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Ameliorative Potential Of *Taraxacum Officinale* Leaves Against Carbon Tetrachloride Mediated Hepatopathy In Rats.

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ABSTRACT

Background: There is an increasing insist for natural drugs as continuous use of synthetic ones can culminate into many side effects and toxicity. Therefore, plant resources are of main concern for the scientists to search the alternate drugs for the liver ailments. Keeping in view the non-toxic nature of medicinal plant extracts, the present study was undertaken to evaluate the hepatoprotective potential of ethanol extract of *Taraxacumofficinale* (EETO) leaves in the rats intoxicated with the carbon tetrachloride.

Results: The crude extract was given to the rats at two doses of 100 mg/kg and 200 mg/kg body weight for the duration of 30 days. The elevated levels of the serum biomarkers (by carbon tetrachloride intoxication, i.p.) such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), acid phosphatase (ACP) and total bilirubin were found to be significantly less and the level of total protein was significantly elevated in groups treated with ethanolic extract of *T. officinale* alongside carbon tetrachloride at two doses of 100 mg/kg and 200 mg/kg body weight. The histopathological studies also confirmed our biochemical results.

Conclusion: The results of both biochemical and histopathological studies revealedthat the ethanolic extract of *T. officinale*is decent enough to combat hepatic damage.

Key words: *Taraxacum officinale,* carbon tetrachloride, hepatotoxicity, ALT, AST, ALP.

INTRODUCTION

India is known for its rich traditional system of medicine mainly Ayurvedabesides a vast knowledge of living traditions of ethnomedicine. The world health organization (WHO) estimates that more than 3/4th of the people of developing countries rely on traditional medicines, mostly plant-derived drugs for their primary health needs. Varieties of plants are used as an

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indispensablesource of medicinal preparation in medicinal systems. Hundreds of species are identified as having medicinal value. A large number of plants have been recognized which possess remarkable protective properties viz. *Curculigo orchioides*(Veenukumar M R and Latha, 2002), *Colocasia antiquoram*(Tuse et al. 2009)' *Opuntia dillenii* (Bouhrim et al. 2018)etc.

Liver is one of the largest organs in the human body and the principal site for intense metabolism and excretion. So, it has an astonishing role in safeguarding, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, wrestle against disease, nutrient supply, energy stipulation and reproduction(Ward FM and Daly 1999). But, it is incessantly and variedly open to the elements, to environmental toxins, battered by poor drug habits and alcohol which can ultimately lead to diverse liver complaints like hepatitis, cirrhosis and alcoholic liver disease (Subramonium and Pushpangadan 1999). Since there are not many drugs accessible for the treatment of liver disorders,therefore, many folk remedies from plant origin are tested for their potential antioxidant and hepatoprotective properties in experimental animal models (Chaterrjee, 2000; Mechchate et al. 2020).

Taraxacum officinale commonly known as dandelion is a medicinal plant especially effective and valuable as a diuretic because it contains high levels of potassium salts and therefore can replace the potassium that is lost from the body when diuretics are used. All parts of the plant especially the roots are slightly aperient, cholagogue, depurative, strongly diuretic, hepatic, laxative, stomachic and tonic. The root is also experimentally cholagogue, hypoglycemic and a weak antibiotic against yeast infections. Dandelion is used as a medicinal plant especially by tribal people for liver (Mahesh et al. 2010), kidney, joint ailments and inflammatory diseases (Seo et al. 2005)). It is a common vegetable relished in Kashmir, Himalaya and is considered to be very good for ladies after childbirth. It is also reported to have decent antioxidant potential (Sheikh et al. 2015)

Liver diseases remain as one of the serious health problems. It is well reported in the literature that many plants have a hepatoprotective property such as *Phyllanthusniruri, Eclipta alba*, *Aloe vera, Solanum indicum* etc. (Parmar 2010). Thus search for the crude drug of plant origin with antioxidant property has drawn the attention of the scientists with the main focus on the study of hepatoprotection. Carbon tetrachloride induces hepatotoxicity and increases the levels of serum variables i.e. SGOT, ALT, ACP, ALP, bilirubin and Cholesterol. The level of these enzymes was reported to decrease due to the use of the methanolic extracts of *Casuarina equisetifolia*, *Cajanus cajan, Glycosmis pentaphylla, Bixa orellana, Argemone mexicana* (Ahsan et al 2009). Keeping in view the above mentioned medicinal properties of the herbs, the present study has been undertaken to evaluate the hepatoprotective property of *Taraxacum officinale* leaves.

