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ORIGINAL ARTICLE

Effect of Mercuric Chloride on Hepatic Enzymes and Their Modulation by Flower Extract of *Tageteserecta* flower Extract in Albino Rats (*Rattus norvegicus*)

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ABSTRACT

Mercuric chloride intoxication may initiate many disorders in humans and animals. This study investigated the role of Tageteserecta in protecting rats against mercuric chloride exposures. The results showed that the administration of Tageteserecta efficiently protected albino rats against the mercuric chloride caused injury to the hepatic enzymes sach as serum alkaline phosphatase and serum alanine phosphatase activities. Thus, this suggests the possibility of Tageteserecta flower extract usefulness in limiting toxicant induced by environmental heavy metals.

Key words: *Tageteserecta , mercuric chloride , oxidative stress, liver, ACP, ALP*

INTRODUCTION

Human exposure to heavy metals has risen dramatically in the last fifty years as a result of an exponential increase in the use of heavy metals in industrial process and products. Also environmental pollution is the contamination of the ecosystem that causes instability, disorder, and harm on discomfort to the physical systems or living organisms. Environmental factors have important links with infectious as well as non-infectious disease of both acute and chronic nature (Ernst and Christensen, 1990). Mercuric chloride is an organic compound multi-targeted toxicant that causes alterations in different organs of the body, including the liver s. The absorbed mercuric chloride is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in various body organs and interferes with their functions, specially the liver as a target site for mercuric chloride toxicity (Uthus*et al*., 1990). Mercuric chloride once absorbed in blood stream combines with proteins in the plasma or enters the red blood cells.Acid phosphatase and alkaline phosphatase are known as inducible enzyme and their activities in animal tissues are accelerated when there is a toxic effect and enzyme being to counteract any alteration to their activity may give information of organ dysfunctioning.

Herbal medicine was practiced by ancient culturein many country including Asia. *Tageteserecta* (Asteraceae) also called marigold. This plant reaches heights of between 50 and 100 cm. Different parts of this plant including flower are used in folk medicine to cure various diseases like fever, epileptic fits (Ayurveda), astringent, carminative, stomachic, scabies and liver complaints and also employed in diseases of the eyes. The leaves are good for piles, kidney troubles and muscular pain. Their juice is used for earache and ophthalmia. The flowers are employed in disease of the eyes and for unhealthy ulcers, internally they are said to purify the blood; their juice is given as remedy for bleeding piles (Ben-Amotz. 1997). The study aimed to evaluate the influence of mercuric chloride toxicity in hepatic enzymes of albino rats and to estimate the productive role of the extract of Tageteserecta flowers against this induced toxicity.

MATERIALS AND METHODS

Experimental Animal:

The experimental rats (*Rattusnorvegicus*) 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+50C,

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 ($HgCl_2$). *Tageteserecta* (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 steamdistillation of fresh leaves offer 0.3% of essential oil with a strong lasting odour and contains dLimonene, ocimene, l-Linalyi, I-linalyl acetate, Ilinalooptagetone and N-nonyl aldehyde.

Dose of Mercuric Chloride:
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 *Tageteserecta*:
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 was 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 (HgCl2) 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: served as control.
Group II: received mercuric chloride (for both acute and sub-acute treatment).
Group III: received Tagetes extract (for both acute and sub-acute treatment)
Group IV: rats received mercuric chloride after Tagetes extract administration (for both acute and sub-acute treatment)

Biochemical Estimation:
The rats were directly anaesthesized with ether. The blood was collected through ventricle with the help of hypodermic syringe.the blood in centrifuge tube was allowed to stand vertically at an angle to clot for about three minutes. It was then centrifuged at 3000 rpmfor fifteen minutes. Thesupernatant; the serum was separated in the test tube.The serum samples were used for the estimation of biochemical parameters.
Serum ACP was estimated by the method of (King andJagatheeson1959), and serum ALP was estimated by the method of (King and king 1954). The experimental data were statistically analyzed after Fisher and Yates (1963).

RESULTS AND DISCUSSION

Mercuric chloride showed a dose response relationship pattern in the experimental animal. This dose response relationship has been marked in the form of elevation in phosphatases activities. Acid phosphates (ACP) were showed non- significantly increased after acute (1d) and significantly increased after sub-acute (7and14d) and non-significantly increased after (21 d) treatment of mercuric chloride in albino rat due to a toxic effect on hepatic tissue.Alkaline phosphatase (ALP) were showed non-significantly increased after acute (1d) and significantly increased after sub-acute (7 d) and non-significantly increased after (14 d) and significantly increased after (21 d)

treatment of mercuric chloride. While the result of a group which dosed by *Tageteserecta* before mercuric chlorideshowed positive control in both ACP and ALP activities.

