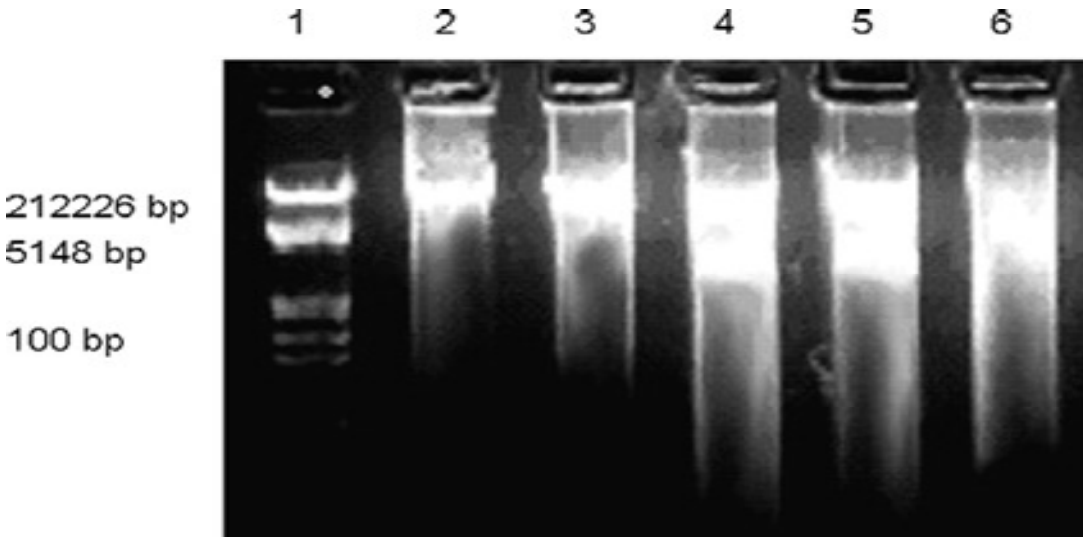

Figure 8. Agarose gel showing DNA damage by CCl_4 and preventive effect of extracts *S. nigrum* in different groups. Lanes (from left) high-molecular weight marker (1), control (2 and 3), CCl4 (4), Alcoholic (5) and Aqueous (6).

The group of rats, which was administered with plant alcoholic extract prior to the liver injury to see whether any alcohol soluble active ingredients have hepato-protective action. In this part of experiment all the prerequisites were the same as that of aqueous extract. Acoholic extract of the plant showed serum AST, ALT and ALP values as 370.8 ± 33.75, 307.6 ± 45.07 and 518.6 ± 29.10 respectively. Comparison of plant alcoholic extract group and pre-treated sham control group shows no significant change in values of these enzymes. Thus it is suggested that plant alcoholic extract does not have any hepato-protective activity i.e. active principals of the plant remained insoluble in alcohol or not lipid soluble. Histological changes like increase in congestion of liver, fatty change of the parenchymal cells were similar to the sham control group. A toxic dose of CCl_4 produced a central necrosis involving one third to one half of each lobule. Only parenchymal cells are damaged and sinusoids remain intact so blood circulation in liver is not affected (Fawcet and Bloom, 1994). Mean decrease in the body weight was 8.5% which was also close to sham control group that is 8%.

In Aqueous extract experiments, Animals were sluggish, less hungry and response to stimulus was slow. In this group, standard control group showed the serum AST, ALT and ALP levels 120.6 ± 15.13, 36.6 ± 9.97 and 271 ± 21.08 respectively where as sham control group showed the same values as 346.8 ± 27.98, 246.8 ± 39.00 and 522.0 ± 66.45 respectively. The above results show that CCl_4 hepatotoxicity was effectively produced in sham control group. The results of ours study show that CCl_4 hepatotoxicity was effectively produced in all cases. Similar observations have been earlier reported (Gilani and Janbaz, 1995).

The group of rats which was administered plant aqueous extract prior to the liver injury and in which serum AST, ALT and ALP values were later measured showed the activity of these enzymes as 101.8 ± 8.90, 44 ± 9.23 and 279.6 ± 18.48 respectively. These results show that the extent of the liver damage is reduced as the enzyme values are lower than sham control group. The aqueous extract showed significant protective effect by lowering the serum AST, ALT, ALP activities in carbon tetrachloride (CCl_4) induced hepatotoxicity. On histological examinations, there were minimal lesions and fatty change was almost absent which reavels that extract contain the active ingredient (s) which protects the liver. Although there was decrease in body weight but mean decrease of absolute body weight was less than sham control group. It appeared likely that hepato-protective gradients are readily available in the aqueous extract to produce their protective action against the injury. Obviously, it is also possible that active gradients are soluble in aqueous extract.

The present study shows that when rats were administrated with aqueous extract of *S. nigrum* the AST, ALT and ALP levels significantly decreased ($P < 0.001$) when compared with sham control group.

Increase in liver weight could be due to increased blood contents due to the dilatation of the central veins and Sinusoids, swelling of the hepatocytes due to increase in water transport in the cell and fatty liver due to increase accumulation of fat in the hepatocytes which was obvious on examination liver enlarged and pale and also on histological examination same observation were made by Robbin et al, 1989.

DNA fragmentation percentage and DNA ladder assay were intimately correlated with each other. Treatment of

aqueous extract showed marked repairing potential against CCl_4 and alcoholic extract.

This study, as a part, rationalizes the traditional use of the aqueous extract of the *S. nigrum* plant to treat the liver disease in humans as it already employed in the unani system of medicine. Alcoholic extract shows no protective effect in this study, suggesting that active principals may not be present in the alcoholic extract of the plant.

The results of the study show that a significant protection from toxin "CCl_4" damage may be achieved through the aqueous extract, pre-treatment, which suggests that plant's protective constituents, may be present only in aqueous extract. These functions may be mediated through the action of the chemical constituents that acts through hepatic MDME inhibition mechanism and/or presence of certain anti-oxidants in the plant constituents.

Further studies with other parameter are needed to establish its efficacy as hepato-protective agent along with its possible side effects and to give firm footing to traditional systems of medicines employing this as a herbal drug against hepatitis. Toxicity studies also need be performed to establish its safety for human consumption as a liver herbal medicine. The active ingredient (s) should be isolated and purified to elucidate the exact mechanism of action.

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