International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.4, pp 1377-1382, Oct-Dec 2012

Efficacy of Tagetes erecta on lead acetate induced oxidative injury in rat kidney

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Abstract : To evaluate the effect of ethanolic extract of *Tagetes erecta* on the tissue antioxidant status in lead acetate-induced hepatotoxicity in male albino rats.Rats were divided into four groups. Groups 1 and 3 received distlled water.Groups 2 and 4 received 160mg/kg bodyweight every day. Groups 3 and 4 received ethanolic extract of *Tagetes erecta* (100 mg/kg bodyweight) dissolved in distilled water and given orally.The results showed significantly elevated levels of tissue thiobarbituric acid reactive substances and lipid hydroperoxides, and significantly lowered enzymic and non-enzymic antioxidant activity of superoxide dismutase, catalase and glutathione-related enzymes such as glutathione peroxidase, reduced glutathione, vitamin C and vitamin E in lead acetate-treated rats compared with the control. Ethanolic extract of *Tagetes erecta* administration to rats with lead acetate-induced liver injury significantly decreased the levels of thiobarbituric acid reactive substances and lipid hydroperoxides and significantly elevated the activity of superoxide dismutase, catalase, glutathione peroxidase, and the levels of reduced glutathione, vitamin C and vitamin E in the tissues compared with those of the unsupplemented lead acetate-treated rats. The histological changes observed in the liver and kidney correlated with the biochemical findings. Ethanolic extract of *Tagetes erecta* offers protection against free radical-mediated oxidative stress in rats with lead acetate-induced liver injury.
Keywords : Antioxidant, lead acetate, liver, *Tagetes erecta.*

Introduction

Heavy metals are persistent environmental contaminants since they cannot be degraded or destroyed. Heavy metals are chemical elements capable of spreading in the environmental compartments and circulating between them. Indeed, heavy metals emitted to the atmosphere with the composition of fine particles or in the gaseous from are transported by atmospheric fluxes to considerable distances and enter ecosystems of remote region. Many heavy metals are urgently necessary for functioning of the body of humans and living organisms in small amounts and belong to the range of nutrients other, when passed on to the living organisms cause poisoning or death[1.]

Lead is a natural compound that exists in elements resistant to corrosion but oxidized and blacker when it comes into contact with air. Lead is being ubiquitous environmental contaminants to its significant role in modern industry[2]. However both occupational and environmental exposures remain a serious problems in many developing and industrializing countries[3].Lead has many undesired effect, including neurological[4], behavioral[5], immunological[6], renal[7], hepatic[8] and especially haematological dysfunctions[9] .One of the prime targets to lead toxicity is the heme synthesis pathway. Lead affects this system by, inhibiting the heme and hemoglobin synthesis and changing the RBC morphology and survival[10].Lead- induced oxidative stress is on the antioxidant defense systems of cells. Several studies have shown that lead alters the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and antioxidant molecules like GSH in animals[11]and human beings[12]. These findings suggest a possible involvement of oxidative stress in the pathophysiology of lead toxicity.

Tagetes erecta L. (Asteraceae) the Mexican marigold, also called Aztec marigold, is a species of the genus *Tagetes* native to Mexico and Central America. Despite its being native to the Americas, it is often called African marigold. This plant reaches heights of between 50 and 100 cm[13]. 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[14].The leaves are good for piles, kidney troubles and muscular pain. Their juice is used for earache and ophthalmia. The flower is bitter astringent,carminative, stomachic, good for the teeth and the gums; 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[15].The study aimed to evaluate the influence of lead acetate toxicity in renal tissue of albino rats and to estimate the productive role of the ethanol extract of *Tagetes erecta* flowers against this induced toxicity.

