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Tagetes minuta Herbal Extract: a Promising Prevention Strategy for the Treatment of Nephropathy.

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Abstract : The aim of present study was to evaluate nephroprotective potential of ethanolic extract of *Tagetes minuta* (EETM). Thirty six rats were used in this experiment and they were divided into six groups (n=6).Group I (control) received distilled water for 30 days orally. Nephrotoxicity was induced by carbon tetrachloride (11 % v/v with olive oil) 2 ml/kg b. wt. once a week for 30 days. Group III and IV received only herb in two doses 100 and 250 mg/kg of body weight respectively. Group V and VI received ethanolic extract of *Tagetes minuta* (100 and 250 mg/kg of b.wt.) along with carbon tetrachloride (11 % v/v with olive oil) 2 ml/kg b. wt. once a week for the time period of 30 days. Blood urea nitrogen, urea and creatinine levels were studied in various groups of experimental animals. It was observed that CCl_4 treatment induced significant elevation ($P<0.001$) in the levels of blood urea nitrogen, urea and creatinine. However, treatment with ethanolic extract of said plant significantly ($P<0.001$) restored the altered levels of these kidney biomarkers in a dose dependant manner. This finding powerfully supports that ethanolic extract of *Tagetes minuta* acts as potent scavenger of free radicals to prevent the toxic effect of carbon tetrachloride and hence validate its ethnomedicinal use.

Key Words: Nephrotoxicity, *Rat,* antioxidant, *Tagetes minuata,* creatinine, blood urea nitrogen.

Introduction

A number of environmental contaminants, chemicals and drugs including antibiotics dramatically alter the structure and function of various tissues and produce multiple adverse effects in the liver, kidney, heart and intestine (Kohn *et al.,* 2005). The kidney and the liver are the major target organs of accumulation and intoxication. Exposure to chemical reagents like ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals such as lead, mercury, cadmium and arsenic also induces nephrotoxicity. Carbon tetrachloride (CCl_4) is known to be nephrotoxic as well as hepatotoxic to humans (Abraham *et al.,* 1999). High exposure to CCl_4 can cause liver, kidney and central nervous system damage, and liver is especially sensitive to CCl_4 because of its role as the body's principal site of metabolism (Sakata *et al.,* 1987, Sheikh *et al.,* 2015, Akram *et al*., 2016).

Measurement of serum creatinine is a valuable indicator of renal dysfunction. Creatinine is a non-protein waste product and continuously and proportionally formed during creatine phosphate metabolism by skeletal muscle tissue. Creatinine is freely filtered and therefore the serum creatinine level depends on the Glomerular Filtration Rate (GFR). Renal dysfunction reduces the ability to filter creatinine and the serum creatinine rises. Increased level of creatinine is considered to reflect loss of kidney function viz. Impaired renal function, Chronic nephritis, Urinary tract obstruction, Muscle diseases such as gigantism, acromegaly, and myasthenia gravis, Congestive heart failure and Shock (Iriada *et al*., 2006). Urea is a waste product formed in the liver when protein is metabolized. Urea is released by the liver into the blood and is carried to the kidneys, where it is filtered out of the blood and released into the urine. However, when the kidneys cannot filter wastes out of the blood due to disease or damage, then the level of urea in the blood will rise (Shalaby and Hammouda, 2014). Drugs of plant origin have served through the ages as the mainstay in the treatment of various ailments. Herbal drugs used in traditional system are found to be more effective with minimal or no side effects. In the recent times, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, due to their natural origin, cost effectiveness and lesser side effects (Naik *et al.,* 2003). The importance of natural antioxidants has been clarified by numerous studies which have demonstrated that the consumption of foods rich in such phytochemicals can exert beneficial effects upon human health, possibly by interfering in the processes involved in reactive oxygen and nitrogen species mediated pathologies (Ahirwar *et al*., 2016). This has resulted in resurgence in phyto-pharmacognosy with extensive attention upon the role that plant secondary metabolites may have in preventative medicine (Hertog *et al.,* 1993; Halliwell *et al.,* 1999).

Tagetes minuta also known as southern cone marigold, stinking roger or black mint, is a tall upright marigold plant from the family Asteraceae. It is an erect, woody annual herb, usually 0.5-2 m tall with strongly odorous foliage. The strong smelling essential oils of *T. minuta* have enabled it to be used for many purposes, including as a relish, laxative, diuretic, flavouring, insect repellent, stimulant and snuff (Holm *et al*., 1997). *T. minuta* (var. vanphool) which has been derived from the open population in northern India has a high yield and quality of essential oil (Kumar *et al*., 1999). It is grown as a vegetable in parts of Peru, dried leaves are used as condiments and flavouring in different food products. It is also used for the treatment of coughs, stomach cramps and rheumatism. However, no pharmacology data regarding the nephro-curative effect of *Tagetes minuta* is available. Hence, the present study was focused to evaluate urea and

nephrocurative activity of ethanolic extract of *Tagetes minuta* in CCl_4 treated rats.

