# PROTECTIVE EFFECT OF TAGETES ERECTA FLOWER EXTRACT AGAINST MERCURIC CHLORIDE INDUCED HEPATIC TOXICITY IN RATTUS NORVEGICUS

**Prabhu N. Saxena and Agam Gupta**

**Toxicology Laboratory, Department of Zoology, Khandari Campus, Dr. B.R. Ambedkar University, Agra-282002, India.**

**ABSTRACT**

Heavy metal poisoning is a serious threat in the world of industrialization as almost all the industries use heavy metals in one or the other way. These heavy metals do harm to vitals such as liver, kidney, lung and blood. Liver is the site of biotransformation of xenobiotic substances. Liver is thus the most affected affected organs of the body. The present study highlights toxic stress of mercury on hepatic of AST and ALT and its modulation through the flower extract of Tagetes erecta, a member of family Asteraceae.

**Keywords :** Heavy metal, mercury, hepatoprotective

## INTRODUCTION

Metals have been poisoning man since the day he first learned to make use of them. Heavy metal toxicity is bearing a world wide problem as they intervene in detoxification pathway (Uthus *et al*., 1990). Virtually all metals can produce toxicity, when ingested in sufficient quantities, but there are several which are specially important because either they are too pervasive or produce toxicity at such low concentration. They are generally, lead, copper, cadmium, arsenic, mercury, nickel and aluminium. The toxicity of metals depend upon a number of factors including its mode of entry into body, its particle size interaction with other metals and also with the constituents of diet (Ernst and Christensen, 1990). Since the industrial revolution of late $18^{th}$ and early $19^{th}$ centuries, anthropogenic sources have become a significant contributor to the environmental distribution of mercury and its derivatives.

Mercury chloride ($HgCl_2$) also known as quick silver, is a naturally toxic element found in air, water and soil. It is widely used in skin-lighting soaps and creams, due to ability of mercury cation to block the production of melanin pigment in the skin. Such uses have resulted in reports of toxicity in number of cases. Mercuric chloride once absorbed into blood stream, combines with proteins in the plasma or enters the red blood cells (Clarkson, 2002). Metallic compound accumulate in liver and destruct the enzymatic chain. Some major enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are normally localized within the cells of liver. These aminotransferases are key enzymes from protein to carbohydrate metabolism. They also help in providing necessary intermediates for gluconeogenesis. Any alteration in their activities may give information of organ dysfunctioning. Medicinal plants play a key role in the human health care. *Tagetes erecta* commonly known as Marigold is a common garden plant, erect, branched and annual growing. The leaves are very strong scented, pinnately with lanceolate-serrate segment. Marigold has been occasionally used in south Asia as medicine. It's leaves and flowers in particular have been used for blood purification and to reduce blood flow. The juice of flower contains antioxidant property and also support the HDL oxidative protection. Keeping in view the pharmacological properties of *Tagetes erecta* (Marigold), this work has been carried out to investigate the effect of *Tagetes erecta* flower extract against mercuric chloride induced hepatic toxicity in albino rats.

***Corresponding author:***
*Email:agamgupta1111@gmail.com*

## MATERIALS AND METHODS

### Experimental animal

The experimental rats (*Rattus norvegicus*) of both the sexes selected from inbred colony were of almost of equal size and weight (110±10). They were maintained at the temperature $27\pm5^0$C, relatively humidity 60±5% and photoperiod of 12 hours per day. The rats were fed on Gold Mohar brand rat feed. The water was provided *ad libitum*.

### Experimental compounds

The experimental compound used in acute and sub-acute studies is inorganic mercuric chloride ($HgCl_2$). *Tagetes erecta* (family Asteraceae) has been selected to reveal its potential as hepatoprotectant. After taxonomical authentication, fresh flowers of the same were collected during early summer. Extraction was done by soxhlet apparatus and its chemical contents were identified by GC-MS. The steam

**Table – 1 Toxicity evaluation of Mercuric chloride for albino rats specifying fiducial limits**

| Experimental individual | Compound | Regression equation | $LD_{50}$ (in mg/ kg b.w.) | Variance | Fiducial limits |
|---|---|---|---|---|---|
| *Rattus norvegicus* | Mercuric Chloride | Y=5.146+3.410 (x-1.009) | 9.26 mg | 0.006 | m1=(±)0.972<br>m2=(-)0.960 |