Materials and methods

Lead acetate was used as the hepatotoxicity inducer in animals and was procured from pharmacy at Thanjavur. All other chemicals used for the experimental were of analytical grade.The fresh flowers of *Tagetes erecta* was collected from the local gardens of STET Women's college,Mannargudi, Tamilnadu,India and vocchur specimens are deposited in the STET Herbarium at the Department of Botany and Microbiology, S.T.E.T. Women's College, Mannargudi, Tamilnadu, India.Measured amount of air dried powdered plant material of *Tagetes erecta* flowers were separately soxhlet extracted with 50 ml of 70 % ethanol. The ethanol mixture were evaporated at 55^{o}C by using heating mantle, the collected extract were subjected for nephrotoxicity studies. Male albino rats (130gm-150gm) were purchased from animalhouse, Thanjavur. The rats were housed in polypropylene cages and kept under standard laboratory conditions (temperature 25±2^{0}C; natural light-dark cycle).The animals were kept individually for feeding in pellet diet (Sai Durga feeds and foods, Chennai, India) with water adlibitum. The rat feed contained 20-21% crude protein, 4-5% ether extract, 4% crude fiber, 8% ash, 1-2% Calcium and 0-6% phosphorous.In this experiment, a total of 24 rats were used. The rats were randomly divided into 4 groups of 6 rats in each group.

Group- I : Rats were kept as control

Group-II : Rats were given lead acetate 160mg/kg body weight orally for 21 days.

Group -III : Rats were given ethanol extract of *tagetes erecta* flowers 100mg/kg body weight orally for 21days.

Group –IV: Rats were given *tagetes erecta* (100mg/kg body weight) along with lead acetate orally for 21days.

At the end of the experimental period, animals in different groups were sacrificed by direct cardiac puncture under diethyl ether

anaesthesia. The kidney tissues were dissected out, weighed and washed using ice cold saline solution. Tissues were minced and homogenized (50% w/v) in sodium phosphate buffer (0.05 M; pH 7.0) and centrifuged at 700 × g for 10 min at 4^0C. The resulting supernatant was used for various biochemical assays. Level of SOD was assayed by the method of Kakkar *et al*.,[16] and the activity of CAT by the method of Sinha[17] and the activity of GPx by the method of Rotruck *et al* .,[18], GSH by the method of Ellman[19] .Vitamin C was measured according to the method of Roje *et al*.,[20] , and Vitamin E in tissues by the method of Zaspel and Csallany[21] .Data were analyzed by the One way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using the statistics software package SPSS for Windows,V.13.0;Chicago,IL,USA.

Results

The levels of enzymatic and nonenzymatic antioxidant systems in the liver tissue of rats treated with lead acetate were significantly ($p<0.05$) lesser than control rats (Table1 and Figure 1). Administration of *Tagetes erecta* to lead treated rats significantly ($p<0.05$) increased the level of enzymic and nonenzymic antioxidants in tissues. In contrast, lead with *Tagetes erecta* treated rats showed a significant ($p<0.05$) increase in the activities of these antioxidants.The result of the present study indicated that the levels antioxidant enzymes got increased when the animals were admininistrated with *T.erecta*, and *T.erecta* along with lead acetate with significant elevations in the activities of SOD, CAT, GPx, GSH, Vitamin –E and Vitamin-C in the liver tissue when compared to those of the kidney tissue when compared to those of the unsupplemented lead acetate treated rats.

Discussion

Antioxidant defense system includes enzymes such as superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GPx) and also non-enzymatic agents with endogenous and exogenous sources such as Reduced glutathione (GSH), Vitamin-E, Vitamin C [22,23] . In recent years, antioxidants have been subjected to many epidemiological studies that have related their consumption to a reduction in the incidence of oxidative damage related diseases. Therefore, much attention has been focused on the use of antioxidants-specially natural antioxidants-for improvement of human health[24].

Table 1: Changes in the activities of enzymatic and nonenzymatic antioxidant rats.

Groups	Group I	Group II	Group III	Group IV
SOD	7.74±1.21^{c}	6.80±0.91^{a}	7.75±0.45^{c}	7.63±1.27^{b}
CAT	76.26±1.54^{c}	54.88±0.80^{a}	72.99±0.80^{c}	66.41±1.03^{b}
GPx	9.48±1.37^{c}	6.64±1.28^{b}	8.52±1.33^{c}	7.55±1.44^{a}
GSH	100.38±1.15^{c}	80.75±4.26^{a}	99.56±1.25^{c}	86.29±2.49^{b}
Vitamin E	128.75±4.34^{c}	90.5±2.88^{a}	115.25±4.75^{c}	109.75±4.11^{b}
Vitamin C	60.75±3.59^{c}	31.25±4.78^{a}	59±3.91^{c}	49±0.06^{b}

Values are mean ± SD for 6 rats in each groups. $^{a-c}$ In each rows means with different superscript letter different significantly at P< 0.05(DMRT).