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	m_1 = (±)0.972 m_2 = (-)0.960

Table 2: Serum acid phosphatase (KAU) in albino rats after acute (1d) and sub-acute (7, 14 and 21ds) treatment with mercuric chloride, *Tageteserecta* and mercuric chloride + *Tageteserecta*

Treatment Day(s)	No. of rats	Control	Mercuric Chloride	T. erecta	Mercuric Chloride + T. erecta	F – value Significance
		Mean ± S. Em.	Mean ± S. Em.	Mean ± S. Em.	Mean ± S. Em.	
Acute (1d)	5	21.41 ± 1.81	32.11 ± 1.00*	20.80 ± 0.88	19.93 ± 0.89*	21.41 ± 1.81
Sub – acute (7ds)	5	23.31 ± 1.11	43.50 ± 1.30**	20.88 ± 1.25	18.97 ± 0.89*	23.31 ± 1.11
Sub – acute (14 ds)	5	23.40 ± 1.85	52.51 ± 1.38**	22.21 ± 0.92	19.11 ± 1.00*	23.40 ± 1.85
Sub – acute (21ds)	5	22.35 ± 1.01	38.62 ± 1.97*	26.32 ± 1.30	21.97 ± 0.89*	22.35 ± 1.01

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

Table 3: Serum alkaline phosphatase (KAU) in albino rats after acute (1d) and sub-acute (7, 14 and 21ds) treatment with mercuric chloride, *Tageteserecta* and mercuric chloride + *Tageteserecta*

Treatment Day(s)	No. of rats	Control	Mercuric Chloride	T. erecta	Mercuric Chloride + T. erecta	F – value Significance
		Mean ± S. Em.	Mean ± S. Em.	Mean ± S. Em.	Mean ± S. Em.	
Acute (1d)	5	16.31 ± 0.38	20.16 ± 0.61*	14.61 ± 0.35	14.95 ± 0.51*	16.31 ± 0.38
Sub – acute (7ds)	5	15.85 ± 0.38	29.16 ± 0.66**	14.36 ± 0.52	15.21 ± 0.34*	15.85 ± 0.38
Sub – acute (14 ds)	5	16.22 ± 0.30	38.02 ± 0.75**	17.76 ± 0.11	15.83 ± 0.35*	16.22 ± 0.30
Sub – acute (21ds)	5	17.08 ± 0.25	33.33 ± 0.09*	15.61 ± 0.35	15.35 ± 0.52*	17.08 ± 0.25

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

Acid phosphatase and alkaline phosphataseare complex enzyme, which performs multiple cellular and metabolic functions such as growth differentiation, protein synthesis of certain enzymes and transport of phosphorylated intermediates across cell membranes and bone mineralization. Phosphatases are present in most tissues; the richest sources being osteoblasts in the bone, the bile canaliculi in the liver, the small intestinal epithelium etc. in all these sites it seems to be involved in the transport of phosphatase across cell membranes.

The elevation in activity of acid phosphatases in the liver of mercuric chloride treated rats suggested the increase in the secretion of hydrolytic enzymes from lysosomes. The lysosomal system has been shown to be very sensitive to changes in the intra and extracellular environment and sub-sequently many physiological and pathological processes. At the cellular level lysosomes are important in the up-take, sequestration and bioaccumulation of various heavy metals.

In the present investigation elevation in alkaline phosphatase activity because the toxic effect of mercuric chloride enhances with the time duration of intoxication and it may be due to the presence of low molecular weight protein like metallothionein in the liver, because Hg++ readily reacts and forms complexes with organic ligands notably sulfhydryl groups. Metallothionein is an

important intracellular sequestration site for toxic elements such as mercury, particularly in the liver. Continuous intoxication of mercuric chloride elevates, the concentration of metallothionein in the liver and for release of this effect, the alkaline phosphatase, a hydrolytic enzyme gets increased.
The increased enzyme activity of acid and alkaline phosphatase in liver of mercuric chloride treated rats could be due to damage to the cell membrane of tissues, where this enzyme is firmly attached to the cell membrane joining the binary canalicules and sinusoidal border of parenchyma cells (Gomez, *et al*., 1997; Subramaniam, *et al*., 1995; Ochmanski, *et al*., 2000; Rajkumar, *et al*., 2002; Nair, 2006 and Saxena and Mahour, 2006). Present findings gain support by Mehra and Kanwar (1986) and Dortmen, *et al*., (1978) who observed activity of liver alkaline phosphatase in rats after cadmium chloride intoxication. Again, increase in liver alkaline phosphatase is in affirmation to Mehta, *et al*., (2002) following chromium VI and beryllium toxicity in albino rats respectively. Bhawara, *et al*., (1997), Koch, *et al* (2004), Rao, *et al*., (1995).
Hepatic hydrolytic enzymes are towards increase after acute and sub-acute studies reflecting hyper lysosomal activity. Further, destruction of the cell membrane of lysosomes under the stress of mercuric chloride could be considered as a possible reason for elevation in hepatic hydrolytic enzymes. Thus, evaluating acid and alkaline phosphatase activity useful information on the mode of action of mercuric chloride is ascertained which highlights its toxicity in albino rat.
Though the oxidative stress can be minimize by endogenous antioxidants present every time within the hepatocyte,however their activity is further increased if exogenous antioxidant derivative are which in the present investigation are none other then the lutin and zeaxenthin present in the flower extract of *Tageteserecta*.
Tagetes extract possesses antioxidant as well as hepatoprotective action is reflected by the values of ACP and ALP almost same to control group when administered alone. In combination it protects the liver cells through its unique activity against the toxicity of mercuric chloride. This significant decrease in elevated level of ACP and ALP due to free radical scavenging property and lipid peroxidation property(Wang *et al*.,2006) The results clearly indicate that supplementation of *Tageteserecta* flower extract show marked ability to protect liver cells against mercuric chloride induced toxicity, Kumar, *et al* (2005), Sharma, *et. al.* (2002).

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