**Table- 2 AST in albino rats after acute (1 day) and sub-acute (7, 14 and 21 days) treatment of $HgCl_2$ and *Tagetes* flower extract separately and in combination**

| S.No | | Treatments | Dose mg/ kg b.wt | No. of individuals | Treatment time | Ranges (Units/ml) | Mean±S.Em |
|---|---|---|---|---|---|---|---|
| **1** | | CONTROL | - | 3 | 1 | 108-115 | 111±2.082 |
| **2** | ACUTE | $HgCl_2$ | 0.926 | - | | 120-130 | 126±3.055*** |
| | | *Tagetes* extract | 10 | - | | 110-119 | 113±3.003* |
| | | *Tagetes* extract+$HgCl_2$ | | - | | 113-122 | 118.33±2.81** |
| **3** | | CONTROL | - | 3 | 7 | 115-120 | 117.67±1.452 |
| **4** | SUB-ACUTE | $HgCl_2$ | 0.132 | - | | 179-186 | 182±2.081*** |
| | | *Tagetes* extract | 10 | - | | 109-119 | 114.67±3.11*3 |
| | | *Tagetes* extract+$HgCl_2$ | | - | | 155-163 | 158±2.511*** |
| **5** | | CONTROL | - | 3 | 14 | 112-121 | 116±2.649 |
| **6** | SUB-ACUTE | $HgCl_2$ | 0.066 | - | | 175-182 | 179±2.082**** |
| | | *Tagetes* extract | 10 | - | | 110-121 | 115.67±3.643* |
| | | *Tagetes* extract+$HgCl_2$ | | - | | 134-152 | 145±5.574** |
| **7** | | CONTROL | - | 3 | 21 | 112-130 | 119.67±5.371 |
| **8** | SUB-ACUTE | $HgCl_2$ | 0.044 | - | | 162-176 | 170±4.168*** |
| | | *Tagetes* extract | 10 | - | | 110-119 | 114.67±2.691* |
| | | *Tagetes* extract+$HgCl_2$ | | - | | 112-134 | 128±3.464*** |

* (P>0.05) ** (P<0.05) ***(P<0.01) **** (P<0.001)

distillation of fresh leaves offer 0.3% of essential oil with a strong lasting odour and contains d-Limonene, ocimene, l-Linalyi, I-linalyl acetate, I-linaloop tagetone and N-nonyl aldehyde.

**Dose of $HgCl_2$*:*** 0.926 mg/kg body weight for acute (1d) and 0.132, 0.066, 0.044 mg/kg body weight for sub-acute 7, 14 and 21ds respectively.

**Dose of *Tagetes erecta:*** 10 mg/kg body weight for acute (1d) and sub-acute (7, 14 and 21ds).

**Determination of $LD_{50}$**

The albino rats were divided into four groups. Each group consisted of 10 individuals. Mercuric chloride was dissolved in distilled water and was introduced in albino rats per os. The number of dead and survived rats were recorded after 14 days. The data were analyzed statistically by log-dose probit regression line method (Finney, 1971). The regression line was used to determine the expected probit necessary for $LD_{50}$ determination which has been calculated as 9.26 mg/kg body weight (Table-1).

**Experimentation**

To determine the effect of *Tagetes* extract following mercuric chloride ($HgCl_2$) toxicity, the albino rats were grouped into four sets, one acute and three sub-acute having three and nine rats respectively. The controls were run simultaneously.

**Group I$^{st}$:** served as control.

**Group II$^{nd}$:** received mercuric chloride (for both acute and sub-acute treatment).

**Table- 3 ALT in albino rats after acute (1 day) and sub-acute (7, 14 and 21 days) treatment of $HgCl_2$ and *Tagetes* flower extract separately and in combination**

| S.No | | Treatments | Dose mg/kg b.wt | No. of individuals | Treatment time | Ranges (Units/ml) | Mean+S.E |
|---|---|---|---|---|---|---|---|
| 1 | CONTROL | | - | 3 | 1 | 70-79 | 74.67±2.603 |
| 2 | ACUTE | $HgCl_2$ | 0.926 | - | | 80-87 | 83±2.081** |
| | | *Tagetes* extract | 10 | - | | 76-80 | 78±1.154* |
| | | *Tagetes* extract+$HgCl_2$ | | - | | 75-79 | 76.66±1.69** |
| 3 | CONTROL | | - | 3 | 7 | 74-82 | 78±2.451 |
| 4 | SUB-ACUTE | $HgCl_2$ | 0.132 | - | | 115-150 | 131.67±10.138*** |
| | | *Tagetes* extract | 10 | - | | 76-79 | 77.33±1.104* |
| | | *Tagetes* extract+$HgCl_2$ | | - | | 94-100 | 96.67±2.213** |
| 5 | CONTROL | | - | 3 | 14 | 72-83 | 77.67±3.178 |
| 6 | SUB-ACUTE | $HgCl_2$ | 0.066 | - | | 120-132 | 125.67±3.479**** |
| | | *Tagetes* extract | 10 | - | | 74-78 | 76±1.156* |
| | | *Tagetes* extract+$HgCl_2$ | | - | | 90-96 | 93.33±2**** |
| 7 | CONTROL | | - | 3 | 21 | 70-88 | 78±5.26 |
| 8 | SUB-ACUTE | $HgCl_2$ | 0.044 | - | | 115-120 | 117.67±1.452*** |
| | | *Tagetes* extract | 10 | - | | 70-78 | 74.67±2.912* |
| | | *Tagetes* extract+$HgCl_2$ | | - | | 73-92 | 84.33±5.86*** |