Materials and methods

Plant material - The leaves of the *Taraxacum officinale* were collected in the month of June from the Kachan area of Ganderbal District of Kashmir valley (J & K), India. The herb was

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taxonomically identified by Akhter H. Malik (Curator, KASH center for Biodiversity and Taxonomy, Department of Botany, University of Kashmir). A voucher specimen bearing number 1747 KASH was deposited in the same department for further reference.

Chemicals and reagents – All chemicals were of analytical grade obtained from Merck, Mumbai, India and HiMedia, Mumbai, India. All Biochemical investigations were performed using commercial diagnostic kits of SPAN Diagnostic, Gujarat, India.

Preparation of crude extract

The collected leaves were washed with purified water and shade dried for a week. These leaves were ground to powder. The powder was extracted in 90 % ethanol by using the Soxhlet extractor. The ethanol extract was then concentrated and dried with the help of rotary vacuum evaporator under reduced pressure. The ethanolic extract of leaves of *Taraxacum officinale* (EETO) was thereafter stored in air tight closed container in the refrigerator at -4^{0}C for further use.

Experimental animals

Thirty-six male Albino Wistar rats weighing 120-150g were obtained from animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal Madhya Pradesh, India. Animals were selected at random from animal house. These were housed in the polypropylene cages maintained in controlled temperature 22 ± 2 ºC and light cycle (12hours light and 12 hours dark). The animals were fed with the pellet diet manufactured by Golden feeds Delhi, India and water *ad libitum.* Housing condition and all animal experiments were performed as per CPCSEA guidelines. Animal experiments were performed with prior permission from Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal, India (1283/PO/C/09/CPCSEA).

Carbon tetra chloride induced liver damage

Hepatopathy was induced in the animals by the intraperitoneal injection of CCl_4 once a week for thirty days at the dose of 2 ml per kg body weight in the same volume of olive oil.

Experimental procedure

Body weight of the animals was recorded and they were divided into 6 groups with 6 rats in each group. Group first consisted of rats administered with vehicle (distilled water) and pallet diet only for 30 days which served as control. Animals of group 2nd were administered with vehicle (distilled water) for 30 days and with CCl_4 (2ml/kg, i.p.) once a week. Animals of group 3rd and 4th were administered with ethanolic extract of *Taraxacum officinale* dailyat the doses of 100 mg/kg and 200 mg/ kg, p.o. respectively using oral gavage for 30 days. In group 5th and 6th, animals were administered with ethanolic extract of *Taraxacum officinale* daily at doses of 100 mg/kg and 200 mg/kg respectively for 30 days along with CCl_4 (2ml/kg, i. p.) once a week. All the animals were maintained at the laboratory conditions for the duration of 30 days.

Biochemical Estimations

The animals were fasted overnight on the 30th day. On the next day, the body weight of the animals was recorded and blood was collected by heart puncture in previously labeled centrifuge

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tubes and allowed to clot for 30 minutes at room temperature. The collected blood was centrifuged in Remi centrifuge at 3000 rpm for 15 minutes so as to get the serum. The serum was used for the estimation of levels of various biochemical markers like aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP), total bilirubin and total protein using commercially available kits. A portion of liver tissue from the animals of each group was carefully dissected out. Then the liver was transferred to 4% formalin solution for fixation and later on processed for histopathological studies following the standard procedure (Raghuramulu et al., 1983).

