INT. J. BIOL. BIOTECH., 16 (2): 307-312, 2019.

ROLE OF *TARAXACUM OFFICINALE* WIGG. AGAINST EXPERIMENTALLY INDUCED RENAL DAMAGE THROUGH CARBON TETRACHLORIDE IN RATS

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ABSTRACT

Kidneys are essential in eliminating toxic by product from body and likewise prone to damage caused by toxins, chemicals and therapeutic drugs during their elimination. Herbal therapies have gained a lot of attention during these days due to least reported side effects. *Taraxacum officinale* Wigg. commonly known as Dandelion found globally and well known for its diuretic effects due to sesquiterpene lactone which is richly found in its leaves. The objective is investigation of the protective effects of Dandelion leaves extract against renal toxicity induced by carbon tetrachloride (CCl_4) in male Wistar rats. Healthy age and body weight matched albino Wistar rats were allotted in random manner into three experimental groups (n=6) named as control; CCl_4 treated and CCl_4+Dandelion treated. Control group remained untreated whereas CCl_4 treated and CCl_4+Dandelion treated received CCl_4, 1mL/Kg of body weight, subcutaneously, twice in a week for 15 days. CCl_4+Dandelion treated group in addition received Dandelion leaves water extract (DLWE) orally for 15 days. Blood and tissue samples were obtained and preserved from each treated group on the 16^{th} day of experiment for biochemical and histological analysis. The body weights of control and CCl_4 treated rats increased after 15 days of treatment whereas decreased in group B. Treating rats with CCl_4 caused renal toxicity as indicated by significantly enhanced Urea, Creatinine and blood urea nitrogen (BUN)levels as compared with control. Dandelion treatment given to rats along with CCl_4 treatment showed reduced renal damage as indicated by non-significant rise in Urea, Creatinine and BUN levels as compared with control. Histological examination revealed only slight glomerular and tubular alterations in renal cortex of rats in CCl_4 treated group which were completely absent in control and CCl_4+Dandelion treated groups. Dandelion treatment decreased nephrotoxic effects of CCl_4 possibly by increasing antioxidant defense system hence this study concludes that regular intake of Dandelion along with conventional medicine can be beneficial in healing kidney injury and would improve working efficiency of kidney while preventing any damage and side effects.

Keywords: Nephrotoxicity, Dandelion Leaf Extract, *Taraxacum officinale* and Carbon Tetrachloride

INTRODUCTION

Taraxacum officinale Wigg. Is usually called as Dandelion which is a French name "Pissenlit" that literally means "wet thebed". Medically it houses many properties such as antioxidant, diuretic, liver protective and anti-inflammatory which render it as a remarkable herb (Park *et al*., 2011). *Taraxacum* species have been considered as a diuretic for over two thousands year in both Ayurvedic and Chinese medicine (Bevin *et al*., 2009).

In oxidative stress there is an inequality between development of free radicals and antioxidants that potentially lead to damage (Betteridge, 2000). Enzymatic and non-enzymatic sources of Reactive Oxidative Species (ROS)are present in the kidney such as peroxides, mitochondrial respiratory chain deficiency, xanthine oxidase, nitric oxide synthase (NOS) and NAD(P)H oxidase. Noxs, (NADPH oxidases) is abundantly found in kidney responsible for the formation of ROS (Cifuentes-Pagano *et al*., 2012) (Lassègue *et al*., 2012). A study revealed its involvement in "angiotensin II-induced ROS production" in renal cells as it enhances O_2 levels and reduces medullary blood flow (Katherine *et al*., 2012). Three categories in which renal damage caused by oxidative stress can be characterized are glomerular alterations which occur due to ROS mediated reaction on cells and elevated lipid and protein in blood (Klahr, 2001). Tubulointerstitial alterations which occur due to LDL exposure to tubule cells as pro-oxidant environment is developed (Agarwal, 1996). Interstitial macrophage infiltration is initiated and chemo tactic cytokines, chemokines, and other inflammatory mediators are produced, the gene expressions of which are triggered by ROS attracting number of leukocytes (Guillermo and Albert, 2015). Endothelial dysfunction which occurs when proliferation of vascular smooth muscle cells are caused as an effect of increased ROS production and cause renal hypertension (Jin., 2015). Major way Carbon tetrachloride (CCl_4) intoxication causes death is by inducing renal failure. However, there exists scarce published data on CCl_4 induced nephrotoxicity. Experimentally induction of renal injury due to oxidative stress by CCl_4 in rats has been proved (Khan *et al*., 2009).

