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Research Article

# Evaluation of Protective Effect of *Tagetes erecta* Against Mercuric Chloride Induced Nephrotoxicity

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**ABSTRACT**

The toxicity of various forms of mercury to the living organisms is well documented. Mammalian kidney is the main site of decomposition of inorganic mercury as well as the target organ for its toxicity in majority of the animals including human beings. Present study has been aimed to investigate the role of *Tagetes erecta* (family-Astereacea) as a possible modifier of mercury induced renal damages. Experimentation was conducted on one hundred female albino rats, divided into five equal groups, each group again sub-divided into four sub-groups having five rats each:
Control group- Orally administrated distilled water only.
*T. erecta* flower extract treated group-10mg/kg b. wt./day for 1, 7, 14, 21 days was administrated orally.
Mercuric chloride treatment groups-A dose of 0.926 mg/kg b.wt. for 01 day, 0.132 mg/kg b.wt. for 7 days, 0.066 mg/kg b.wt. for 14 days and 0.044 for 21 days was administrated through oral route.
Oral administration of *T. erecta* flower extract followed by mercuric chloride treatment for 1, 7, 14 and 21 days.
Oral administration of Mercuric chloride followed by *T. erecta* flower extract administration for 1, 7, 14 and 21 days.
These animals were then sacrificed after 1, 7, 14 and 21 days treatment respectively. Controls were also run respectively. Mercuric chloride intoxication resulted in pathological alterations in the kidney of albino rats, such as degradation of glomerulus, proximal and distal tubules. Combined pre and post treatment of *T. erecta* with mercuric chloride has been found to reduce the pathological alterations in the kidney. Thus, the results from the present study suggest that *T. erecta* can modify the renal damages against mercuric chloride induced toxicity.

**Keywords:** Mercuric chloride, *T. erecta*, kidney, histopathology, glomerulus, degradation.

## INTRODUCTION

Mercury has long been recognised as a highly toxic metal to man and other living forms, still is used in various domestic, industrial, agricultural and medical applications. The toxicity of mercury depends greatly on the form of mercury compounds *viz.* elemental, inorganic and organic. Inorganic mercury present in the environment is a well established toxicant to human health. On absorption through the alimentary tract, mercury binds with the thio proteins, which plays an important role in the further metabolism of this metal[1-6].

Historically, plants have been used as folk medicine against various types of diseases. Remedies from plant sources (Indian system of medicine the 'Ayurveda') have proved to be very popular in primary health care in India for a long time[7].

In recent years, *Tagetes* is gaining more attention from medical scientists as a source of potential pharmaceutical. This genus is recognised as a source of very interesting biologically active products such as carotenoids used as food colorants and additives, possessing anti-cancer and anti-ageing effects, essential oils known for their anti-bacterial and insecticidal properties, thiophenes with a marked biocidal activity and flavinoids having pharmacological properties[8-10].

The present investigation has thus, been carried out to evaluate the role of *Tagetes erecta* in modifying the mercury induced nephrotoxicity in albino rats on the basis of comparative histopathological evaluations of kidney in various treatment groups.

## MATERIALS AND METHODS

*Rearing of experimental animals*

This study was conducted on one hundred female albino rats, *Rattus norvegicus*(Wistar strain), eight week old, 100±20 gm in weight and selected from an inbred colony. They were kept under appropriate temperature and light conditions, provided standard rat pellet feed and water *ad libitum*. These experimental animals were acclimatized for one week to laboratory conditions prior to experimentation.

*Preparation of plant extract*

The selected plant material (*Tagetes erecta*) was collected from the local Agra (India) region in suitable season. The collected plant material was shade dried for seven days, grinded to coarse powder and subjected to extraction by

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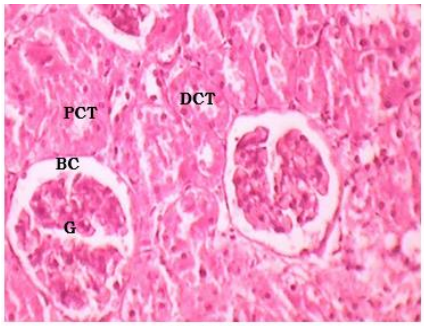