Results

In presentinvestigation, hepatotoxicity was induced by carbon tetrachloride. On comparison of carbon tetrachloride treated group (group 2) with vehicle-treated group (group 1), it was observed that there was a significant increase ($P<0.001$) in SGOT, SGPT, ALP, ACP and bilirubin levels. However, the level of total protein was significantly less ($P<0.001$). In extract treated groups at both doses of 100 mg/kg and 200 mg/kg (group 3rd and group 4th) no significant difference in enzyme level was observed as compared to vehicle-treated group (Table 1 and Table 2). This confirmed that extract was not having any type of side effect on the liver and extract is safe at selected doses. In animals treated with extract (both at 100 mg/kg and 200 mg/kg i.e. group 5th and group 6th) and carbon tetrachloride, levels of SGOT, SGPT, ALP, ACP and total bilirubin were significantly less ($P<0.001$) as compared to that of only carbon tetrachloride treated group of animals. However, the protein level was elevated in the group of rats supplied with extract (both at 100 mg/kg and 200 mg/kg i.e. group 5th and group 6th) and carbon tetrachloride as compared to that of only carbon tetrachloride treated group of rats. In animals treated with extract at 200 mg/kg along with carbon tetrachloride, levels of bilirubin, SGOT, and ALP were significantly less ($P<0.001$) in contrast to those receiving 100 mg/kg and CCl_4 (i.e. group 5th).No significant difference in between these doses was observed for SGPT and ACP level (Fig. 1 and Fig. 2).

Thus extract is showing hepatoprotective activity at both doses of 100 mg/kg and 200 mg/kg against carbon tetrachloride induced hepatotoxicity. The findings of biochemical investigations were fully supported by the histopathological studies. In the present study, the liver sections of the control group showed the fully intact cells with prominent nuclei, hepatic artery, portal vein, central vein and regular sinusoids (Fig. 1.)

In Group 2nd i.e. animals inebriated with CCl_4, there were clear evidences of hepatotoxicity as depicted by centrilobular necrosis, vacuolization of cytoplasm, dilation of sinusoidal spaces, the appearance of a large number of kupffer cells, inflammatory infiltration, the disappearance of cellular boundaries and fatty changes (steatosis) (Fig. 2).

The rats supplied with the EETO at 100 mg/kg and 200 mg/kg i.e. group 3rd and group 4th showed the hepatic architecture similar to that of the control group, thus proving the non-toxic nature of the extract at the selected doses (Fig. 3 and Fig. 4).

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However, in the group administered with the 100 mg/kg of EETO along with CCl_4 i.e. group 5th, histological alterations were mild with hepatocytes retaining their nuclei and reduction in the sinusoidal extension. Yet, the kupffer cells were present in good numbers and inflammatory infiltration was still persistent (Fig. 5).

In group 6th, i.e. rats supplied with 200 mg/kg of EETO alongside CCl_4, comparatively higher protection was noticed than that of group 5th and the hepatic architecture was almost similar to that of the control group (Fig. 6).

Discussion

Liver the largest internal organ is a target for toxicity because of its role in cleaning and metabolizing through the process called detoxification. Being one of the major organs coming in contact with each component reaching blood circulation, chances of toxicity associated with these components on the liver is much. Since ancient times many herbs were used by traditional healers and local dwellers for treating ailments related to the liver. But still, we are lacking scientific evidence in concern of this knowledge. In present investigation hepatoprotective potential of one of the traditionally used herb, *Taraxacum officinale* was investigated in carbon tetrachloride induced hepatotoxicity in rats.

Carbon tetrachloride (CCl_4) is an archetype of hepatotoxin used commonly in experimental models to induce oxidative stress in the liver. CCl_4 is also known to be involved in inducing injuries to other organs (Khan and Zehra 2011). In its first step of metabolism, CCl_4 is converted by cytochrome P_{450} to a carbon-centered radical, the trichloromethyl ($\bullet CCl_3$) which combines with cellular lipids and proteins in presence of oxygen to induce lipid peroxidation by attacking polyenoic fatty acids (Halliwell and Whiteman 2004). The trichloromethyl radical can also react with oxygen to form the peroxy trichloromethyl free radical ($\bullet CCl_3O_2$), which is more reactive than the 3, 2 trichloromethyl radical with the corresponding health disturbances (Rechnagel et al 1989).