Chemical analysis of dandelion has found rich traces of β-carotene, cellular protective component (Khoo *et al*., 2011). The active constituents in the root of plant which imparts an antioxidant property are lactones, inulins, sterols, triterpenes, flavonoids, and phenolic acids (Amim *et al*., 2013). Relatively less has been studied about the therapeutic effects of dandelion leaves on kidney damage. This study emphasizes on the significance of ROS in renal pathophysiology and the therapeutic role of orally administered Dandelion leaves in prevention and protection against nephrotoxicity induced by CCl_4.

MATERIALS AND METHODS

Albino wistar rats weighing 168-199g were purchased from ICCBS (International Center for Chemical and Biological Sciences) for the study. Rats were caged in a quiet temperature controlled room (23 ± 4ºC) and provided water adlibitum and standard rat diet. After animals' acclimatization to the laboratory conditions, experiment was initiated.

Ethical guidelines

All experiments were carried out in accordance with ethical guidelines of institutional ERB (Ethical Review Board) and principles accepted internationally for laboratory use and care in animal research (Health Research Extension Act, 1985).

Extract Preparation

Taraxacum Officinale Wigg. (Dandelion) leaves were purchased from herbal store situated in main Herbal market in Karachi. Dandelion Leaf Water Extract (DLWE) was prepared by first drying leaves at room temperature for 3-4 days. The leaves were then grinded into a homogenized powder. The DLWE was achieved after adding 2.5L water in 250g dandelion leaves powder and then boiled in a double boiler for 4 h until water evaporated. The extract was drained using filter paper and frozen at -2˚C for 2 h and kept as stock.

Study Design

Rats were randomly allotted in three experimental groups (n=6) according to age and body weight. Group A considered as control, group B considered as nephrotoxic group as they received CCl4 subcutaneously; 0.8 mL/kg body weight, subcutaneously twice a week for 15 days. Group C received CCl4; 0.8 mL/kg body weight, twice a week together with Dandelion Leaf Water Extract (DLWE) orally by intubation tube for 15 days. On 16th day animals were decapitated and blood was collected by cardiac puncture through heparinised syringes and centrifuged at 5000 rpm for 5 min to collect plasma. Kidney was excised, rinsed with saline water, dried by blotting and weighed. The tissues were stored at -70 ºC until analysis.

Assessment of Plasma Urea, Creatinine and Blood Urea Nitrogen (BUN)

Plasma Urea was measured using method stated by Fawcett and Scott, 1960, Estimation of plasma creatinine was done as described in the method(Hare, 1950; Kostir and Sonka, 1952) and BUN was estimated using reagent kits of Randox.

Statistical analysis

Results are recorded in mean± standard deviation. Statistical Package for the Social Sciences (SPSS) version 16 is used for the analysis of data. Estimation of statistical comparisons and difference between group means were calculated by using one-way analysis of variance (ANOVA) followed by the LSD (Least Significance Difference Post hoc multiple comparison test) and were found significant when $p<0.05$.

RESULTS

Effect of CCL_4 and Dandelion treatment on Body Weights in control, CCl_4 treated and Dandelion + CCl_4 treated groups

The body weight of control groups increased after 15 days ($p>0.05$), but the results are non-significant. The body weights of CCl_4 treated groups decreased significantly after 15 days of treatment ($p<0.05$) whereas in CCl_4+ Dandelion treated group the body weights of the rats increased non-significantly after treatment ($p>0.05$).CCl_4treatment has shown to decrease body weights significantly ($p<0.05$) (Table1) in CCl_4 treated group.

Table.1. Comparison of body weights in Control, CCl_4 treated and CCl_4 + Dandelion treated rats groups..

