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Green Tea Protective Effect against lead acetate Inducing Reprotoxic Effects in Male Rats.

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ABSTRACT

The purpose of this study was to investigate the effect of GTE on testes damage caused by Pb in male rats. Four groups of male rats were utilized as follows: Control samples, GTE treated, Pb treated and Pb + GTE, treated rats at the same doses. The rats received GTE and/or Pb orally in drinking water. After 4 weeks, the animals were sacrificed and testes were removed for microscopic and biochemical evaluation. The levels of Lipid Peroxides (LPO) and Catalase were detected in the tissue homogenates of rat testes. Marked morphological changes in the form of swelling, congestion, hemorrhage and necrosis were seen in testes of rats treated with Pb alone. Rats treated with Pb+GTE showed milder edema, congestion and minute foci of necrosis in the testes. LPO levels in Pb-treated were significantly higher as compared to control samples. The levels of catalase were significantly lower in Pb-treated rats but, when GTE was co-administrated with Pb, there was an effective reduction in oxidative stress In this study it was found that - animals treated with GTE + Pb appear to have an enhanced antioxidant/ detoxification system that reduced the oxidative stress in rat testes. The beneficial effect of GTE thus may have potential in reducing Pb toxicity in testes.

Keywords: Rat Testes; lead toxicity; Green tea extract; Lipid peroxides; Catalase.

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INTRODUCTION

Lead (Pb) one of the oldest and commonest environmental pollutants, is reported to cause damage in multiple body systems [1]. In developing countries, people are often exposed to lead pollution through air, water and soil [2]. It was shown that Lead toxicity affects the brain and other organ systems, notably male reproduction and fertility both in clinical and animal studies [3]. It may have direct testicular toxicity, indirect effects through targeting the endocrine control of reproduction, or both [4]. Studies in male rats have shown that lead intoxication disrupts testicular steroidogenesis by inhibiting the activities of testicular steroidogenic enzymes [4].

Traditional herbal medicine has more acceptance than prescription drugs in many cultures largely because of a patient perception of greater safety. Green tea (Camellia sinensis) is one such widely used plant-based medicine. Both green and black tea contains flavenoids such as quercetin, kaempferol and myricetin, which have potent antitoxic and anti-carcinogenic effects [5, 6]. Much research shows that green tea contains antioxidants that remove free radicals and thereby reduce the risk of heart disease, stroke, thrombosis and elevated blood sugar levels. Green tea has been found to aid in heavy metal detoxification by inhibiting its absorption and promoting excretion. . Green tea polyphenols, such as catechin, bind with Pb ions to form an insoluble complex –ionic salt that has been used to remove Pb. Catechin normalizes testicular metabolic disorders in Pb – poisoned rats [7]. The antioxidant action of phenol rich tea extracts reduced the ability of humans to utilize dietary iron. Epigallocatechin 3-gallet (EGCG) inhibits type I 5α reductase activity in vitro, which is partially responsible for conversion of testosterone to dihydrotestosterone [8].

The aim of the present study was to throw a light the protective effect of green tea extract as a model of powerful antioxidant against the toxic effects of lead acetate on the testes of male albino rats.

MATERIALS AND METHODS

Green Tea Extracts (GTE) preparation

Green tea used in this study was of the type (TCHICO TEA) which was produced in China and purchased locally. The dispensed green tea extract was prepared according to Khan [9]. By adding 30g of green tea – to 500 ml of boiling water, steeped for 15–20 min. The infusion was cooled to room temperature and then filtered. The tea leaves were extracted a second time with 500ml of boiling water and filtered, and the two filtrates were combined to obtain a concentration of 6.6% green tea extract (6,6 g tea leaves/100 ml water). The resulting clear solution is similar to tea brews consumed by humans. The prepared dietary treatment is equivalent to a daily consumption of 3 cups of green tea by an adult weighing 70 kg. The male rats were given the freshly prepared tea every morning via a feeding bottle.

Animals and treatments

Male Wistar rats (*Rattus norvegicus*) weighing approximately 200 ± 5 g were provided with animal feed and water ad-libitum and maintained in a 12 h light/dark cycles and an environmental temperature of 24 ± 4°C. All procedures performed on animals were approved and conducted in accordance with the National Institute of Health Guide (Reg. No. 488/160/1999/CPCSEA). The experiment was conducted by dividing the male rats into 4 groups of 10 rats per group and caged separately. Each group was then treated as follows:

Group 1 (control): this group of animals was given standard diet and tap water.
Group 2: (Pb): this group of male rats was intoxicated by treating it with lead acetate Pb $(C_2H_3O_2)_2$ in doses of 3% (Pb weight to distillate water volume) for a duration of 30 days.
Group 3: (Pb+GTE): this group of male rats was given lead acetate Pb $(C_2H_3O_2)_2$ and 6.6% green tea extract instead of drinking water for a same period of 30 days.
Group 4: (GTE): this group was treated only with the prepared 6.6% green tea extract.

