



Antioxidant potential of *Taraxacum officinale* against carbon tetrachloride induced oxidative stress in rats.

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ABSTRACT

Free radicals are regularly produced in the body as a result of normal aerobic cellular metabolism. The body's natural in-built antioxidant system plays a vital role in preclusion of any harm due to free radicals. However, excessive production or addition of free radicals from environment to living system and weak defense mechanism of antioxidants often leads to serious consequence leading to oxidative stress and other ailments. Antioxidants from natural sources are now being considered as convincing therapeutic remedy against serious liver injury due to oxidative stress as they have capability to combat by neutralizing free radicals. This study was aimed to evaluate the *in vivo* antioxidant potential of ethanolic leaf extract of *Taraxacum officinale* (EETO) against carbon tetrachloride (CCl₄) induced oxidative-stress mediated hepatotoxicity in rats. For the antioxidant study, the analysis of superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) and lipid peroxidation (LPO) in liver homogenate of wistar albino rats were done. Our results clearly indicated that CCl₄ intoxication decreased the levels of GSH, CAT and SOD, while increased the level of LPO.

The extract both at the doses of 100 and 200 mg/kg b. wt. highly significantly ($P < 0.001$) increased the reduced levels of GSH, CAT and SOD compared to the CCl₄-treated animals, while as decreased the increased level of LPO as compared to CCl₄-treated animals. The findings of the study showed that the leaf extract of *Taraxacum officinale* possess a potential antioxidant activity.

Keywords: Antioxidants, carbon tetrachloride, CAT, free radicals, GSH, LPO, *Taraxacum officinale*, SOD.

I INTRODUCTION

Liver is the main detoxifying organ which receives 75% of blood directly from gastrointestinal organs and then spleen via portal veins which bring drugs and xenobiotics. The excessive load of such compounds is responsible for either inducing hepatic injury or worsening the damage process. Many chemicals and free radicals damage mitochondria and its dysfunction releases excessive amount of oxidants which in turn harm the hepatic cells. In the healthy state of body, there is a balance between formation and release of free radicals and the antioxidants. The

disturbance between the two increases free radicals in the body. However, the body has its natural antioxidant defense system in the form of low molecular weight antioxidants such as vitamins E and C which block free radicals, or in the form of enzymes such as superoxide dismutase, catalase and the glutathione system that reduce the levels of reactive oxygen species^[1]. Carbon tetrachloride induces hepatotoxicity and decreases the level of antioxidants such as Superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH)^[2].

Superoxide dismutases are a family of antioxidant enzymes which are important in the catalytic decomposition of the superoxide radical to hydrogen peroxide and oxygen. Catalase specifically catalyses the decomposition of hydrogen peroxide. Glutathione peroxidases are a family of antioxidant enzymes containing selenium which are imperative in the reduction of hydroperoxides, for example, those that result from lipid oxidation. In addition, antioxidants help sustain lower levels of free radicals; thus they perform beneficial physiological roles^[3]. Many researchers have demonstrated that medicinal herbs like *Asparagus racemosus*^[4] *Leucas aspera*^[5], *Colocasia esculenta*^[6] *Coleus amboinicus*^[7] etc. possess strong antioxidant and hepatoprotective properties. The extracts from different medicinal plants deliver the mother lode of antioxidants to the body, which help fight free radicals^[8]. *Taraxacum officinale*, commonly known as dandelion, is a medicinal plant effective and valuable as cholagogue, depurative, strongly diuretic, hepatic, laxative, stomachic and tonic. Dandelion is used as medicinal plant especially by tribal people for liver^[9], kidney and joint ailments and inflammatory diseases^[10]. It is a common vegetable relished in Kashmir, J and K, India, Himalaya and is considered to be very good for ladies after child birth.

Therefore, it is essential to search for herbal drugs for the treatment of liver disease for better efficacy and safety to replace currently used drugs. With the same objective, the present study was conducted to assess the *in vivo* antioxidant potential of ethanolic leaf extract of *Taraxacum officinale* (EETO) against carbon tetrachloride (CCl₄) induced oxidative-stress intervened hepatotoxicity in rats.

II MATERIALS AND METHODS

Preparation of leaf extract of *Taraxacum officinale*

The leaves of *Taraxacum officinale* were collected during the month of June from the Kachan area of Ganderbal (J&K), India. The herb was taxonomically identified and authenticated by Dr. Akhter H. Malik (Curator, KASH centre 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 references. The collected leaves were thoroughly checked for any foreign matter and thereafter washed in purified water and shade dried for a week at room temperature. The completely shade dried leaves were powdered in a grinder. The powder was extracted in 90% ethanol by using the Soxhlet extractor. The ethanol extract was then dried under vacuum and the semi solid material thus obtained was stored in storage bottles which were kept at -4 °C for further use.