Serum AST and ALT are the most responsive biomarkers used in the diagnosis of liver diseases. During hepatocellular damage, varieties of enzymes normally located in the cytosol are released into the blood flow. Their quantification in plasma is useful biomarkers of the extent and type of hepatocellular damage (Pari and Murugan 2004). ALT and AST are enzymes normally present in the liver, heart, muscles and blood cells. They are basically located within hepatocytes. So when liver cells are damaged or die, transaminases are released into blood stream where they can be measured. Serum ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, serum ALT is more specific to the liver and is thus a better parameter for detecting liver injury (Williamson et al 1996). In concurrence with the reports of other investigators, (Shetti et al 2018), (Asad et al 2012), (Ahirwar and Tembhre 2016), (Ouassou et al 2021). Evaluation of Hepatoprotective Activity of Caralluma europaea Stem Extract against CCl_4-Induced Hepatic Damage in Wistar

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Rats. Advances in pharmacological and pharmaceutical sciences, 2021., data from the present investigation illustrated that CCl_4 caused hepatic damage with a significant increase in serum levels of ALT and AST by 76.87 % and 62.57% respectively. Treatment with ethanolic leaf extract of *Taraxacum officinale* at 100 mg/ kg significantly decreased the levels of ALT and AST by 53.54 and 43.30 % respectively in comparison to group 2nd rats. However, at a higher dose of 200 mg/kg of *Taraxacum officinale*, a further decrease in the levels of these enzymes was noticed viz. ALT 57.33% and AST 50.54% thus demonstrating the protection offered by the extract to maintain the functional integrity of hepatic cell membrane (Table 1).

ALP concentration is related to the functioning of hepatocytes, high level of ALP in the blood stream is related to the increased synthesis of it by cells lining bile canaliculi usually in the response of cholestasis and increased biliary pressure (Muriel and Garcipiana 1992). The significant elevation (61.45%) was observed in the level of ALP by CCl_4 administration. However, the level was significantly lowered by 39.51% and 51.56% when animals were supplied with the ethanolic leaf extract of *Taraxacum officinale* at two chosen doses viz. 100 mg/ kg and 200 mg/kg respectively along with CCl_4 (Table 1) in comparison to group 2nd rats.

ACP is regarded as a key lysosomal enzyme involved in autolytic degradation of tissues. It is used to monitor cell death and lysis (Sangeetha and Krishnakumari 2010). The increased level of ACP in the serum of CCl_4 treated rats could be due to damage to the cell membrane of tissues, where these enzymes are firmly attached to the cell membrane and the damage releases these enzymes from the membrane joining the biliary canaliculus and the sinusoidal border of parenchymal cells. In the present investigation, it was found that CCl_4 inebriation significantly increased the serum level of ACP by 64.98% as compared to that of the control. However, 47.34% and 54.93% inhibition was noticed in the ACP levels of groups of animals administered with ethanolic leaf extract of *Taraxacum officinale* at 100 mg/kg b.w. and 200 mg/kg b.w. respectively alongside CCl_4 (Table 2.) in comparison to only CCl_4 treated group of rats. Our findings corroborate the results of other researchers.

Bilirubin is one of the most valuable medical inklings for the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocytes. Bilirubin, a yellow pigment is formed when heme is catabolised. Hepatocytes render bilirubin water soluble and therefore easily excretable by conjugating it with glucuronic acid prior to secreting it into bile by active transport. Hyperbilirubinemia may result from the production of more bilirubin than the liver can process, damage to the liver impairing its ability to excrete normal amount of bilirubin or obstruction of excretory ducts of the liver (Olaleye et al 2010)**.** Serum bilirubin is considered one of the true tests of liver functions since it reflects the ability of the liver to take up and process bilirubin into bile. Elevated levels may indicate severe illness. High levels of bilirubin in CCl_4 treated rats may be due to CCl_4 toxicity which resulted in hyperbilirubinemia. The results obtained from the present study indicate that CCl_4 intoxication significantly elevated the level of bilirubin by 68.80% in rats as against the control group.

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However, the ethanolic extract of *Taraxacum officinale* leaves at 100 mg/kg b.w. and 200 mg/kg b.w. respectively reduced the levels of bilirubin by 30.27% and 61.46% in dose-dependent manner (when compared with group 2nd), thereby confirming the restorative nature of the said plant extract (Table 2).