On completing the treatment, the animals were lightly anesthesitized with (Chloral 10% at 3ml/kg) and terminated by cervical dislocation. The testes were bilaterally/ excised immediately and divided into two parts, one for biochemical investigation and the other for histological examination.

Body and organ weight

The body weight of all animals was recorded one day prior to commencement of the treatment with GTE (initial weight) and on the day of sacrifice (final weight). The testes were dissected out, trimmed from the attached tissues and weighed. The relative weight of the organs was expressed as a ratio of the animal's body weight.

Sperm count

The sperm count was measured according to the method described by Majumdar and Biswas [10].In which a semen sample of 0.1 ml was placed in 0.9 ml of normal saline for sperm cells to swim out in a Petri-dish equipped with a counting chamber (haemocytometer). After the sperm cells have settled on the grid, the number spermatozoa in five haemocytomete squares was determined microscopically and multiplied by 10^6 to determine the number of sperm per ml.

Biochemical analysis

The removed testicular tissue was homogenized in ice-cold 100mM phosphate buffer with a pH of 7.4. The homogenates were centrifuged at 11,000 x g for 20 min and the resulting supernatants divided into aliquots and stored at –80° C. The levels of lipid peroxides (LPO) were measured in tissue homogenates as Thiobarbituric acid reactivity (TBARS). The product of the reaction between Malondialdehyde and Thiobarbituric acid was measured as described by Thayer [11]. Similarly, Catalase (CAT) activity was measured using the method of Aeibi [12]. This was conducted by adding 20 µl of the supernatant to a cuvette containing 780 µl of 50 mM potassium phosphate buffer (pH 7.4) and then the reaction was initiated at 25 $^{\circ}$C by adding 200 µl of 500 mM H_2O_2 to make a final volume of 1 ml. The decomposition rate of H_2O_2 was measured at 240 nm for 1 min on a spectrophotometer. A molar extinction coefficient of 0.0041 mM–1 was used to determine CAT activity. The activity was defined as an n moles H_2O_2 decrease/min/mg protein.

Histological studies

The histological part of this work was conducted using dissected rats testis after trimming off any excess fat. Then, the testis was fixed in 10% buffered formalin and processed for paraffin sectioning by dehydration in different concentrations of alcohol, cleared with xylol and embedded in paraffin blocks. Sections of about 5 µm thickness were stained with Harris haematoxylin and Eosin (H&E) for histological study [13].

Statistical analysis

The results of the analyzed samples were presented using a one-way Analysis Of Variance (ANOVA). A comparison difference within groups was considered statistically significant at $p<0.05$. All the analyzed data were expressed as mean ±SD of number of experiments.

RESULTS

Body weight changes

Table 1: Body weight (g) of rats treated with/out lead and Green Tea Extract (GTE) for 30 days

Groups	Initial BW (g)	Final BW (g)
Control	201 ± 2,20	250 ± 10,19
Pb	203 ± 5,10	138 ± 6, 21**
Pb+GTE	200 ± 4,17	145 ± 7,19**
GTE	200 ± 6,44	190 ± 3,44*

The values represent the mean (± SD, n =5), * significant difference (P ≤ 0.05), ** very significant difference (p<0.01) for comparison Pb, Pb+GTE, GTE groups versus controls group.

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The results of the experiment after a period of 30 days show that (Table 1) the mean body weight of rats in the Control group had increase by about 25% whereas the animals in the groups that were treated by lead acetate and PB+GTE had lost weight by 31 and 27.5 % respectively ($p<0.01$). It was also shown that the mean body weight loss of rats that received green tea treatment was lower relative to that of animals treated with Pb alone ($P \leq 0.05$).

Organs weight

In Table 2, it was also shown that the index weight of testis was significantly decreased ($P \leq 0.05$) in rats treated with lead compared to the Control group. The administration of GTE to Pb-treated groups also improved the toxic effect (p<0, 01).

Table 2: Weight of testis for rats treated with green tea extract (GTE) for 30 days.