2.1 Experimental Animals

Male albino wistar rats weighing 120-150 g 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 °C and light cycle (12 hours light and 12 hours dark). The animals were fed with the pellet diet manufactured by Golden feeds Delhi and water *ad libitum*. Housing condition and all animal experiments were performed as per the guidelines of committee for the purpose of control and supervision on experiments on animals (CPCSEA). Animal experiments were performed with prior permission from Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal, India (1283/C/09/CPCSEA).

2.2 Experimental Design

Body weight of the animals was recorded and were randomly divided into six groups of six rats each and treated as follows for 30 days: Group 1- Normal healthy control group received distilled water for 30 days orally with canula and served as control. Group 2- Received the dose of CCl₄ (11 % v/v with olive oil, i.p.) 2 ml/kg b. wt. once a week for 30 days to induce chronic liver injury and served as treated control. Group 3- Received the leaf extract of *Taraxacum officinale* orally 100 mg/kg b. wt. daily for 30 days. Group 4- Received the leaf extract of *Taraxacum officinale* orally 200 mg/kg b. wt. daily for 30 days. Group 5- Received the extract of *Taraxacum officinale* 100 mg/kg b. wt. orally daily followed by i.p. dose of CCl₄ 2 ml/kg b. wt. once a week for 30 days. Group 6- Received the extract of *Taraxacum officinale* 200 mg/kg b. wt. daily followed by the dose of CCl₄ 2 ml/kg body weight once a week for 30 days.

2.3 Biochemical Estimations

At the end of experiment, the animals were fasted overnight on the 30th day. On the next day, the body weights of the animals were recorded and then euthanized with chloroform and dissected thereafter. The liver was dissected out, blotted off blood and rinsed in freshly prepared ice cold saline. The fat was freed from the organ and a part of the liver was homogenized in phosphate buffer and certain portion in triss HCl buffer separately in Potter Elvehgen homogenizer fitted with polyteflon plunger at high speed. The homogenate thus obtained was centrifuged at 4500 rpm at 4°C for 10 minutes to get the supernatant fraction, which was used for various biochemical estimations like superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and lipid peroxidation (LPO) by using standard procedures^[11],^[12],^[13] and^[14] respectively. All the analysis was done in triplicates.



2.4 Statistical analysis

The results were expressed as means \pm standard deviation. The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test to determine the level of significance using Biostat 3 software. A value of $P < 0.001$ was considered to be highly significant.

III RESULTS

Effect of ethanolic extract of *Taraxacum officinale* (EETO) on Superoxide Dismutase (SOD) in experimental rats (Table: 1).

The level of SOD in control group of rats was 3.378 ± 0.077 U/mg. In rats inebriated with CCl_4 , the level was reduced ($p < 0.001$) to 2.430 ± 0.086 U/mg against the control group of rats. In the groups of rats administered with EETO at 100 mg/kg b. w. and 200 mg/kg b. w., the levels of SOD were 3.215 ± 0.117 U/mg and 3.265 ± 0.216 U/mg respectively. Supplying the animals with EETO at 100 mg/kg b. w. along with CCl_4 , the level of SOD was elevated in a highly significant ($p < 0.001$) way to 3.048 ± 0.093 U/mg. In the group of rats supplied with EETO at 200 mg/kg b. w. along with CCl_4 , the level of SOD was elevated ($p < 0.001$) to 3.145 ± 0.088 U/mg in comparison to that of treated control group.

Effect of ethanolic extract of *Taraxacum officinale* (EETO) on Catalase (CAT) in experimental rats (Table:1).

The level of CAT in control group of rats was 4.673 ± 0.309 U/mg. CCl_4 intoxication in rats caused a highly significant ($p < 0.001$) decrease in CAT level to 1.841 ± 0.136 U/mg. However, the doses of EETO at 100 mg/kg b. w. and 200 mg/kg b. w. did not show any significant change in the levels of CAT which were 4.139 ± 0.356 U/mg and 4.286 ± 0.182 respectively. In the combination groups of rats treated with EETO at 100 mg/kg b. w. and 200 mg/kg b. w. alongside CCl_4 , we observed a highly significant ($p < 0.001$) elevation in CAT level to 3.033 ± 0.285 U/mg and 3.40 ± 0.407 U/mg respectively in comparison to treated control group of rats.

Effect of ethanolic extract of *Taraxacum officinale* (EETO) on Glutathione (GSH) in experimental rats (Table: 1).