Plate-I(a): 1d

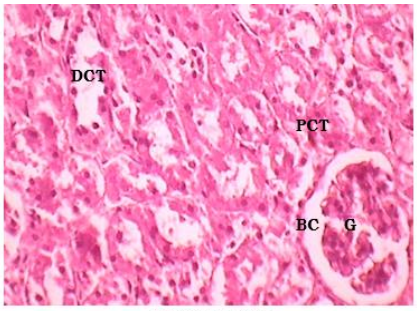



Plate-I(b): 7ds

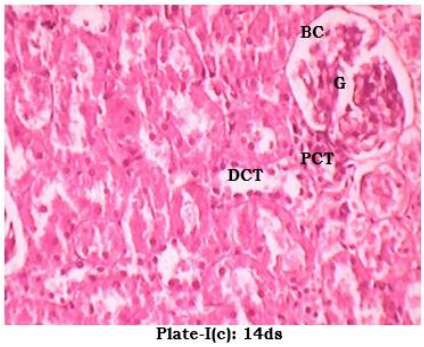



Plate-I(c): 14ds

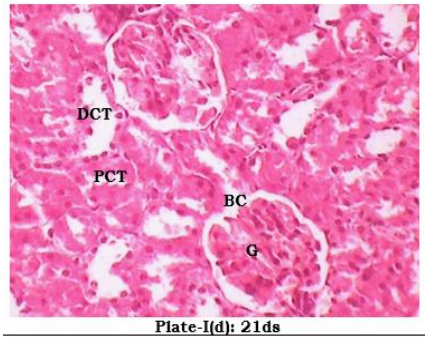



Plate-I(d): 21ds

Group I: Plate-I (a): Section of kidney of control rats for 1 day, (b): Section of kidney of control rats for 7 days, (c): Section of kidney of control rats for 14 days, (d): Section of kidney of control rats for 21 days.
BC: Bowman's capsule, CS: Cell swelling, DCT: Distal convoluted tubule, EG: Enlarged glomerulus, G: Glomerulus, HT: Hypertrophy, IF: Infiltration, N: Nucleus, NG: Necrotic glomerulus, NT: Necrotic tubule, PCT: Proximal convoluted tubule, PN: Pyknotic nuclei.