NO.OF DAYS	CONTROL (n=3)	CCl_4 treated (n=3)	CCl_4+dandelion treated (n=3)
0	179.6 ± 10.11	196 ± 5.19	192 ± 1
7	179.6 ± 22.36	191 ± 10.14	201.3 ± 7.09
15	180.3 ± 17.67	185 ± 5	202.3 ± 13.05

Effect of CCL_4 and Dandelion treatment on Plasma Urea levels in control, CCl_4 treated and Dandelion + CCl_4 treated groups

In our study plasma urea concentration was markedly raised in CCl4 treated group with respect to control group ($p<0.001$) (Table.2). Plasma urea concentration was increased significantly ($p<0.05$) in CCl4+Dandelion treated group as compared with Control but found to be reduced significantly ($p<0.05$) when compared with CCl4 treated group (Table.2).

Effect of $CCL_4$and Dandelion treatment on Plasma creatinine levels

InCCl_4treated rats, a significant increase in serum creatinine level in comparison to control ($p<0.05$) was observed (Table.2). The creatinine concentration was found to be increased significantly in CCl_4+Dandelion treated group with respect to control ($p<0.01$) and CCl_4 ($p<0.05$) treated group.

Effect of CCL_4 and Dandelion treatment on Plasma BUN levels

In CCl_4 treated rats ($p<0.01$) andCCl_4+Dandelion treated group ($p<0.05$) serum BUN level is markedly increased as compared to control (Table.2). However the serum Bun level in CCl_4+Dandelion treated group is remarkably decreased as compared to CCl_4 treated group.

Table 2. Comparison of Plasma Urea, Creatinine and BUN in Control, CCl_4And CCl_4+Dandelion Group.

	Control (n=3)	CCl_4 treated[1] (n=3)	CCl_4 + Dandelion[1'2] treated (n=3)	LSD 0.05
Urea (mg/dL)	4.85 ± 3.24 c	17.64 ± 1.47 a	14.8 ± 0.2 b	1.04
Creatinine (mg/dL)	2.26 ± 0.665 b	3.63 ± 0.15 a	4.43 ± 0 a	1.15
BUN (mg/dL)	2.26 ± 1.51 c	8.23 ± 0.68 a	6.91 ± 0.09 b	1.27

The data is expressed as mean ± standard deviation; Same letters are not significant in each row according to Duncan's Multiple Range Test at $P < 0.05$.

Histopathological findings of Kidney samples

Histology of Kidney samples showed no Mesangial proliferation and Tubulointerstitial fibrosis in control and CCl_4 treated + Dandelion treated group of rats. Whereas, CCl_4 treated group of rats showed tubulointerstitial fibrosis (Fig. 1, 2 and 3). This leads us to interpret that Dandelion treatment has reverted fibrosis which was induced in CCl_4 treated group of rats.

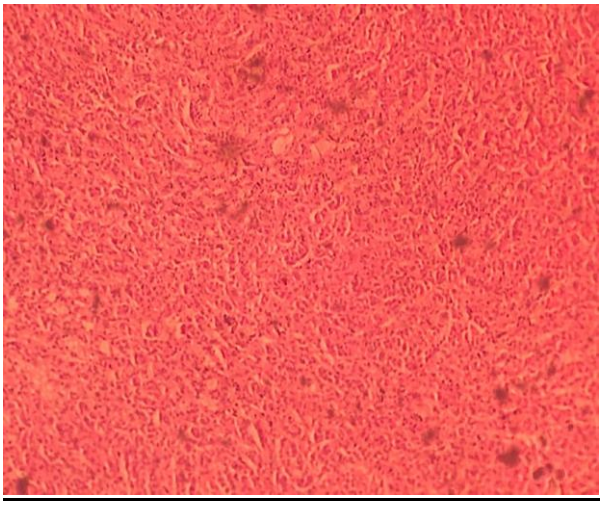

Fig.1.Histopathological characteristics of Control group.