Groups	Control	Pb	Pb+GTE	GTE
Testis weight	1.465 ± 0,031	1.443±0.034*	1.503±0.010**	1.208±0.081

The values represent the mean (± SD, n =5), * significant difference ($P \leq 0.05$), ** very significant difference ($p<0.01$) for comparison Pb, Pb+GTE, GTE groups versus controls group.

Sperm count

Epididymal sperm concentration is shown in Table 3. Pb group had a highly significant ($p<0.001$) lower sperm count than the control group. However, the Pb **+** GTE group was significantly increased the sperm concentration ($p<0.05$) when compared to the Pb group.

Table 3: Green Tea Extract (GTE) induced alteration on sperm count

Groups	Control	Pb	Pb+GTE	GTE
Sperm count (10^6/ml)	112.72 ± 5.23	78±2.84***	89±1.18*	90±1.81

The values represent the mean (± SD, n =5), * significant difference ($P \leq 0.05$), ** very significant difference ($p<0.01$), ***highly significant difference ($p<0.001$), for comparison Pb, Pb+GTE, GTE groups versus controls group.

Biochemical results

The levels of LPO in the tissues homogenates of testes were very significantly higher in Pb-group than the Control group. In the Pb+GTE group, the levels of LPO were significantly reduced, as shown in Fig. 1. Similarly, Fig. 2 illustrates that in GTE+Pb group, the levels of catalase were significantly elevated in comparison with Control group.

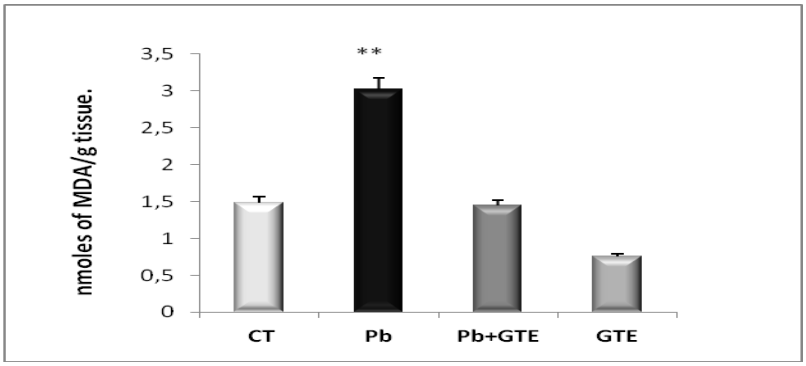

Fig. 1: Levels of lipid peroxidation in tissue homogenates of rat testes of different treated groups.
The values represent the mean (± SD, n =5), * significant difference ($P \leq 0.05$), ** very significant difference ($p<0.01$) for comparison Pb, Pb+GTE, GTE groups versus controls group.

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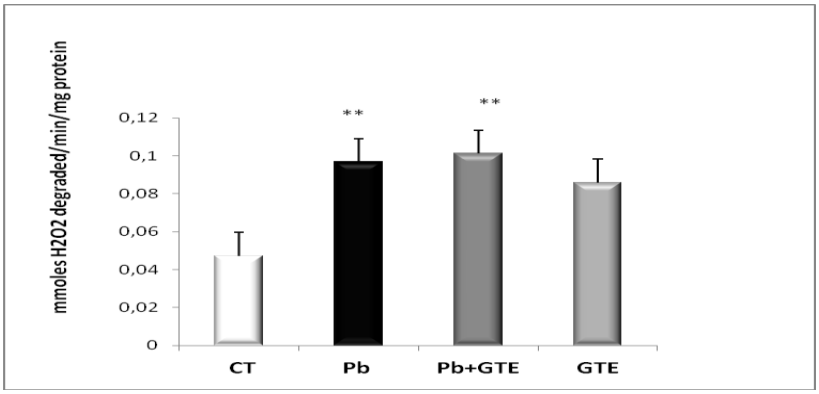

Fig. 2: Levels of catalase in tissue homogenates of rat testes of different treated groups
The values represent the mean (± SD, n =5), * significant difference (P ≤ 0.05), ** very significant difference (p<0.01) for comparison Pb, Pb+GTE, GTE groups versus controls group.

Histopathological results

The histological study in the control and GTE group showed normal appearance of the germinal epithelium and and regular organization of seminiferous tubules primary spermatocytes, spermatids and leydig cells (Figure1,2).The histological study in the Pb traited group showed disorganization of the tubules, an increased intestinal tissue was observed (Figure 3).The treatment with Pb and green tea extract revealed different degrees of amelioration compared to the intervals of Pb group. Interstitial spaces were within normal limits and Leydig cells normal appearance and distribution compared to the Pb group (Figure4).