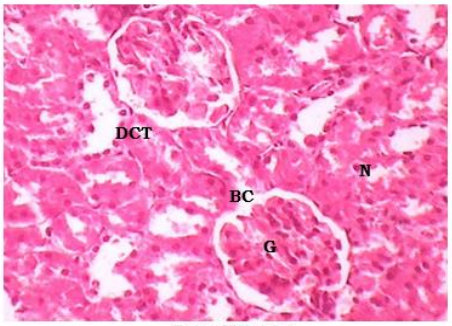



Plate-II(a): 1d

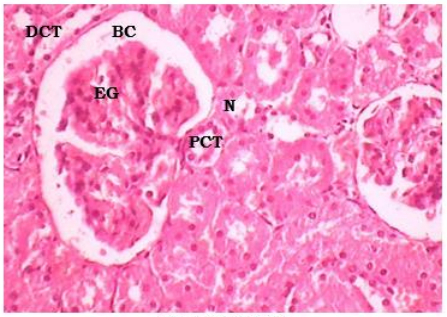



Plate-II(b): 7ds

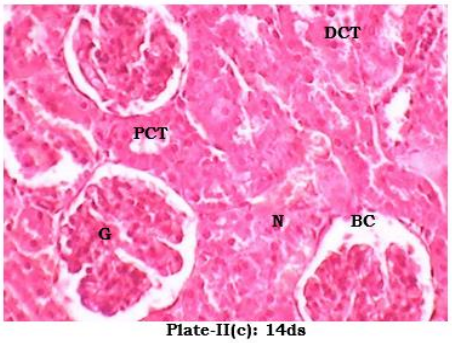



Plate-II(c): 14ds

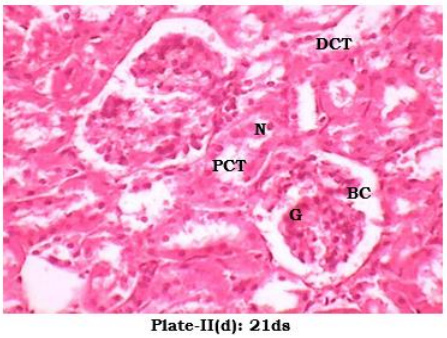



Plate-II(d): 21ds

Group II: Plate-II (a): Section of kidney of *Tagetes erecta* flower extract treated rats for 1 day, (b): Section of kidney of *Tagetes erecta* flower extract treated rats for 7 days, (c): Section of kidney of *Tagetes erecta* flower extract treated rats for 14 days, (d): Section of kidney of *Tagetes erecta* flower extract treated rats for 21 days.
BC: Bowman's capsule, CS: Cell swelling, DCT: Distal convoluted tubule, EG: Enlarged glomerulus, G: Glomerulus, HT: Hypertrophy, IF: Infiltration, N: Nucleus, NG: Necrotic glomerulus, NT: Necrotic tubule, PCT: Proximal convoluted tubule, PN: Pyknotic nuclei.

Soxhlet apparatus in methanol solvent for twenty two continuous cycles. Prepared plant extract was concentrated by rotator evaporator under optimum temperature and pressure conditions.

*Experimental compound*

Mercuric chloride was obtained from Sigma chemicals Ltd., Mumbai (India), and was dissolved in distilled water. The flower extract of *Tagetes erecta* was given on the basis of safety trials.

*Experimental protocol*

The acute oral $LD_{50}$ of mercuric chloride in albino rats came out to be 9.26 mg/kg b.wt.[2,11] Experimental albino rats were divided into five groups with twenty rats each, corresponding to *Tagetes erecta* flower extract administration, Mercuric chloride intoxication, *Tagetes eretca* pre and post mercuric chloride intoxications as well as control corresponding to these administrated groups. All these five groups were again sub-divided into four sub-groups corresponding to acute (1d) and sub-acute (7, 14 and 21 ds) treatments, having five rats each. Rats corresponding to control set were orally administrated distilled water. 10 mg/kg b.wt. of *T. erecta* flower extract was administrated to experimental albino rats for 1, 7, 14 and 21 days as per experimental schedule, where as $1/10^{th}$ of $LD_{50}$ of mercuric chloride i.e. 0.926, 0.132, 0.066 and 0.044 mg/kg b.wt. was administrated by gavage to albino rats for 1, 7, 14 and 21 days respectively.

*Histological studies*

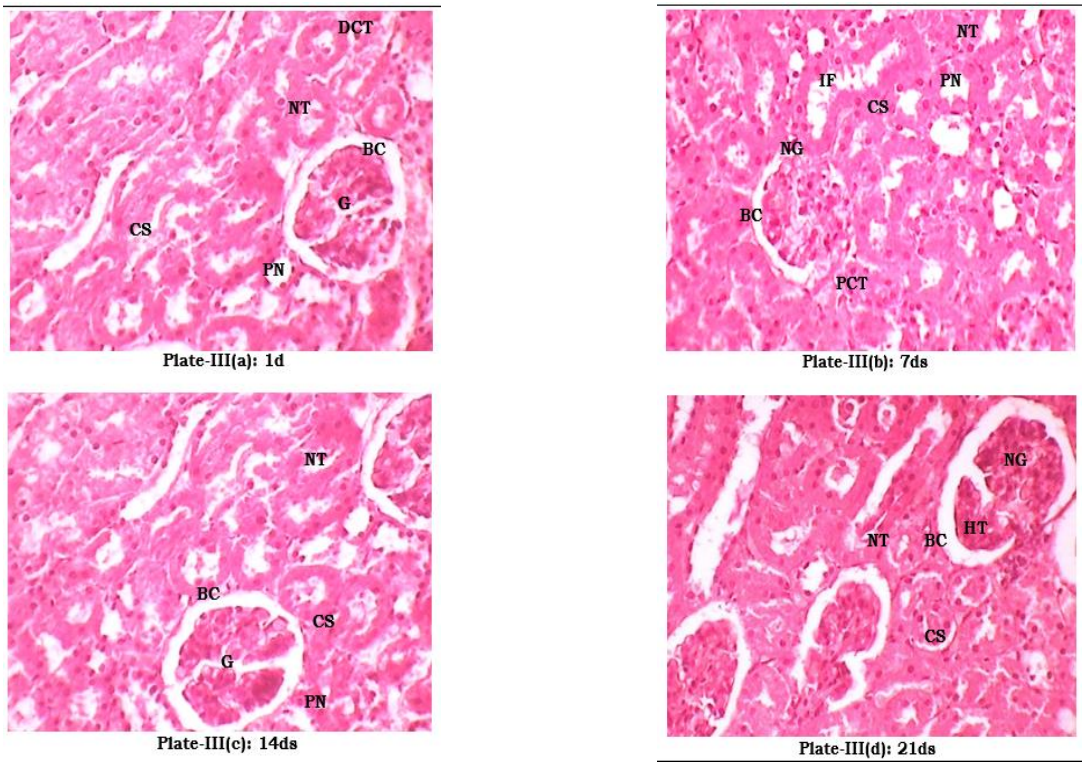
Kidneys from sacrificed animals were excised out and fixed in Bouin's fluid for 48 hrs, dehydrated in ascending alcoholic series and then embedded in paraffin wax. 5µ sections were then-after cut, stained with haematoxylin and eosin to observe histological alterations[12].