* (P>0.05) ** (P<0.05) ***(P<0.01) **** (P<0.001)

**Group III$^{rd}$:** received *Tagetes* extract (for both acute and sub-acute treatment)

**Group IV$^{th}$:** rats received mercuric chloride after *Tagetes* extract administration (for both acute and sub-acute treatment)

**Biochemical estimation**

The liver was excised out and put in physiological saline (ph 7.4) at predetermined time intervals to estimate enzymatic activities of aspartate amino transferase (Reitman and Frankel, 1957) and alanine amino transferase (Reitman and Frankel, 1957). The experimental data were statistically analyzed after Fisher and Yates (1963).

## RESULTS AND DISCUSSION

Mercuric chloride altered the transferase activities (Table-2 and 3. Aspartate amino transferase is localized in the mitochondria and cytoplasm, whereas, alanine aminotransferase is localized in cytoplasm of hepatic cell. These aminotransferases are important links between carbohydrate and protein metabolic pathways. In the present study, both aspartate and alanine which is on increase after mercuric chloride treatment, while no adverse effect with only *Tagetes erecta* administration. In combination set, where mercuric chloride and *Tagetes erecta* were administered, supportive effect of *Tagetes erecta* has been observed which lowered the level of transaminases of mercuric chloride intoxicated rats. Transamination is often involved in the synthesis of amino acids and in the conversion of alpha ketogluterate to glutamate. Alpha ketoglutarate is the usual acceptor of amino groups from most of other amino acids. The glutamic acid serves to channelise amino groups into various biosynthatic pathways as per the cellular demands or into final sequence of reaction by which nitrogen-gas wastes are formed and finally excreted. Any alteration in the level of these enzymes in the serum indicates hepatocellular damage. The increase in AST and ALT in serum may be due to hepatocellular necrosis, which causes increase in the permeability of the cell membrane resulting in the release of transaminases in the blood stream (Vandenberghe, 1995; Rana *et al*. 1996; Sharma *et al*. 2002). The increase in the aspartate and alanine aminotransferase might be due to the leakage of soluble tissue enzyme into the blood as a result of necrosis of the liver tissue (Yarbrough *et al*., 1982). Cadmium and lead also induce hepatocellular damage as evidenced by increase in

the level of aspartate and alanine aminotransferase (Kowalczyk *et al*., 2002). Perhaps necrosis in the present investigation, could be a reason for altering levels of the concerned hepatic enzymes and is in accordance to Jamall *et al*. 1985 and Reus *et al*. 2005 who also revealed similar changes after oxidative stress induced by mercuric chloride, cadmium and lead intoxication. Further, increase in aspartate and alanine amino transferase could also be due to altered membrane permeability (Padma *et al*., 2001). Release of a variety of enzymes normally located in the cytosol following plasma membrane drain into the blood stream and which leads to increase in the activity of aspartate and aminotransferase (Kumar *et al*., 2005).

In the combination group where animals were pretreated with *Tagetes* flower extract, decline in AST and ALT activities after treatments have been observed. This decline has been compared with the animals treated with mercuric chloride and is indicative of reduced rate of liver damage. The efficacy of *Tagetes erecta* flower extract may be due to several active components such as carotenoids (lutin, zeaxanthin). The active components found in *Tagetes erecta* provoke the activity of free radical scavenging enzyme systems which probably render protection against mercury induced liver damage. Metallo-protective role of herbal extracts has earlier been highlighted in the presence of β-carotene (Prescott, 1978, Seshadri *et al*., 1991), vit C, E, enzyme superoxide dismutase (Benamotz, 1987, Henrikson, 1989) and selenium in enhancing the activity of free radical scavenging system, diminution in lipid peroxidation and reduction in mercury toxicity which in turn leads to significant decrease in the activity of AST and ALT. Similarly, extract of *Curculigo orehioides* against carbon tetrachloride produced hepatocellular damage, prevents defame of liver cell plasma membrane, neutralizing the toxic effect and help in regeneration of hepatocyte and maintain the level of AST and ALT in liver (Kumar *et al*., 2005). Reduced level of AST and ALT by *Hibiscus sabdariffa* and *Origanum meyorana* extract against ammonium chloride and lead acetate induced toxicity were observed due to their free radical scavenging property and the presence of natural antioxidants which influence the levels of lipid peroxidation products and liver marker enzymes (Ibrahim *et al*., 2005; Mustafa *et al*., 2005). Saxena *et al*. (2008) also revealed modulatory effect of flower extract of *Tagetes erecta* on renal functions of $HgCl_2$ intoxicated albino rats.

