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ORIGINAL ARTICLE

Testicular toxic effect of lead acetate on adult male rats and the potential protective role of alcoholic extract of ginseng (histological, histomorphometrical and physiological)

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ABSTRACT

Objective: This work aims to evaluate the histological and histomorphometrical changes in the testicular oxidative stress in male rats post exposure to lead acetate and the possible protective role of *Panax ginseng* extract, which reducing heavy metal toxicities has raised worldwide.

Methods: Animals were divided into five groups for 5 and 10 weeks. Each group (n=8). Group II, received (100 mg/kg b. wt/day) lead acetate for 5 weeks. Group III, received (100 mg/kg b.wt/day) lead acetate for 10 weeks. Group IV, received (100mg/kg b. wt/day) lead acetate with (200mg/kg b. wt/day) of *P. ginseng* extract, Group IIV, received (200mg/kg b. wt/day) ethanolic *P. ginseng* extract after exposure to lead acetate for 10 weeks. LH, FSH and testosterone level, body weight, genital organ weight and concentration of lipid peroxidation product (MDA) was estimated.

Results: The result showed clearly impact of lead acetate on testicular tissue of male rats and degenerative changes in seminiferous tubules, vascular congestion, also alteration in spermatogenic layers in many tubules and necrosis dilation of interstitial spaces, leydig cell degeneration, while, the groups receiving *P. ginseng* extract after exposure for lead acetate (PbAc) were ameliorated the damaging effects, however body weight and testes weight, LH, FSH and testosterone level significantly increase in treated groups with *P. ginseng* extract.

Conclusion: show that dose dependent *P. ginseng* extract significantly present adverse testicular toxicity and oxidative stress induced with lead acetate.

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INTRODUCTION

Lead is aubiajutous environmental and industrial. Pollutant that has been detected in every facet of environmental and biological system [1]. Lead is one of the most toxic metals to human, animal and plants, that

important environmental contaminant which affects many body organs including male genital system, practically in workers of lead based factories showed disorders in structure and function of male reproductive system and quality of sperms are principal symptoms of exposure to metal [2]. This substance can be absorbed through respiration, digestion and dermal, and accumulated in body, when, the metal absorption by ingestion depend of factors such as the physical form, particle size, gastro intestinal transit time and nutritional status of as person and patterns of foods intake affect absorption [3]. Lead can show their toxic effects through the inhibition of antioxidant enzyme activity, and generation of reactive oxygen species (ROS) with subsequent stimulation of lipid peroxidation [4]. Many researchers have documented the antioxidant property of Ginseng [5]. It was reported that ginseng roots extract has been recognized as the most prized medicine among all herbal medicine, that are rich in saponins (ginsenosides), phenolic compounds polysaccharides, and poly acetylenes, tannin, flavonoids, alkaloids and glycoside, estrogenic materials, as gonad stimulating compounds that improve male infertility and exhibit gonadotrophin activity in the animals [6].

In addition, *Panax ginseng* has been demonstrated to have various pharmacological activities including anticancer, antiaging, anti-inflammatory and various phytotherapeutic properties, the preventive effects of testicular damage [7].

The aims of this study investigate the changes of testicular structure in lead acetate rats, additionally; we have also further demonstrated the possible beneficial effect of ginseng on lead toxicity in male rats.

MATERIALS AND METHODS

Animals: About forty male adult rats weighing (200-250) gm and aged (8-10) weeks (*Rattus norvegicus*), were obtained from the animal house, the animals were divided into 5 groups (N=8). The rats were collected in the isolated cages in the experimental house of the biological department, college of sciences, Basrah university, inderstrict hygienic and standard management condition at temperature 20-25^{0}C and humidity of 5%, when the animals given free access commercial feed pellets and water.

Preparation of chemical material: Lead acetate trihydrate [$(C_2H_3O_2)_2$ Pb.3H_2O], PbAc solution was prepared in normal saline and replaced every day to minimize lead precipitates. The *P. ginseng* roots was obtained from the local market shop cut into tiny parts and then dried carefully at room temperature for two days under the shade, after that ground into powder.