SOD - Enzyme concentration required to inhibit the chromogen produced by 50 % in one minute understandard condition.

CAT - µmole of H_2O_2 consumed / minute.

GPx - µg of GSH utilized / minute.

GSH - mg/g tissue

Vitamin- C&E - mg/dl

SOD is thought to be one of the major enzymes, which protects against tissue damage caused by the potentially cytotoxic reactivities of radicals. Superoxide anion is known to inactivate enzymes and initate the damaging chain reactions of lipid peroxidation. Cellular defense mechanisms against superoxides include a series of linked enzyme reactions, which remove the toxic radicals and repair radical induced damage. Superoxide dismutase converts superoxide anion to hydrogen peroxide. Hydrogen peroxide, also toxic to cells, is removed by catalase.Catalases are most efficient enzyme in the cells compartment. It protects our red blood cells. The enzyme is composed of four identical sub units and uses a heme/iron group to perform the reaction. Catalase is an unusually stable enzyme, it fights against reactive molecules. It catalyses the decomposition of hydrogen peroxide to oxygen and water. Each catalase molecule can decompose millions of hydrogen peroxide molecules every second. Catalase performs its rapid destruction of hydrogen peroxide in two steps. First, a molecule of hydrogen peroxide binds and is broken apart. One oxygen atom is extracted and attached to the iron atom, and the rest is released as harmless water. Then, a second hydrogen peroxide molecule binds. It is also broken apart and the pieces are combined with the iron bound oxygen atom, releasing water and oxygen gas[25].

Glutathione peroxide (GPx) has a well established role in protecting cells against oxidative injury. GPx is a selenium containing metalloenzyme, partially located within cellular membranes, which can remove hydrogen peroxide by converting, reduced glutathione into oxidized glutathione. GPx can also terminate the chain reaction of lipid peroxidation by removing lipid hydroperoxides and H_2O_2 from the cell membrane[26] .

Glutathione belongs to antioxidant defence systems and prevents harmful effects of free radicals by scavenging hydroxyl radicals and singlet oxygen.GSH is a tripeptide consisting of glutamate, cysteine and glycine. It acts as an antioxidant both intracellularly and extracellularly in conjunction with various enzymatic processes that reduce hydrogen peroxide and hydroperoxides as GSH is oxidized to GSSG and other mixed disulfides. GSH is produced in the liver and maintained at a higher concentration in most tissues[27] . GSH is a critical determinant of tissue susceptibility to oxidative damage. The depletion of GSH levels has been shown to be associated with enhanced lead toxicity[28].The present study showed a significant reduction in GSH levels in liver of lead treated rats. This may be due to the increased free radical generation, which may react with GSH resulting in its decreased levels.

Vitamin E is the major lipid soluble antioxidant found in cells.The main function of Vitamin E prevents the peroxidation of membrane phospholipids, and avoids cell membrane damage through its antioxidant action. The lipophilic character of tocopherol enables it to locate in the interior of the cell membrane bilayers[29].

Vitamin C also contributes to the regeneration of membrane bound oxidized vitamin E. It will react with the α-tocopheroxyl radical, resulting in the generation of tocopherol in this process itself being oxidized to dehydroascorbic acid Vitamin C supplementation in animals leads to increased plasma and tissue levels of vitamin E[30]. In our study we observed decreased level of total antioxidant level of superoxide dismutase, catalase,Glutathione peroxidase,Reduced glutathione, vitamin C and vitamin E in lead treated group and administration of *T.erecta* improved the total antioxidant status.

Conclusion

The present study indicated exposure to lead acetate could generate free radicals which resulted in the decreased level of antioxidant levels. The protective effects of *T.erecta* extract may be due to radical scavenging activity of its components. Moreover, the protective role was more pronounced when the extract administrated after lead acetate intoxication.Concequently, *T.erecta* extract is quite useful and reasonable in the treatment of lead toxicity.

Acknowledgements

The authors are grateful to the management of STET Women's college, Mannargudi for their encouragement and support and also thankful to Dr.P.Krishnamoorthy, Assistant Professor, Dept.of Zoology for providing laboratory facilities.

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