## RESULTS

The control kidney revealed glomerulus having a network of densely packed capillaries inside the capsule. The tubular cells had abundant granular cytoplasm with distinct cell borders and centrally placed nuclei. The distal convoluted tubule exhibited wide lumen and also centrally placed nuclei on day 1, 7, 14, 21(Plate Ia-Id).

Histopathological alterations in the kidney of rats following acute (1 day) and sub-acute (7, 14 and 21 days) mercuric chloride intoxication has been observed in the present investigation. Following acute (1d) mercuric chloride intoxication, widespread proximal tubule necrosis with absence of brush border and desquamated necrotic epithelial cells have been observed. Remnants of cellular debris were also observed in the lumen of both proximal and distal convoluted tubules. Epithelial cell nuclei indicated progressive death (Plate IIIa).

Severity of nephrosis has been pronounced following sub-acute (7, 14, 21 ds) mercuric chloride intoxication, however the magnitude was less compared to that of acute 1(d) dose of mercuric chloride. Glomerulus showed degenerative changes, distortion and obliterations of the




Group III: Plate-III (a): Section of kidney of mercuric chloride treated rats for 1 day, (b): Section of kidney of mercuric chloride treated rats for 7 days, (c): Section of kidney of mercuric chloride treated rats for 14 days, (d): Section of kidney of mercuric chloride treated rats for 21 days.

BC: Bowman's capsule, CS: Cell swelling, DCT: Distal convoluted tubule, EG: Enlarged glomerulus, G: Glomerulus, HT: Hypertrophy, IF: Infiltration, N: Nucleus, NG: Necrotic glomerulus, NT: Necrotic tubule, PCT: Proximal convoluted tubule, PN: Pyknotic nuclei.