The level of GSH in control group was 500.980 ± 16.770 nMol/mg. In the treated control group of rats, the level of GSH was reduced to 321.900 ± 18.460 nMol/mg than that of the control group in a highly significant ($p < 0.001$) way. In EETO supplied groups of rats at 100 mg/kg b. w. and 200 mg/kg b. w., the levels of GSH were 451.400 ± 26.959 nMol/mg and 464.350 ± 16.578 nMol/mg respectively. However, EETO at 100 mg/kg along with CCl_4 increased ($p < 0.001$) the level of GSH to 415.140 ± 25.350 nMol/mg than that of the treated control group. Supplying the animals

with the EETO at 200 mg/kg+CCl₄ elevated (p< 0.001) the level of GSH to 458.430 ± 28.456 nMol/mg as against the treated control group.

Effect of ethanolic extract of *Taraxacum officinale* (EETO) on Lipid Peroxidation (LPO) in experimental rats (Table: 1).

In control group, the level of LPO was 0.235±0.021 nM/mg. However, in treated control group of rats, a highly significant elevation in the level of LPO was observed viz.0.533±0.040 nM/mg as against the control. In the groups which received EETO at 100 mg/kg b. w. and 200 mg/kg b. w., the levels of LPO were 0.244±0.025 nM/mg and 0.237±0.019 nM/mg respectively. The level of LPO was reduced to 0.285±0.023 nM/mg (p< 0.001) when the rats were supplied with EETO at100 mg/kg b. w. along with CCl₄ in contrast to treated control group of rats. Supplying the rats with EETO at 200 mg/kg+CCl₄ decreased the level of LPO to 0.254±0.024 nM/mg in comparison to treated control group in a highly significant (p< 0.001) way.

Table: 1 - Effect of ethanolic extract of *Taraxacum officinale* (EETO) on the levels of Superoxide dismutase (SOD), Catalase (CAT) Glutathione (GSH) and Lipid Peroxidation (LPO) in CCl₄ induced liver damage in rats.

Control	3.378 ± 0.077*	4.673±0.309*	500.980 ±16.770*	0.235 ± 0.021*
CCl₄ (2 ml/kg b. wt.) (Treated control)	2.430 ± 0.086** (-28.06%)	1.841±0.136** (-60.60%)	321.900 ± 18.460** (-35.74%)	0.533 ± 0.040** (+55.90%)
EETO 100 mg/kg	3.215 ± 0.117* (+17.40%)	4.139±0.356* (+55.52%)	451.400 ± 26.959* (+28.68%)	0.244 ± 0.025* (-54.22%)
EETO 200 mg/kg	3.265 ± 0.216* (+19.54%)	4.286±0.182* (+57.04%)	464.350 ± 16.578* (+20.07%)	0.237 ± 0.019* (-55.53%)
EETO 100 mg/kg + CCl₄	3.048 ± 0.093* (+13.81%)	3.033±0.285* (+39.30%)	415.140 ± 25.350* (+22.45%)	0.285 ± 0.023* (-46.52%)
EETO 200 mg/kg + CCl₄	3.145 ± 0.088* (+16.47%)	3.4±0.407* (+45.88%)	458.430 ± 28.456* (+29.78%)	0.254 ± 0.024* (-52.34%)

All data presented in Mean ± SD (n=6) and * P<0.001 as compared to CCl₄ treated group. ** P<0.001 as compared to Control group.

+ = % increase, - = % decrease, CCl₄ treated group was compared with control and rest of the groups were compared with CCl₄ treated group.

IV DISCUSSION

In traditional practice, several plants have been used to treat health disorders, including liver diseases. Rich heritage of Indian medicinal system has shown that plants contain active compounds that have become new sources to investigate for the pharmaceutical industry. The assessment of the protective action in liver damage induced by CCl₄ has been extensively used for hepatoprotective drug screening^[15]. CCl₄ is a widely used experimental hepatotoxicant, which is metabolically activated by the liver cytochrome *P*-450 enzymes to highly reactive toxic metabolites such as trichloromethyl radical (CCl₃[•]) and peroxy trichloromethyl radical (CCl₃OO[•]). Both of these metabolites covalently bind to different proteins or lipids of cell membranes and organelles, abstracting H⁺ atoms from polyunsaturated fatty acid, initiating lipid peroxidation (LPO) thus causing damage to cell membrane, disturbing Ca²⁺ homeostasis, changing enzyme activities and ultimately inducing hepatic damage or necrosis^[16] and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals^[17]. The findings of our investigation with control group of rats revealed the normal levels of SOD (3.378±0.077 U/mg), CAT (4.673±0.309 U/mg), GSH (500.980±16.770 nM/mg) and LPO (0.235±0.021 nM/mg). However, CCl₄ intoxication to the rats resulted in a significant decrease in the levels of SOD by 28.06%, CAT by 60.60%, GSH by 35.74% and elevated the level of LPO by 55.90% (P<0.001) compared to healthy control animals.