Prepare *Panax ginseng* extract (PGE): Sixty grams of ginseng powder were put in round bottle flask, then 500 ml of ethanol 70% were added to flask and extracted for 24 hr. in 50^{0}C.the extract was filtered by whatman filter paper No.1 and the extract is distributed into clean sterile petri-dishes and left to dry at lab temperature under the shade, the dry extract is collected in black and stored at 4^{0}C [8].

Specific chemical indicators: Qualitative chemical tests were carried out for the extract of ginseng, as described in Table 1.

Table 1: **specific chemical indicators for 70% ethanolic extract of *P. ginseng*.**

Phytoconstitute	Reagents	Results
Phenols	1ml extract+1ml$FeCl_3$	Brown precipitate
Saponins	1ml extract+$HgCl_2$	Yellow precipitate
Tannins	1ml extract+1ml lead acetate	Yellow to brown precipitate
Alkaloids	1ml extract+1ml mayers	White to brown precipitate
Flavonoids	1ml extract+1ml KoH alcoholic	Red precipitate
Glycosides	1ml extract+1ml Bendict	Brown precipitate
pH	10mg extract+50ml distal water	pH meter French origin

Experimental protocol: The duration of administration were 5 weeks and 10 weeks, the animals were divided into 5 groups

- Group I (8 animals): were used as control, orally given distilled water.
- Group II (8animals): was orally administration with PbAc in a dose of (100mg/kg) daily for 5 weeks.
- Group III (8animals): Was orally administration with PbAc in a dose of (100mg/kg) daily for 10 weeks.
- Group IV: Was orally administration with (100mg/kg) PbAc with ethanolic ginseng extract (200mg/kg B.W.) for 5 weeks.
- Group IIV: was orally administration with (100 mg/kg B.W) PbAc with ethanolic ginseng extract (200 mg/kg B.W) for 10 weeks.

The oral LD50 of PbAc for male rats is 100 mg/kg B.W. that used 1/100 LD50 of PbAc, the dose was chosen of according to [9], also the dose of extract was chosen of according to [10].

Methods:

- Testicular histology study: the specimens were fixed in 10% formalin in neutral buffered for haematoxylin and eosin stain by microscopic slides exam for general histology.
- Testicular histomorphometrical study.
- Estimation of function state of testes: parameters were used following diameter of seminiferous tubules was obtained by measuring across the minor and major axes. Also analyzed, germinal epithelium height in testes, was measured for the same tubules, was calculated as the space between the tunica propria and the edge of the lumen, when, image analysis of testes from all rats were examined under light microscopy using an objective me measuring menu of image analyzer.
- Biochemical parameter study: at the end of the experiment, blood sample were collected via cardiac puncture by using disposable syringe, blood were poured into plan tubes to be centrifuged at 3000 cycle/min for 10 min, to obtain the serum was used to estimate markers of testicular damage.

- Estimation of serum malondial aldehyde (MDA): MDA serum as one of the main endo product of lipid peroxidation was measured according to the method originally described by.
- Estimation of FSH, LH and testosterone hormone: measurement of serum FSH, LH and Testosteron hormone concentration are regarded as valuable tool in the diagnosis of hemostasis of fertility regulation by the hypothalamic-pituitary-gonad axis (ELISA, kit monobind Inc, USA and CVSA Bio, USA).

Statistical Analysis: Data are presented as the mean±standard deviation (SD). One way ANOVA flowed by Tukey's post boc test Pvalues<0.05 were considered as significant.

RESULTS

Essential chemical detection of ethanol *P. ginseng* extract: The results of tests showed according to Table 2. The presence of saponins, tannin, alkaloids, phenols, glycoside, flavonoids and the pH was 5.4.

Table 2: **Quantitative chemical tests performed in the 70% ethanolic extract of *P.ginseng*.**

Chemical constituents	Tannin	Saponins	Phenolic compound	Flavonoids	Glycoside	alkaloids	pH
Results	++	++	+	+	+	++	5.4

Serum biochemical parameter

Serum MDA level: MDA level in the serum was found to be significantly higher in rats treated with PbAc alone than those in the normal control group while, serum MDA was significantly declined followed PbAc+*P. ginseng* medication of rats at a dose of 200 mg/kg for 5 weeks and 10 weeks as compared with those of PbAc-control rats.