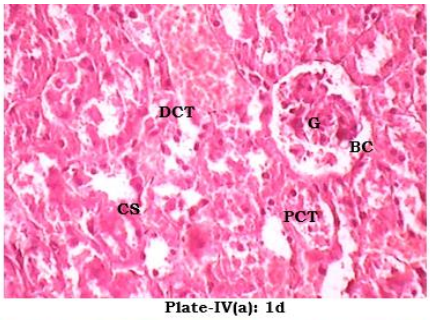



Plate-IV(a): 1d

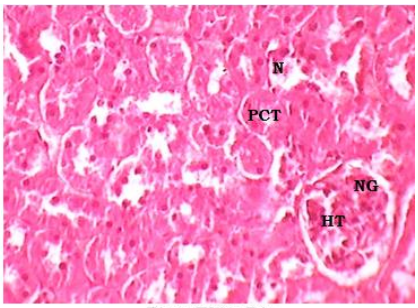



Plate-IV(b): 7ds

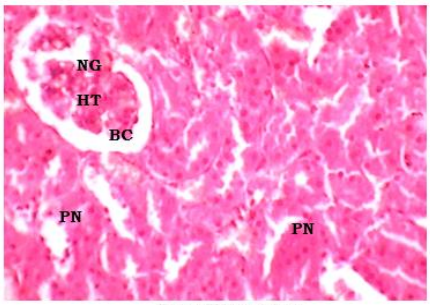



Plate-IV(c): 14ds

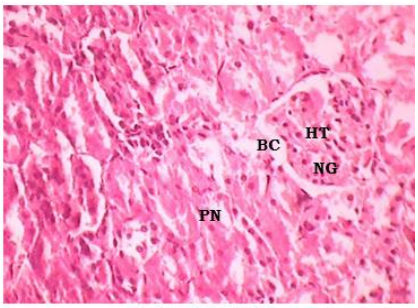



Plate-IV(d): 21ds

Group IV: Plate-IV (a): Section of kidney of *Tagetes erecta* flower extract followed by mercuric chloride treated rats for 1 day, (b): Section of kidney of *Tagetes erecta* flower extract followed by mercuric chloride treated rats for 7 days (c): Section of kidney of *Tagetes erecta* flower extract followed by mercuric chloride treated rats for 14 days, (d): Section of kidney of *Tagetes erecta* flower extract followed by mercuric chloride treated rats for 21 days.
BC: Bowman's capsule, CS: Cell swelling, DCT: Distal convoluted tubule, EG: Enlarged glomerulus, G: Glomerulus, HT: Hypertrophy, IF: Infiltration, N: Nucleus, NG: Necrotic glomerulus, NT: Necrotic tubule, PCT: Proximal convoluted tubule, PN: Pyknotic nuclei.