Living tissue possess a major defense mechanism involving antioxidative enzymes that convert active oxygen molecules into non-toxic compounds^[18]. Superoxide dismutase (SOD) is a major endogenous antioxidant which counterbalances free radical mediated damage. It is well known that SOD is involved in the protection of normal cell structure and function by maintaining the redox homeostasis, quenching of free radicals and by participating in detoxification reactions^[19]. SOD is the omnipresent cellular enzyme that dismutates superoxide radicals to H₂O₂ and oxygen and is one of the main cellular defense mechanisms. The H₂O₂ formed by SOD and other processes is scavenged by catalase, an enzyme responsible for the dismutation of H₂O₂ into H₂O and molecular oxygen.

Glutathione (GSH) is one of the most copious tripeptide, non enzymatic biological antioxidant present in the liver^[20]. GSH is helpful for the removal of free radicals such as H₂O₂ and superoxide radicals, detoxification of foreign chemicals, alkoxy radicals and biotransformation of drugs. The decrease in liver GSH levels represents increased utilization due to oxidative stress^[21]. Therefore, measurement of SOD, CAT, GSH and LPO levels in the liver provides an indication about the extent of cell injury caused by a certain compounds. The results obtained for the group 3rd and 4th groups i.e. the animals which received EETO at 100 mg/kg b. w. and 200 mg/kg b. w., were near to the control levels. These results clearly proved the non toxic nature and thus the safety of the EETO at the two selected doses. In current study in group 5th i.e. animals treated with EETO at 100 mg/kg b. w. along with CCl₄, a highly significant restoration of the altered levels of SOD by 13.81%, CAT by 39.30%, GSH by 22.45% and LPO

by 46.52% was observed in comparison to that of group 2nd i.e. CCl₄ exposed group. Our finding goes in accordance with reports of extract of *Piper guineense* that showed the similar restorative effects in SOD, CAT and GSH while same LPO-depleting effects for [22]. In current investigation slightly higher protection was offered by EETO when the dose was increased to 200 mg/kg in CCl₄ treated rats as indicated by elevated levels of SOD, CAT and GSH by 16.47%, 45.88%, 29.78% respectively. But the level of LPO was decreased by 52.34% (P<0.001). These results clearly showed that EETO produced the dose dependent protective effect in the rats challenged with CCl₄. Similar sort of results were shown by the methanolic leaf extract of *Caesalpinia bonducella* at 50 mg/kg and 100 mg/kg doses [23] and by the leaf extract of *Aloe vera* at 250 mg/kg and 500 mg/kg doses in rats challenged with CCl₄ showing decreased level of LPO and increased levels of GSH, SOD and CAT in a dose dependent manner [24].

The protective components of the EETO may have exerted their action against CCl₄- induced liver injury by the impairment of CCl₄- mediated lipid peroxidation via a decreased production of CCl₄- derived free radicals. Moreover, *Taraxacum officinale* has been reported to possess fair amount of phytochemicals, decent *in vitro* antioxidant activity, total phenolic and total flavonoid contents. In the present study, the ethanolic extract of leaves of the plant showed a potential *in vivo* antioxidant activity as it elevated the otherwise reduced levels of liver SOD, CAT and GSH, while as, decreased the level of LPO. These results may be due to lipotropic substance present in the extract which helps to improve the functions of liver cells. Also, dandelion is reported to contain cichoric acid, luteolin, luteolin-7- oglucoside, flavonoids, alkaloids, steroids and high content of polyphenols with potential application as radical scavengers (ROS), play a vital role in controlling oxidation and prevent DNA from ROS-induced damage [25].

V CONCLUSION

The findings of the present investigation adequately proved the antioxidant potential of ethanolic extract of *Taraxacum officinale* leaves in rats challenged with CCl₄, by restoring the tissue biochemicals (SOD, CAT, GSH and LPO). The therapeutic potential shown by the extract of *Taraxacum officinale* in the management of hepatic dysfunction may be due to its phytochemical constituents acting synergistically. Extraction, isolation and characterization of the constituents responsible for the therapeutic efficacy of *Taraxacum officinale* leaves followed by evaluation of their pharmacological action against liver damage can be carried out in order to have a more accurate picture about the possible mechanism of action of the components of the extract so as to identify an even efficient antioxidant drug.

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