Table 3: **Serum MDA concentration of control groups and groups treated with lead acetate and ethanolic PG. extract.**

Period	Groups	MDA (um/L)
5 weeks	Control	4.22±0.06
	PbAc (100 mg/kg)	6.32±0.04
	PbAc+PG (200 mg/kg)	5.52±0.04
10 weeks	Control	4.22±0.06
	PbAc (100 mg/kg)	8.31±0.04
	PbAc+PG (200 mg/kg)	7.27±0.06

Serum FSH, LH and Testosteron level: The results presented in Table 4 showed that PbAc significantly decreased the serum FSH, LH and testosterone level (P<0.05) as compared with control group. However, the rats in group that received ethanolic *P. ginseng* extract show a significant increase in averages of hormons levels compared with PbAc groups during 5 weeks and 10 weeks.

Table 4: **Effect of ethanolic *P. ginseng* extract and PbAc on FSH, LH and testosterone in male rat.**

Period	Groups	FSH (MIU/ml)	LH (MIU/ml)	Testosterone (MIU/ml)
5 weeks	Control	1.36±0.02	1.16±0.01	1.73±0.02
	PbAc (100 mg/kg)	0.75±0.02	0.46±0.02	0.72±1.001
	PbAc+PG (200 mg/kg)	0.96±0.01	0.74±0.2	1.01±0.01
10 weeks	Control	1.36±0.02	1.16±0.02	1.74±0.02
	PbAc (100 mg/kg)	0.36±0.009	0.27±0.01	0.31±0.01
	PbAc+PG (200 mg/kg)	0.70±0.02	0.60±0.02	0.60±.02

Body and testicular weight: Data presented in Table 5 clarified that administration PbAc only was obvious on the body and testes weight. At the end of the experiment (5 weeks and 10 weeks), PbAc-treated group showed high significant decrease (P<0.05) in their body weight, particularly during 10 weeks. When *P. ginseng* extract were offered as treatment they caused elevation in body weight and testes, but the values still less compared to the body and test weights of control group.

Table 3: **The effect of PG extract on body and tests weight in lead acetate treated male rats.**

Periods	Groups	Body weight (g)	Testes (g)
5 weeks	Control	232±2.37	1.83±0.02
	PbAc(100mg/kg)	194.3±1.97	0.96±0.02
	PbAc+PG(200mg/kg)	210.2±1.84	1.44±0.03
10weeks	Control	243±2.28	1.88±0.05
	PbAc(100mg/kg)	179.3±3.83	0.75±0.02
	PbAc+PG(200mg/kg)	197±2.53	1.35±0.02

Testicular histological and morphometric observations: Examination of control section related to control testes showed normal histological structure of seminiferous tubules associated with complete spermatogenic series (Figure1). After 5 weeks, when treated animals with PbAc showed mild of seminiferous tubular are markedly distorted with depletion of the spermatogenic, many vacuolated with deeply stained pyknotic nuclei (Figure 2) many of seminiferous tubules were thickening and irregular in basement membrane are observed (Figure 3) after 10 weeks, alteration in testes structure were observed including marked degenerative changes in most of the seminiferous tubules, that, appeared necroticdedris, shrunken with more irregularity of tubular basement and lined with one or two layers of small acidophilic cells and atrophy (Figure 4). Different spermatogenic cells especially mature sperms were absence seen in most of the seminiferous tubules, also appeared edema with dilated interstitial spoces, numerous vacuoles in the sertoli cells and interstitial

cells (Figure 5). In rats that received PbAc with *P. ginseng* extract, the testes showed restoration of the structure of seminiferous tubules appearance of most tubules, leyding and interstitial space during 5 weeks and 10 weeks (Figure 6).

Morphometric results of testicular tissue structure showed that significant decline in the mean diameter of the seminiferous tubules in PbAc treated group 242.03±0.87 um at 5 weeks and 223.04±1.70 um at 10 weeks at ($P<0.05$) compared with control group when, in lead acetate treated with P. ginseng extract, the mean diameter of the seminiferous tubules were significantly different from control in dose 200mg/kg and in both periods 253.75±0.99 for 5 weeks and 240.76±1.69 at 10 weeks. While, a significantly analysis of data of germinal epithelium height in testicular tissue, which lower in the PbAc treated group 46.05±0.73 um at 5 weeks and 36.46±0.72 um at 10 weeks than control groups. When treated with PbAc with combined *P. ginseng* extract, the mean height of the germinal epithelium was increase 51.10±0.45 after 5 weeks and 44.64±0.47 after 10 weeks than in the PbAc group (Table 6).