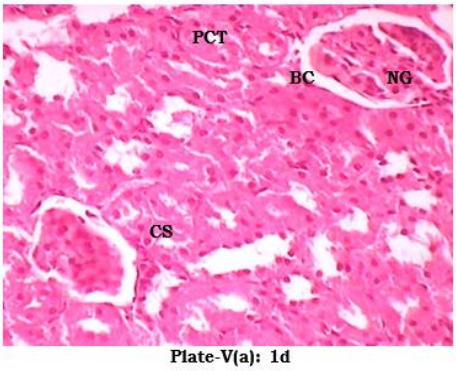



Plate-V(a): 1d

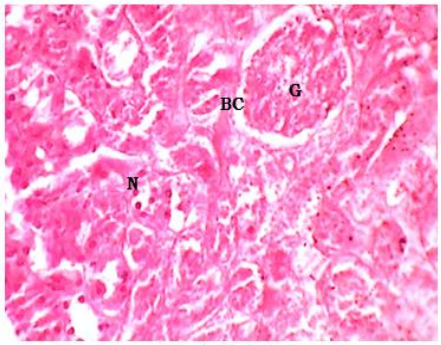



Plate-V(b): 7ds

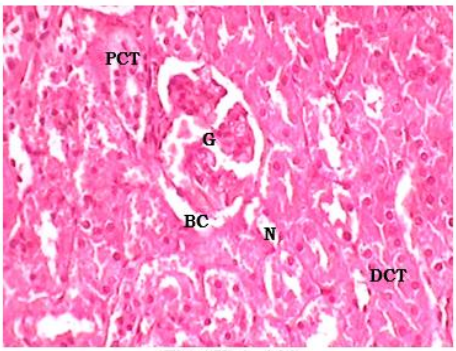



Plate-V(c): 14ds

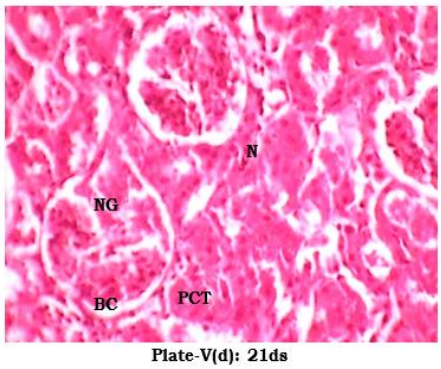



Plate-V(d): 21ds

Group V: Plate-V (a): Section of kidney of mercuric chloride followed by *Tagetes erecta* flower extract treated rats for 1 day, (b): Section of kidney of mercuric chloride followed by *Tagetes erecta* flower extract treated rats for 7 days, (c): Section of kidney of mercuric chloride followed by *Tagetes erecta* flower extract treated rats for 14 days, (d): Section of kidney of mercuric chloride followed by *Tagetes erecta* flower extract treated rats for 21 days.
BC: Bowman's capsule, CS: Cell swelling, DCT: Distal convoluted tubule, EG: Enlarged glomerulus, G: Glomerulus, HT: Hypertrophy, IF: Infiltration, N: Nucleus, NG: Necrotic glomerulus, NT: Necrotic tubule, PCT: Proximal convoluted tubule, PN: Pyknotic nuclei.

capsular space due to swelling of its epithelial lining after 7, 14 and 21 days of treatment (Plate III b-IIId).

Thus, time dependent nephritic changes were observed in the mercuric chloride intoxicated rats. These changes were more prominent on day one (Plate IIIa-IIId).

In *Tagetes erecta* treated animals, kidney showed almost normal histoarchitecture (Plate IIa-IId), whereas, reparative tendencies in kidney in *Tagetes* pre and post treated groups have been observed. On day 1, proximal convoluted tubules and distal convoluted tubules showed slight degeneration at a few places. Upto day 21$^{th}$, the sign of preparation was observed with normal epithelial lining and normal nuclei as compared to mercuric chloride treated group. From day 1 upto day 21th, glomerulus appeared normal without any epithelial damage (plate IVa-Vd).

## DISCUSSION

Kidneys are the primary target organ for accumulation and toxicity of the inorganic mercury. In the present investigation widespread proximal tubular necrosis with absence of brush border and desquamated necrotic epithelial cells in the lumen following mercuric chloride exposure has been observed. Proximal tubule has been considered as the most common site of toxicant induced cell injury. This might be due to the selective accumulation of xenobiotic substance into the segment of nephron through co-transport of mercury with an endogenous ligand such as glutathione, cysteine and albumin, or through some plasma membrane mercury ligand complex. Shortening of the brush border, pycnotic nuclei, cytoplasmic debris in the vicinity of brush borders as a result of the ruptured apical membrane of proximal and distal tubular cells in the mercuric chloride treated rats have been observed. The toxic effects of mercuric chloride may thus be due to the production of oxidative stress on kidneys as well as generation of reactive oxygen species producing a number of toxic reactions[13-14].

Morphological alterations in kidney of rats following mercury intoxication have earlier been reported. These alterations were characterized by blebbing of brush border and sloughing of microvilli with desquamation into the lumens and PCT's necrosis was evident. It is plausible to speculate that the amount of free radicals produced by mercury actions exceeds antioxidant enzyme activities, and thus kidney functions are disrupted and tissue damage develops[15-16].

There has been a significant regeneration of tubular epithelium of kidneys after the administration of *T. erecta* flower extract due to antioxidant potential.

It has been affirmed that the flower extract of *T. erecta* when given in combination reduces the mercury induced nephro-toxicity. Although there have been individual differences, yet the damage to the kidney ranged from moderate to minimal in the albino rats administrated with mercuric chloride followed by *T. erecta* flower extract, while the damage in the kidney was minimal only in the albino rats administrated with T. erecta flower extract followed by mercuric chloride. It has been found earlier that several active components such as β-carotene in *S. fusiformis* scavenge free radicals generated by mercury and renders protection against mercury induced renal damages. Free radicals scavenging activity is normally attributed to phenolic compounds present in the plant. Earlier treatment of mercuric chloride followed by *Panax ginseng* extract and Panax ginseng extract followed by mercuric chloride resulted in significant improvement in kidney functions due to its free radical scavenging property and its antioxidant property[13-14].

Hence on the basis of nephropathological analysis it is evident that *T.erecta* extract followed by mercuric chloride is more potent than mercuric chloride followed by *T. erecta* extract treatment with regard to protection.

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