It is therefore concluded that *Tagetes* extract possesses antioxidant as well as hepatoprotective action is reflected by the values of AST and ALT almost equal to control group when administered alone. In combination it protects the liver cells through its unique activity against the toxicity of mercuric chloride. The results clearly indicate that supplementation of *Tagetes erecta* flower extract show marked ability to protect liver cells against mercuric chloride induced toxicity.

## REFERENCES


1. Bagenstose, L.M., P. Salgame and M. Monesteir. 1999. Cytokine regulation of a rodent model of mercuric chloride induced autoimmunity. Environ. Health Perspect., 5: 807-810.
2. Ben-Amotz. 1997. Presentation of polysaccharides from MicroAge workshop, Duke University.
3. Henrikson, R. 1989. Earth food Spirulina, cited from Recolina Ltd. Ronor enterprises Inc., Launa Beach, California, pp 27-65.
4. Ibrahim, M., El-Ashmawy, Abeer F. El-Nahas and Osama M. and Salama. 2005. Protective effect of volatile oil, alcoholic and aqueous extracts of *Origanum majorana* on lead acetate toxicity in mice. Basic and Clinical Pharmacology and Toxicology, 97: 238.
5. Kowalczyk, E., A. Kopff, P. Fijalkowski, M. Kopff, J. Niedworok, J. Blaszczyk and P. Tyslerowicz. 2003. Effect of anthocyanins on selected biochemical parameters in rats exposed to cadmium. Acta Biochemica Polonica. 50(2): 543-548.
6. Kowalczyk, E., Jankiwaski A., Niedwork J., Snigielsk J., Tyslerowicz P. 2002. Effect of long term cadmium intoxication on selected biochemical parameters in experimental animals. Pol. J. Environ. Stud., 11:599-601.
7. Mercado, R. and Blumberg. 2004. Leutin and Zeaxanthin and their potential roles in disease prevention. J. An. Coll. Nutr., 23 : 567-587.
8. Mustafa Mohammad Essa, P. Subramanian, G. Sulthakar. 2005. Influence of *Hiscuss sabdarijja* (Gongura) on the levels of circulatory lipid peroxidation products and liver marker enzymes in experimental hyperammnomemia. J. Appl. Biomed., 4: 30.
9. Padmaja S. and T. Nagasaju. 2001. A study of selenium toxicity on aspartate alanine aminotransferase in wistar rats. Poll. Res., 20(4): 571-573.

10. Piotrowski, J.K., J.M. Trojanowska, J.M. Wisniewskaknypl and W. Bolanowska. 1974. Mercury binding in the kidney and liver of rats repeatedly exposed to mercuric chloride: induction of metallothionine by mercury and cadmium. Toxicol. Appl. Pharmacol., 27: 11-19.

11. Prescott, G.W. 1978. How do know the fresh water algae? 3rd ed. Wn. C. Brown Company Publishers, Iowa, U.S.A., pp 182.

12. Rana, S.V.S., S. Rekha and V. Seama. 1996. Protective effects of few antioxidants in liver function in rats treated with cadmium and mercury. Ind. J. Exp. Biol., 34 : 177-179.

13. Reus, I.S., I. bando, D. Anres and M. Cascales. 2003. Relationship between expression of HSP-70 and metallothionine and oxidative stress during mercury chloride induced acute liver injury in rats. J. Biochem. Mol. Toxicol., 17(3): 161.

14. Seshadri, C.V., Umesh B.V. and Mandran R. 1991. β-carotene studies in spirulina. Biores. Tech., 38: 111-113.

15. Sharma, M.K., Kumar M., Kumar A. 2002. *Ocimum sanctum* aqueous leaf extract provides protection against mercury induced toxicity in swiss albino mice. Indian J. Exp. Biol., 40(9): 1079-82.

16. Uthus, E.O., Nielson, F.H. Magnes. 1990. Trace Elem., 9(4): 219-226.

17. Walsh, C.T. 1982. The influence of age on the gastrointestinal absorption of mercuric chloride and methyl mercury chloride in the rat. Environ. Res., 27: 412-420.

18. Yarbrough, J.D., Chambers and R.J. Randall. 1982. Alterations in liver structure and functions resulting from chronic insecticide exposure. Animal system. Raven Press. New York., 1 (5) : 25-59.