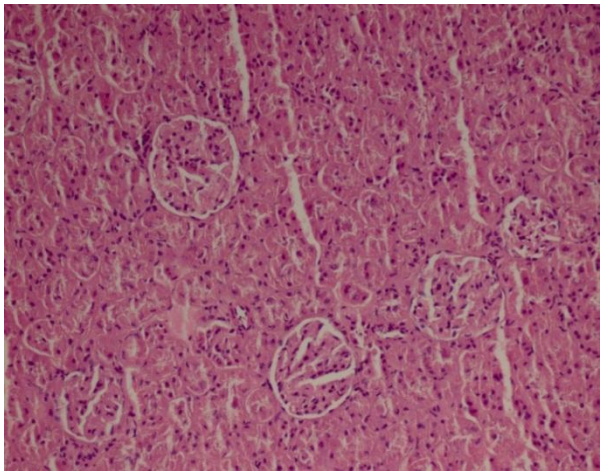

Fig.2.Histopathological characteristics of CCl_4 treated group.

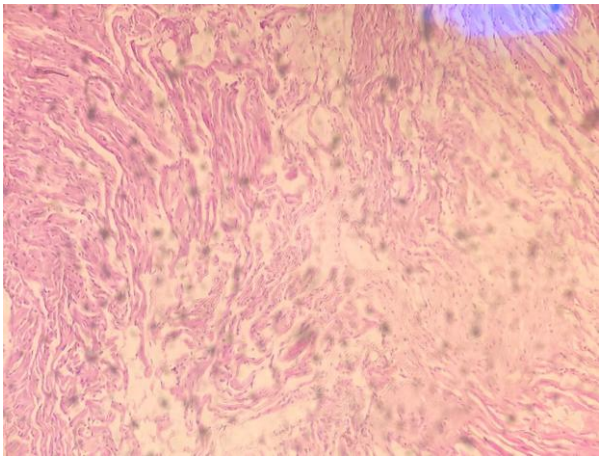

Fig.3.Histopathological characteristics of CCl_4 +Dandelion treated group.

DISCUSSION

Toxicity caused by CCl_4 has been experimentally proven to damage liver and kidney. Cytochrome P450 2E1 metabolize it to trichloromethyl which is highly reactive radical responsible to cause lipid peroxidation eventually leading to renal as well as hepatotoxicity (Naz and Mehboob, 2015). CCl_4 treated rats showed elevated urea,

creatinine and BUN concentration which mark the incidence of renal impairment (Table 2). The increase in urea is possibly due to enhanced peroxidation caused by CCl_4 leading to kidney damage in CCl_4 treated group. However, there is marked reduction in urea observed in CCl_4 + Dandelion treated group that is possibly because of the therapeutic action of Dandelion which promotes diuresis and decreases body water content. Some studies (Hu and Kitts, 2005) have confirmed the therapeutic effectiveness of dandelion leaves in eliminating body fluids through diuresis but the exact mechanism of renal damage prevention is yet to be identified. The increase in creatinine in CCl_4 treated group is due to poor clearance of creatinine by kidney because of structurally damaged glomerulus. The increase in creatinine concentration in CCl_4+Dandelion group may be due to excessive filtration in glomerulus because kidneys are responsible to eliminate drugs or other toxic agents from body. The ingestion of drugs may probably increase the burden on kidneys function and may enhance the creatinine levels in blood. Since the leaf extracts of Dandelion are found to be effective hydrogen donors, scavengers of hydrogen peroxides, and also strong reducing agents (Czinner *et al*., 2001). Histopathological features of Kidney samples of CCl4 + Dandelion treated rats showed that Dandelion is effective in reversing the changes caused in CCl4 treated rats, by ameliorating tubulointerstitial fibrosis when it was compared with histologic features of control group which showed no mesangial proliferation and tubulointerstitial fibrosis. These attributes show that Dandelion has potency to significantly decrease the elevated levels of serum BUN possibly defending kidney tissue against oxidative damaged by CCl_4 and contributing its role in healing the injury. Thus, in present study we found that damage caused by CCl4 in the kidneys was effectively combated by the antioxidant effects of leave extract of *Taraxacum officinale.*

CONCLUSION

As dandelion has shown in this study to exert healing and protective effect against kidney damage, it is therefore concluded that daily usage of Dandelion can improve physical state of the consumers because of the presence of various vital compounds essential forkidney's health. Further studies are to be warranted to evaluate its safety, efficacy and mechanism of action.

ACKNOWLEDGEMENTS

We would like to thank Dean, Faculty of Science, and University of Karachi for financial support of the project.

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(*Accepted for publication March 2019*)