Table 6: **The effect of PG extract on body and tests weight in lead acetate treated male rats.**

Period	Groups	Diameter of seminiferous tubules (um)	Germinal epithelium height (um)
5 weeks	Control	264.78±0.47	57.82±0.70
	PbAc (100 mg/kg)	242.03±0.87	46.05±0.73
	PbAc+PG (200 mg/kg)	253.75±0.99	51.10±0.45
10 weeks	Control	265.19±0.77	58.09±0.66
	PbAc (100 mg/kg)	223.04±1.70	36.46±0.72
	PbAc+PG (200 mg/kg)	240.76±1.69	44.64±0.47

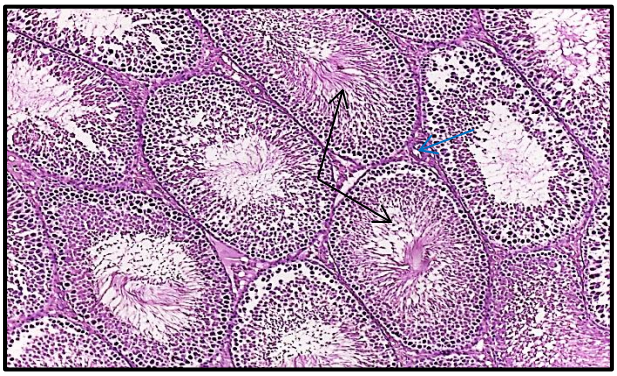

Figure 1: **Section in testis of rat control group showing normal structure of seminiferous tubuleswith complete spermatogenic series (black arrow), little interstitial region (blue arrow). H&E. 100X.**

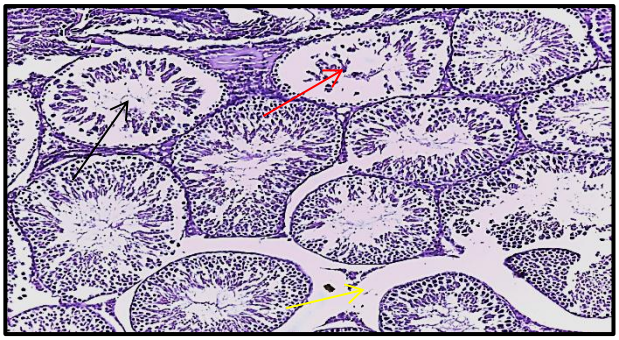

Figure 2: **Section in testis of rat treated with (PbAc) for 5 week showing marked degeneration of most seminiferous tubules (black arrow) with loss of spermatogenic series in tubular lumen (red arrow), and increase interstitial space (yellow arrow). H&E. 100X.**

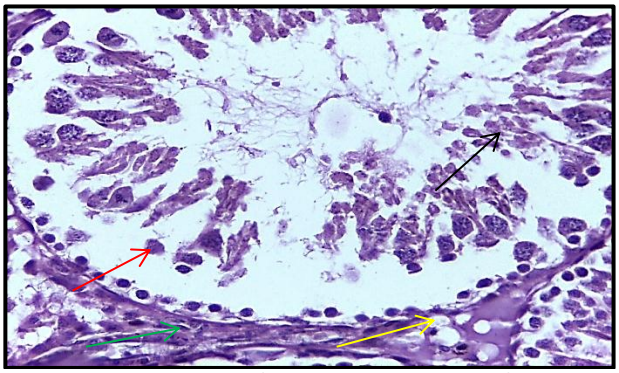

Figure 3: **Section in testis of rat treated with (PbAc) for 5 weeks showing spermatogenic cell with shrunken pykontic nuclei (red arrow), and necrosis with numerous vacuoles (black and yellow arrow arrow) and thickening in basement membrane (green arrow). H&E. 400X.**