A reduction in total protein (TP) as observed in the CCl_4 treated animals may be linked with the decrease in the number of hepatocytes which in turn may result inthe decreased hepatic capacity to synthesize protein. Hence, the decline in total protein content can be believed as a useful index of the severity of cellular dysfunction in chronic liver diseases which indicates hepatopathy (Gandhare et al 2012). Our investigation revealed that CCl_4 significantly reduced the serum level of total protein in rats by 44.08%. The decreased values of total protein were elevated when animals were supplied with ethanolic leaf extract of *Taraxacum officinale* at 200 mg/kg and 100 mg/kg by 32.51 % and 44.62% respectively when compared with group 2nd (Table 2).

Histopathology of liver ascertained the effect of plant extracts and carbon tetrachloride on the microstructure of the liver. The histopathological investigation of liver sections of control group of rats revealed typical cellular architecture with well defined nucleated hepatocytes, portal triad, sinusoids and well brought out central vein (Fig 1). On the other hand, CCl_4 inebriated rats illustrated disarrangement of hepatic cells with centrilobular necrosis, vacuolization of cytoplasm, presence of hepatic plates, large number of kupffer cells and fatty degeneration (Fig 2). The groups treated with the ethanolic extracts of *Taraxacum officinale* (EETO) at 100 mg/kg and 200 mg/kg for 30 days, have not shown any alteration in the histology of the rat liver as disclosed by well arranged hepatocytes with prominent nucleus, normal sinusoids and central vein (Fig 3 and 4). However, in group 5th, treated with 100 mg/kg of EETO along with CCl_4, the changes were mild with hepatocytes retaining nucleus, reduction in the sinusoidal expansion but kupffer cells were still persistent (Fig 5). However, moderate changes were noticed in rats treated with extract at 200 mg/kg of EETO alongside CCl_4 with hepatic architecture similar to that of the control group (Fig 6). These results were in concurrence with the study of other investigators (Gumaa et al 2017).

CONCLUSION

Our data manifestly illustrated that ethanolic leaf extract of *Taraxacum officinale* possessed the hepatoprotective effect when the rats were inebriated with CCl_4 which may be attributed to the presence of various phytochemicals like alkaloids, flavonoids, saponins, phenolic compounds and glycosides in it. When ethanolic leaf extract of *Taraxacum officinale* (EETO) was given orally to the normal rats, no significant alteration in biochemical parameters and histology occurred proving its safety at selected doses.

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Declarations

Acknowledgments

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Funding

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Availability of data and materials

The manuscript contained all the necessary data obtained during the study. However, on the recommendation or request addition information will be provided by the corresponding author.

Authors' contributions

The work was conceived and planned by MAS, MAA. The experiment was carried out by MAS. MAS, DMB and MAAanalysed the data. The compilation of the first draft, as well as editing, was done by MAS and DNB. The manuscript so finalized was read as well as approved by all authors.

Ethics approval and consent to participate

Animal experiments were performed with prior permission from Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal bearing Approval No. PBRI/13/IAEC/PN-296a.

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest

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Figure title and Legend

Fig 1: Photomicrograph of control rat (40xHaematoxylin and Eosin stain)

Fig 2: Photomicrograph of CCl_4 exposed rat(40xHaematoxylin and Eosin stain)

Fig 3: Photomicrograph of EETO 100 mg/kg treated rats (40xHaematoxylin and Eosin stain)

Fig 4: Photomicrograph of EETO 200 mg/kg treated rats (40xHaematoxylin and Eosin stain)

Fig 5: Photomicrograph of EETO 100 mg/kg+CCl_4 treated Rats (40xHaematoxylin and Eosin stain)

Fig 6: Photomicrograph of EETO 200 mg/kg+CCl_4 treated rats(40xHaematoxylin and Eosin stain)

Table 1: Effect of ethanolic extract of *Taraxacum officinale* (EETO) leaves on ALT, AST and ALP in CCl_4 induced liver damage in rats.

Table 2: Effect of ethanolic extract of *Taraxacum officinale* (EETO) leaves on ACP, Total Protein (TP) and Total Bilirubin (TB) in CCl_4 induced liver damage in rats.