Materials and Methods

Chemicals and reagents

All the chemicals use were of analytical grade obtained from Merck, Mumbai and HiMedia, Mumbai. All Biochemical investigations were performed using commercial available diagnostic kits of Erba Mannheim, Germany.

Preparation of *Tagetes minuta* extract

Whole plant of *Tagetes minuta* was purchased from Indian institute of integrative medicines Srinagar J&K, India. The plant material was washed with double distilled water and thereafter shade dried for the period of 2 weeks at room temperature. The fully dried plant material was powered with the help of mechanical 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 vials which were kept at -4^{0}C for further use. Phytochemical screening of the extract was also carried out according to the standard procedures (*Trease and Evans, 1989 and Kokate et al., 2006).* The fresh stock solution of *Tagetes minuta* 80 mg/ml was prepared in double distilled water just before use.

Experimental Animals

Wistar albino rats, weighing (235±15 gms) were used in the study. They were obtained from animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal Madhya Pradesh. Animals were maintained under standard conditions of temperature 23±1^{0}C and with regular 12:12 light/dark cycle and allowed free access to standard laboratory food (Golden feeds Delhi) and water *ad libitum*. 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 (1283/C/09/CPCSEA).

Animal Treatments

The animals were divided at random into six groups of 6 animals each and treated as follows. Group I (control) received distilled water for 30 days orally. The II group, received carbon tetrachloride (11 % v/v with olive oil) 2 ml/kg b. wt. once a week for 30 days. The III and IV groups received only herb EETM at the doses of 100 mg/kg and 250 mg/kg of b. wt. for 30 days respectively. The V group received EETM orally 100 mg/kg of b. wt. daily followed by dose of carbon tetrachloride 2 ml/kg body weight once a week for 30 days. The VI group received 250 mg/kg b. wt. of EETM daily fallowed by dose of carbon tetrachloride 2 ml/kg b. wt. once a week for 30 days. At the end of experiment, animals were fasted overnight, blood samples were collected by cardiac puncture, under light diethyl ether anesthesia into previously labeled EDTA retaining tubes and centrifuged in Remi centrifuge for 10 minutes at 5000 rpm as to get the plasma. The obtained plasma was used for the measurement of various biochemical markers like blood urea nitrogen (BUN), urea and creatinine levels by using commercially available kits.

Biochemical Parameters

Blood urea nitrogen and urea was determined by GLDH-Urease Method, Initial Rate (Talka and Schubert, 1965; Tiffany *et al*., 1972) while Creatinine was estimated by Jaffe's method (Bowers, 1980; Slot, 1965; Young, 1975).

Statistical Analysis

Data were expressed in MeanSD. Statistical comparison between different groups were done by using One Way ANOVA followed by Benferroni's test. $P<0.05$ and $P<0.001$ were considered as levels of significance.

Results

Observations of Phytochemical Investigation

The phytochemical analysis of ethanolic extract of *Tagetes minuta* whole plant showed the presence of alkaloids, terpenoids, flavonoids, saponins, carbohydrates, glycosides, tannins, phenolic compounds and amino acids (Table: 1)**.**

Effect of Ethanolic Extract of *Tagetes minu*ta on CCl_4 induced changes

The levels of BUN, urea and creatinine in control group of rats was 17.99±1.20 mg/dL, 38.54±1.55 mg/dL and 0.88±0.14 mg/dL respectively. Intra-peritoneal administration of CCl_4 2ml/kg b.w. once a week for 30 days caused abnormal renal functions in all experimental animals. Blood urea nitrogen (BUN), urea and creatinine levels were highly significantly ($P<0.001$) elevated to 39.62±3.30 mg/dL, 84.85±4.73 mg/dL and 2.08±0.21 mg/dL i.e. increased by +54.59%, +54.57% and +57.69% respectively of their control values. However, the animals which received ethanolic extract of *Tagetes minuta* at 100 mg/kg and 250 mg/kg, no significant variations in the levels of BUN, urea and creatinine were noticed and the values of these parameters in EETM 100 mg/kg treated group of rats were as BUN 19.20±1.96 mg/dL, urea 41.13±2.69 mg/dL and 1.00±0.07 mg/dL of creatinine and the percentage inhibition for these kidney markers against control group of rats was (+6.30%), (+6.29%) and (+12%) respectively. In group of rats supplied with EETM 250 mg/kg, the levels of BUN, urea and creatinine were 19.55±1.26 mg/dL, 41.88±1.22 mg/dL and 0.95±0.16 mg/dL with percentage inhibition (+7.97%), (+7.97%) and (+7.36%) against control group of rats respectively. Thus, these results revealed that the EETM was not having any type of side effect on kidneys. Hence, the extract was