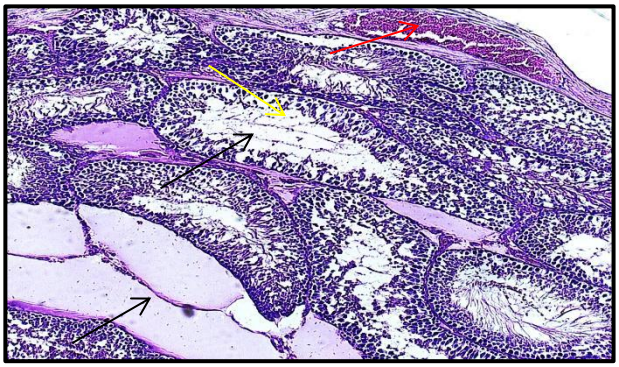

Figure 4: **Section in testis of rat treated with (PbAc) for 10 weeks showing most of seminiferous tubules are marked degeneration (black arrow), vascular congestion (red arrow) with loss of spermatogenic series in tubular lumen (yellow arrow). H&E. 100X.**

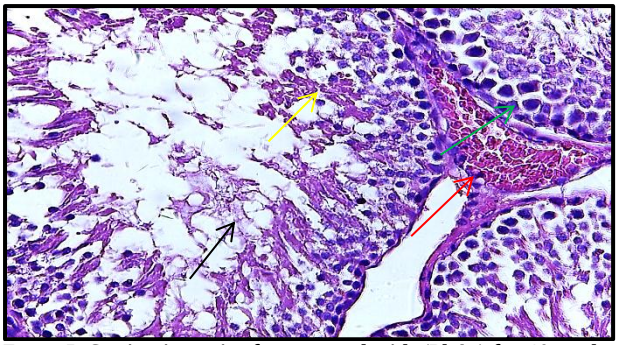

Figure 5: **Section in testis of rat treated with (PbAc) for 10 weeks showing less spermatogenic cell (black arrow) and thickening and irregular of basement membrane (red arrow), necrosis (yellow arrow), high congestion (green arrow). H&E. 400X.**

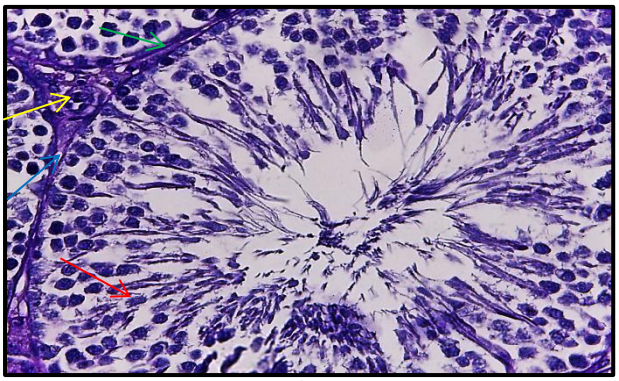

Figure 6: **Section in testis of rat treated with (PbAc plus (200mg/kg) *P.genseng* extract) for 5 weeks showing more restoration spermatogenic layers (red arrow) and leyding cells (yellow arrow) and reduced of interstitial space (blue arrow), less thickening of basal membrane (green arrow). H&E. 400X.**

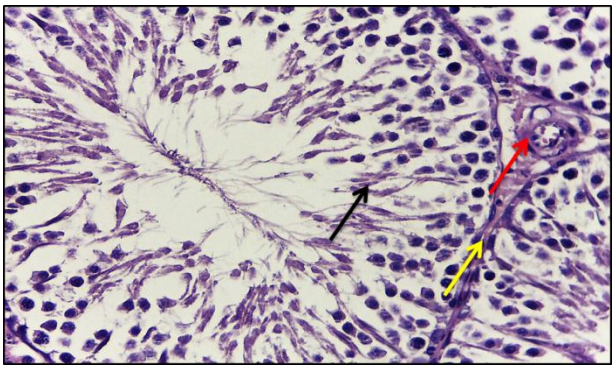

Figure 7: **Testis section of rat treated (PbAc) with (200mg/kg) *P.genseng* extract post10 weeks showing more rebuilding of the spermatogenic layers and their cells of seminiferous tubules (black arrow), more restored of leyding cell (red arrow) and less thickening basement membrane (yellow arrow). H&E. 400X.**

Discussion

Lead is one of the most widely used in many manufacture such as, in the production of lead acid batteries, water pipes a paint, also in leaded gasoline and in the auto mobile [11]. The lead environmental causes represent one of the major factors affecting male fertility [12]. Pb was related to a broad range of physiologic and biochemical dysfunction that induced oxidative stress involves an imbalance between generation and removal of reactive oxygen species (RGS) like lipid peroxides that, play an important role in the pathophysiology of lead poisoning including damage in testicular tissue [13].