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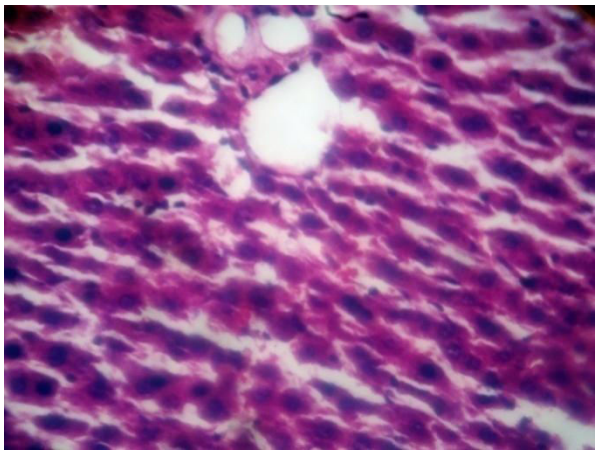

Fig. 1 Photomicrograph of control rat

(40xHaematoxylin and Eosin stain)

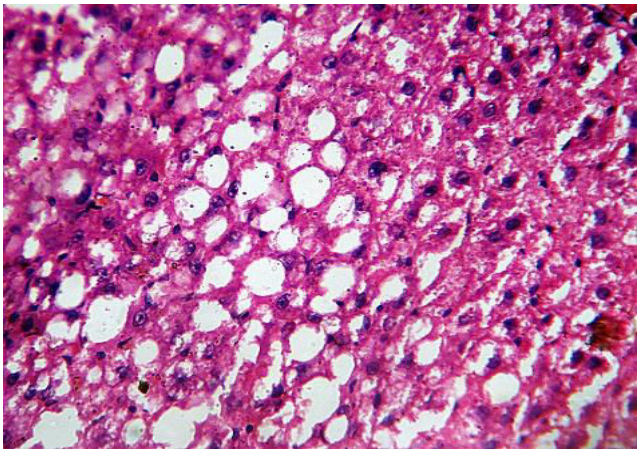

Fig. 2 Photomicrograph of CCl_4 exposed rat

(40xHaematoxylin and Eosin stain)

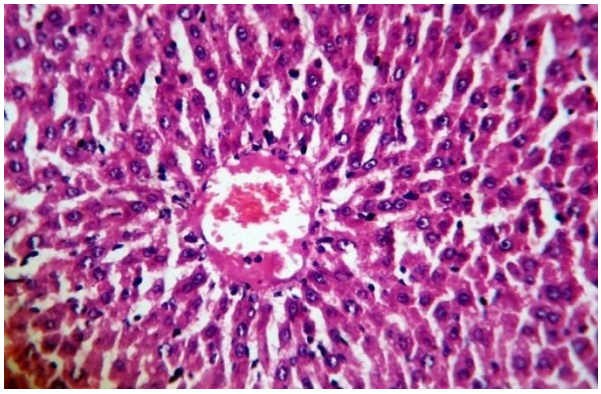

Fig 3Photomicrograph of EETO 100 mg/kg treated rats,

(40xHaematoxylin and Eosin stain)

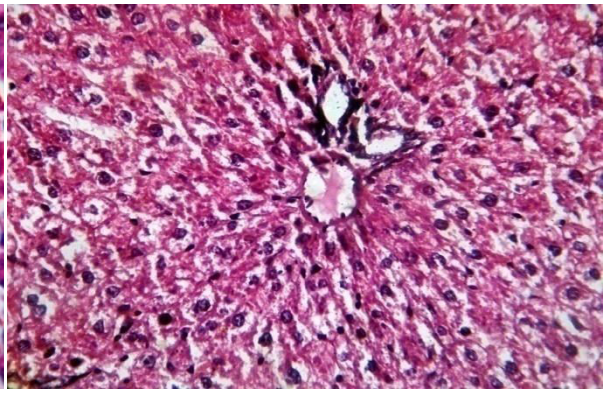

Fig. 4Photomicrograph of EETO 200 mg/kg treated rats.