safe at selected doses. Pretreatment with EETM at 100 mg/kg along with CCl_4 restored the altered levels of BUN, urea and creatinine to 20.29+2.00 mg/dL, 43.46±4.05 mg/dL and 1.120.18 mg/dL and their percentage inhibition was -48.78%,-48.78% and -46.15% respectively, were highly significant as compared to 2nd group i.e. the rats which were intoxicated with CCl_4. However in group sixth i.e. animals which received EETM at 250 mg/kg along with CCl_4, the levels of BUN, urea and creatinine were further reduced by -35.68%, -53.65% and -47.59% respetively (Table: 2). The results were highly significant as compared to that of 2nd group. Thus the extract produced nephro-protective effect at both doses i.e. 100 and 250 mg/kg against carbon tetrachloride induced nephrotoxicity and the protection was offered in a dose dependant manner.

Discussion

As predicted, administration of CCl_4(2 ml/kg i.p.) resulted in an overt nephrotoxicity as evident by significant increase in the levels of renal biomarkers such as blood urea nitrogen (BUN), urea and creatinine. The observed nephrotoxic effect CCl_4 were similar those of previously reported (Choie *et al.*, 1981; Anderson *et al.,* 1989; Heidemann *et al.*, 1989; Ozturk *et al.*, 2003; Ogeturk *et al.* 2005; Khan and Ahmed, 2009 and Khan *et al.,* 2009). In renal diseases, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance (Mayne, 1994). Also the concentration of creatinine is known to correlate inversely with the degree of glomerular filtration. Hence, creatinine is considered to be among the useful markers of the filtration task of kidneys, predominantly that creatinine is excreted only via the kidneys (Pietta, 2000). Evaluation of urea and creatinine levels in the serum was taken as index of nephrotoxicity (Bennit *et al*., 1982; Anwar *et al*., 1999; Ali *et al*., 2001). In our study the increased level of BUN, urea and creatinine was highly significant restored near to normal levels when CCl_4 intoxicated rats were given access to EETM and 100 mg/kg and 250 mg/kg of b. wt. in a dose dependent manner. In agreement with the results of present study various investigators reported that the increased levels of BUN, urea and creatinine as a result of toxicities were restored when the rats were treated with herbal extracts (Harlalka *et al.,* 2007; Adikay *et al*., 2010; kannappan *et al*., 2010; Kore *et al*., 2011 and Hiremath *et al*., 2012). The protective effect of EETM may be credited to its antioxidant properties, as it contains fair amount preliminary phytoconstituents such as alkaloids, terpenoids, flavonoids, saponins, carbohydrates, glycosides, tannins, phenolic compounds and amino acids.

Conclusion

The findings suggested that the ethanolic extract of *Tagetes minuta* possessed the nephroprotective property. Therefore, further studies to elucidate the mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases.

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Table: 1 - Showing the presence of different phyto-chemicals in *Tagetes minuta*.

Phytochemicals	Tests	Presence(+)/ Absence(-)
Alkaloids	**Mayer's Test**	+
	Wagner's Test	+
	Hager's Test	+
	Dragendroff's Test	+
Terpenoids	Salkowski test	+
	Libermann Burchards Test	+
Flavonoids	Lead Acetate Test	+
	Alkaline reagent test	+
	Shinoda test	-
Carbohydrates	Molish test	+
	Fehling's Test:	+
	Benedict's Test:	+
	Barfoed's Test	+
Glycosides	Killer Killians test	+
	Borntrager's Test	-
	Legal's Test	+
Tannins and Phenolic compounds	$FeCl_3$ test	+
	Dilute Iodine Solution test	+
	Lead Acetate Test	+
	Gelatin Test	+
Saponins	Froth test	+
Amino acid Proteins	Biuret's Test	+
	Millon's Test	+
	Ninhydrin test	-

Table: 2 - Effect of ethanolic extract of whole plant of *Tagetes minuta* (EETM) on BUN, urea and creatinine in CCl_4 induced nephrotoxicity in rats.

Groups	BUN(mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Control	17.99±1.20	38.54±1.55	0.88±0.14
CCl_4	39.62±3.20 (+54.59%)	84.85±4.73 (+54.57%)	2.08±0.21 (+57.69%)
EETM 100 mg/kg	19.20±1.96* (+6.30%)	41.13±2.69* (+6.29%)	1.00±0.07* (+12%)
EETM 250 mg/kg	19.55±1.26* (+7.97%)	41.88±2.12* (+7.97%)	0.95±0.16* (+7.36%)
EETM 100 mg/kg + CCl_4	20.29±2.00* (−48.78%)	43.46±4.05* (−48.78%)	1.12±0.18* (−46.15%)
EETM 250 mg/kg + CCl_4	18.35±1.43* (−53.68%)	39.29±2.64* (−53.65%)	1.09±0.19* (−47.59%)

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