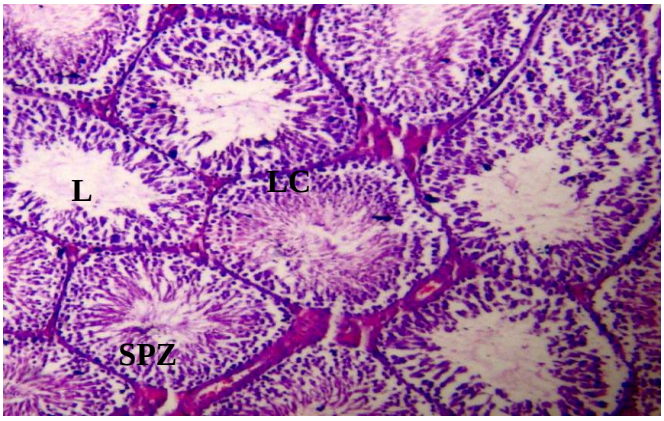

Fig 3: Micrograph of testis section in control group, showing normal architecture of semniferous tubules (S.T.) Leydig cells have normal appearance (L.C.) (H&E., 400X).

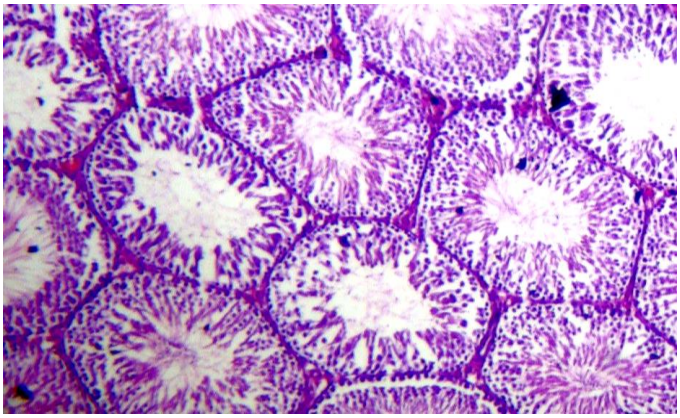

Fig 4: Micrograph of testis section treated with green tea showing a normal testis structure, complete spermatogenesis (S.T.) and normal appearance of Leydig cells (arrow) H &E, 400 X).

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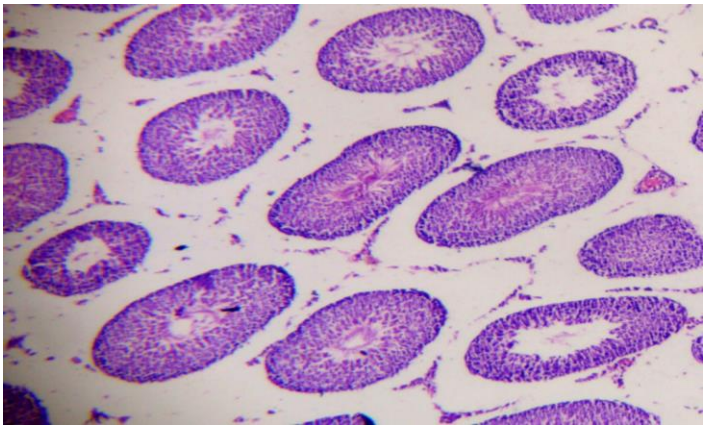

Fig 5: Micrograph of testis section of a rat treated with Pb for four weeks showing damage of seminiferous tubules , moderate reduction in spermatogenic series and few number of Leydig cells are appeared(L.C.). (H&E., 400X).

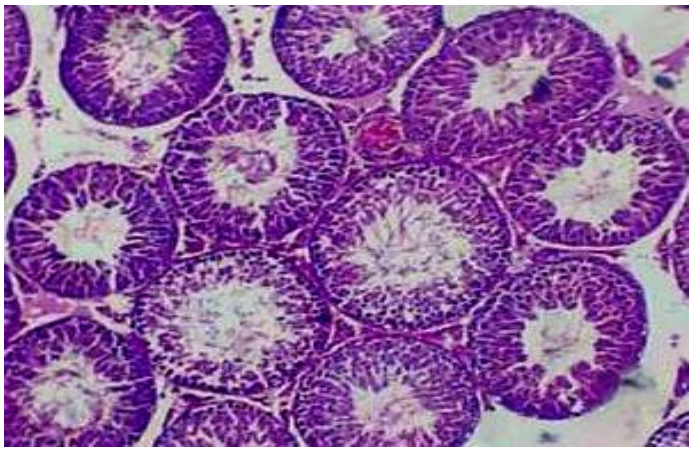

Fig 6: Micrograph of testis section of a rat treated with green tea and Pb for four weeks showing a little change in architecture of somniferous tubules with normal and well-ordered sperm (S.T.). (H&E, 400X).