(40xHaematoxylin and Eosin stain)

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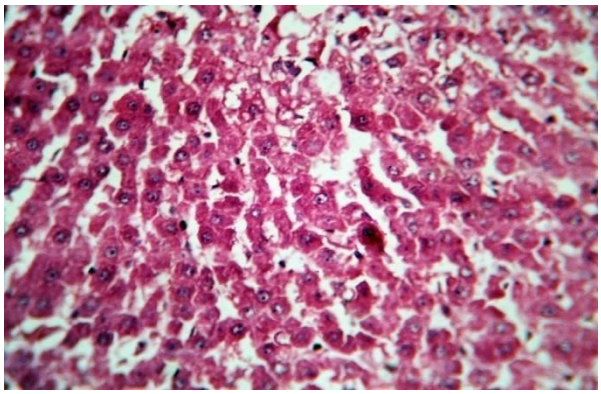

Fig.5Photomicrograph of EETO 100 mg/kg+CCl_4 treated Rats, (40xHaematoxylin and Eosin stain) treated rats,

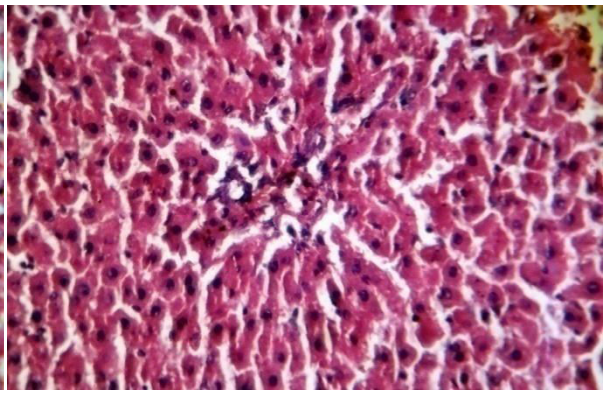

Fig. 6Photomicrograph of EETO 200 mg/kg+CCl_4

(40xHaematoxylin and Eosin stain)

Table: 1 - Effect of Ethanolic Extract of *Taraxacum officinale* (EETO) Leaves on ALT, AST and ALP in CCl_4 Induced Liver Damage in Rats.

Groups	ALT IU/L	AST IU/L	ALP IU/L
Control	36.14±4.745	101.94±6.868	114.36±5.618
CCl_4	156.29±10.83 (+76.87%)	272.36±10.904 (+62.57%)	296.73±16.46 (+61.45%)
EETO 100 mg/kg	37.98±3.633* (-75.69%)	94.97±4.894* (-65.13%)	109.9±8.443* (-62.96%)
EETO 200 mg/kg	45.88±2.79* (-70.64%)	95.7±4.77* (-64.68%)	119.86±5.931* (-59.60%)
EETO 100 mg/kg + CCl_4	72.61±3.699* (- 53.54%)	154.42±3.521* (-43.30 %)	179.49±13.185* (- 39.51%)
EETO 200 mg/kg + CCl_4	66.68±4.6119* (- 57.33%)	134.7±8.4153* (-50.54%)	143.72±10.98* (- 51.56%)

All data presented in Mean±SD (n=6) and * P<0.001 as compared to CCl_4 treated group.

+ = %increase, - = % decrease, CCl_4 treated group was compared with control and rest of the groups were compared with CCl_4 treated group.

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Table: 2 - Effect of Ethanolic Extract of *Taraxacum officinale* (EETO) Leaves on ACP, Total Protein (TP) and Total Bilirubin (TB) in CCl_4 Induced Liver Damage in Rats.

Groups	ACP IU/L	TP g/dL	TB mg/dL
Control	5.68±0.838	10.32±0.474	0.34±0.063
CCl_4	16.22±1.63 (+64.98%)	5.77±0.977 (-44.08%)	1.09±0.067 (+68.80%)
EETO 100 mg/kg	7.12±1.037* (-56.10%)	9.2±0.600* (+37.28%)	0.38±0.06* (-65.13%)
EETO 200 mg/kg	6.16±0.57* (-62.02%)	10.45±0.668* (+44.78%)	0.46±0.083* (-57.79%)
EETO 100mg/kg + CCl_4	8.54±0.750* (- 47.34%)	8.55±0.825* (+32.51%)	0.76±0.097* (- 30.27%)
EETO 200 mg/kg + CCl_4	7.31±0.77556* (- 54.93%)	10.42±1.007* (+44.62%)	0.42±0.04* (- 61.46%)

All data presented in Mean±SD (n=6) and * P<0.001 as compared to CCl_4 treated group.

+ = % increase, - = % decrease, CCl_4 treated group was compared with control and rest of the groups were compared with CCl_4 treated group.