The current study showed that exposure of male rats to 100mg/kg B.W of PbAc daily for 5 weeks and 10 weeks induced disorganization, degeneration and atrophy in most seminiferous tubules raise interstitial spaces and decrease leyding cells. Reduction spermatogenic cells from the basement membrane, thickening and irregular basement membrane of most seminiferous tubules with less spermatogenic layers [14]. In the present study oral administration male rats of lead acetate affected the testes and caused histological and morphometric changes [2]. Exposure for longer time may have negative effects on the sperm abnormality and male fertility [15]. In agreement with the present findings Al-Chalabi *et al*. [16] showed that exposure to PbAc caused a significant decrease in the body weight and weight of testes, with diameter and height seminiferous tubules epithelial, when, reduction in body weight may result from reduced food intake and increase catabolic state because the raise duration of exposure, also imbalance in the metabolism produced by changing znic-deppendent enzymes [17].

In addition Elgawish and Abdrlrazek [15] showed reduced weight of the testis is dependent on the size of differentiated spermatogenic cells, that concluded, the lower of the weight may be due to less tubular size. In the current research, many seminiferous tubules basement membrane showed increase thickening in the PbAc treated group during two periods, this thickening to an to arise produce collagen by stimulant myoid cell and extracellular matrix which are important responsible for increase basement membrane thickness [18].

Numerous studies have shown that PbAc cause increased lipid peroxidation, which was manifested by an high in MDA levels. When level of MDA is commonly used as a measure of the oxidative stress in cell, moreover, lead affects membrane-related process such as the activity of membrane and accumulated in testicular tissue causes oxidative and damage, this result in agreement with previous findings showing an increase in serum MDA level in testes in lead acetate rats [19]. Form the results, that exposure to PbAc significant decrease in the levels of LH, FSH and testosterone.

Concentration in rats as compared with normal control LH and FSH activity depends on the quantity of these hormones and the number of specific receptors in the testes. As the metal opposite effect on the leydig cells responsible for the secretion testosterone hormone via influence on the hypothalamus-pituitary axis, these results agree with [20, 21]. In this work we found that ethanolic alcohol extract of root *P. ginseng*, which active compounds and made its piecipitated or be analyzed like, tannins, saponins, alkaloids, flavonids, phenols and glycosides, have bioactivity antioxidant, anti-inflammatory, immunostimulating, also affects the synthesis of RNA and DNA, which preserve the safety of the cell membrane and resistance entrance of toxic substances and raise protein synthesis by stimulating RNA polymerase I activity [5]. Therefore, this extract has more effective against lead toxicity, administration of *P.*

ginseng extract after exposure to PbAc showed more clear testicular architecture where most of the seminiferous tubules, sertoli cells, leyd cells and less interstitial space, also tubular lumen are filled with sperms, this current observations come in agreement with the previous reports of who reported that extract can protect living cells from toxicity damage by ameliorating the deleterious effect of free radicals.

Co-administration of *P. ginseng* extract to male rats in the present experiment showed more improvement weight body and testes weight compared to that treated group with PbAc, that, antioxidant compounds can decreases in oxidative stress. So that increase body weight and testes weight, in similar studies, it has been reported the *P. ginseng* extract, which presents oxidative stress, and increase body weight and testes reported by. Present results showed that male rats received lead acetate with *P.ginseng* extract showed activity as manifested by reduction in the MDA levels, these observations in agreement with previous findings [22, 23] showing a decrease in serum MDA level in PbAc administrated rats. The results show the positive effects of ginseng extract on increasing the level of LH, FSH and testosterone hormones in male rats through effects on the pituitary and hypothalamus and stimulate secretion the FSH and LH hormones is saponins, flavonoids, ascorbic acid and polyphenolic present in ginseng and known characteristics antioxidant and detoxification resulting from heavy metal.

Conclusions

The present experiment demonstrated the protective effect of *P. ginseng* extract against lead acetate induced damage in rat testes and deleterious impact on blood LH, FSH and testosterone level through the induction of oxidative stress further investigations are encouraged to explore the full potential of.

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