DISCUSSION

Herbal medicines derived from plant extracts are widely used as an inexpensive method to treat a wide variety of clinical diseases. More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities [14]. Nowadays, tea is considered as a source of dietary constituents endowed with biological and pharmacological activities with potential benefits to human health. The increasing interest in the health properties of tea extract and its main catechin polyphenols have led to a significant rise in scientific investigation for prevention and therapeutics in several diseases[15,16]. Crespy and Williamson [17] reported that green tea extract (GTE) displays antioxidants and free radicals scavenger properties. Mohamadin et al found that supplementation of GTE attenuates cyclosporine A induced oxidative stress in rats [18].

The present study demonstrates the protective effect of green tea against testicular damage induced by Lead Acetate toxicity in experimental animals. The effect of Lead Acetate on the mean body weight of intoxicated rats was significantly lower than that of the healthy normal control group. These results clearly indicated that Lead caused a significant decrease in body weight. Nabil et al. found that lead caused decrease in growth rate in rats when fed lead[19].

The action of green tea in reducing body weight might result from inhibition of the catechol-o- methyl transferase (COMT) enzyme by Epigallocatechin 3-gallet (EGCG) of tea [20,21]. It has been shown that thermogenesis and fat oxidation are stimulated by norepinephrine, the action of which is degraded COMT [20]. Therefore, the inhibition of COMT enzyme activity by EGCG reduces the body weight.

Along with the decrease in body weight, a significant reduction in testicular weight was also found in lead-treated animals. The weight of the testis is largely dependent on the mass of differentiated spermatogenic cells. Hence a reduction in testes weight might be due to the decreased number of germ cells and elongated spermatids [22].

The present study demonstrated that the levels of catalase in the tissue homogenates of testes were significantly declined in Pb-group comparing with controls. Various mechanisms may be suggested to be responsible for the Pb toxicity. One of these mechanisms includes Pb binding to–SH groups from cell membrane proteins, cytoplasmic proteins, and enzymes. In addition, Pb can reduce activities of several enzymes including enzymeatic antioxidants and increase lipid peroxidation [23]. In agreement with previous results, we found that the levels of LPO were significantly higher in Pb group than control group in the tissues homogenates of testes. Aruldhas et al. reported that oxidative stress by free radical toxicity caused by Pb affected fertility [24]. Moreover, Gupta et al. found a significant elevation of LPO in testis tissue of rat treated with Pb [25]. Stajn and Patra [26,27] reported that different doses of Pb increase organ lipid peroxidation (LPO) in many organs, including male sex organs, and brought about changes in the antioxidant defense system.

The histological examination of testis sections taken from rats treated with Pb for four weeks showed mild to moderate reduction in spermatogenic series and reduction in sperm count in some seminiferous tubules. This was accompanied with scattered nuclear pyknotic change in the basal cell layers, Prominent degenerative changes were also noticed in few seminiferous tubules , This was in agreement with the work done by Danial [28] who stated that administration of different doses of Pb reduces the weight of the testis, and the number of spermatocytes and spermatids and Sertoli cells accompanied by thickening of the tunica propria with a daily treatment with Pb. the damage in testicular tissue to the direct cytotoxic effects of nicotine on spermatogenic cells or via inhibition of prostaglandin synthesis, which plays a functional role in the initiation and completion of spermatogenesis and steroidgenesis in the testis. Patrick [29] revealed that Pb can inhibit the neural stimulus essential for the release of pituitary gonadotrophins. In the present study the combined treatment of Pb and green tea extract ameliorated the histological changes in testicular tissue induced by Pb alone. This was evidenced by normal appearance of spermatogenic layers and sperm in the seminiferous tubules. The interstitial spaces and Leydig cells appeared normal.

CONCLUSION

In conclusion, the results of the present study indicate that GTE is a potential formulation which can be used for treatment reprotoxicity. It shows more efficient recovery from the toxicant-induced oxidative damage, histopathological changes in rat testes.

More studies, must be conducted to prove that GTE had a reproprotective proprieties on normal and intoxicated rats, beside isolation of the bioactive compound (s) must done to determine its effect separately on animal model.

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