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Research Article

Ameliorative Effect of *Cucurbita pepo* L. Seed Extract against Lead Induced Effects on the Serum and Testicular Oxidative Status: An *In-vivo* Validation

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ABSTRACT

Lead (Pb) exposure is considered to be an alarming public health problem since evidence has mounted regarding its adverse impact on health and reproduction. The current research was intended to evaluate the ameliorative effects of *Cucurbita pepo* L. (pumpkin) against lead toxicity-induced oxidative stress in experimental rats. Before the animal study, a preliminary phytochemical screening was done to detect the presence of various phytoconstituents in the seed extract. Thirty adult male wistar rats were selected and randomly divided into five groups for the experimental study. Group 1 served as the control while groups 2, 3, 4 and 5 were treated with 30 mg/kg lead acetate, 1000 mg/kg seed extract alone (high dose), 30 mg/kg lead acetate and 100 mg/kg seed extract (low dose) and 30 mg/kg lead acetate and 1000 mg/kg seed extract (high dose), respectively. Enzymatic antioxidant concentrations in serum and testis were found to check the response of antioxidants to lead toxicity. In lead treated group, increased oxidative stress was observed which was indicated by a significant ($p<0.001$) decline in the concentration of the enzymatic antioxidants (SOD, CAT and GPx) coupled with a significant increase in lipid peroxidation marked by high MDA level. Interestingly, at high dosage of *C. pepo* seed extract, enzymatic antioxidant concentration was comparable to control and significantly higher compared to other experimental groups. The study revealed that even in low dosage, *C. pepo* administration could improve the antioxidant status in the lead-treated group. This investigation recommends *C. pepo* seeds as a potent natural product promising strong protection against lead toxicity-induced oxidative stress which could be pharmacologically explored for drug synthesis.

Introduction

Free radicals are natural unfavorable byproducts in our body created by various biological processes such as respiration, digestion, metabolism of alcohol and drugs, and the lipids conversion into energy. They can initiate multiple reactions in the body, including damage to cell membranes, block the functioning of key enzymes, hamper vital cellular processes, impair normal cell division, DNA, and obstruct energy generation.[1] If the natural antioxidant mechanism in our bodies that normally eliminates free radicals cannot cope properly, it may lead to oxidative stress, which is the underlying cause of a wide spectrum of diseases and disorders. The occurrence of oxidative stress can be accelerated by modern lifestyle, which includes an unhealthy diet, sedentary work, and exposure to a variety of toxic chemical substances, including pesticides, heavy metals, food additives, and environmental pollutants.[2]

Lead (Pb) is a heavy metal and a well-known environmental contaminant that poses a significant threat to mankind. The extensive use of lead in metal products, paints, batteries, medical appliances, and varnishes makes human exposure inevitable.[3] Lead has received considerable attention due to its bioaccumulative,

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nonbiodegradable, and immutable characteristics. Lead poisoning causes unusual physiological, biochemical, and behavioral changes in the circulatory, metabolic, cardiovascular, neurological, excretory, and reproductive systems.[4] Conversely, little is known about the exact mechanism of action through which lead affects male fertility. Despite the fact that different theories have been put forth to elucidate the pathogenesis of lead toxicity, several studies declare that lead may partially intercede oxidative stress to repress male reproduction either by producing reactive oxygen species (ROS) or by affecting antioxidant enzymes, or sometimes both.[5]

The body possesses several enzymatic mechanisms to reduce radically generated damage and safeguard against the excess production of free radicals. Antioxidants play a crucial part in these defense mechanisms. A delicate balance between oxidants and antioxidants helps healthy organisms defend against reactive oxygen species' harmful effects. Therefore, the steady formation of free radicals must be balanced or neutralized by a constant intake of antioxidants. Antioxidants, irrespective of enzymatic or non-enzymatic, are chemicals that prevent the production of free radicals and strive to scavenge, neutralize, or repair the damage caused by them.[6] It is widely accepted that antioxidant supplementation against lead-induced oxidative stress is an established method to alleviate lead toxicity.[7] According to reports, antioxidant enzymes are essential for shielding cells from the oxidative stress brought on by lead exposure.[8] Natural antioxidants are available in various substances categorized as secondary plant metabolites, such as polyphenols (phenolic acids, flavonoids) and terpenoids (carotenoids) and consuming foods rich in these substances in significant amounts appears to play a crucial role in disease prevention.[9]

Since the dawn of human civilization, plants have served as a local repository of raw resources to isolate or produce several traditional medications.[10] Pumpkins are one of these plants, and because of its components and health-promoting phytochemicals, it is utilized to treat various diseases and disorders.[11] Pumpkins belong to the Cucurbitaceae family and are comprised of many edible species. They are mostly found in China, India, Pakistan, Argentina, Yugoslavia, America, Mexico and Brazil.[12] The macro-and micronutrient composition of pumpkin seeds-which includes proteins, phytosterols, lignans, triterpenes, polyunsaturated fatty acids, phenolic compounds, tocopherol, carotenoids, and minerals are all thought to be responsible for their health benefits.[13] Pumpkin seeds (*Cucurbita pepo* L.) are also rich sources of unsaturated fatty acids, fibers and antioxidants.[14]

Mechanisms of action must certainly be identified *in-vitro*; nonetheless, it is also necessary to demonstrate the efficacy of these same ingredients *in-vivo*.[15] In view of the scanty studies on the protective effect of *C. pepo* seeds against lead toxicity induced oxidative stress, the present study was designed to investigate on the efficacy of *C. pepo* seed extract in mitigating lead induced effects on the serum and testicular oxidative status in experimental rats and discover whether co-treatment with *C. pepo* seed extract could reverse these adverse physiological changes. The study also hopes it could lay a scientific foundation for exploring on multiple therapeutic applications of *C. pepo* seeds particularly in drug discovery.

Materials and Methods

Sample Collection and Identification

The *C. pepo* seeds were collected directly from farmers at Tudiyalur, locale in Coimbatore, Tamilnadu, India. The sample was confirmed and authenticated (voucher specimen number BSI/SRC/5/23/2021/Tech/282) at Botanical Survey of India, Southern Regional Centre, Tamilnadu Agricultural University, Coimbatore.

Preparation of *C. pepo* Seed Extract

The seeds were washed, cleaned to remove any debris followed by dehulling and shade drying for a week. Approximately 100 g of dried *C. pepo* seeds were coarsely powdered using mortar and pestle. Total of 50 gm of powder was subjected to extraction in 300 mL distilled water and kept in an incubator for 48 hours at 37°C. The slurry was intermittently stirred for 2 hours and kept overnight. The mixture was collected and filtered and the filtrate was dried in room temperature. Residue was collected and suspended in water at a fixed dose and used for experiments.

Phytochemical Screening of the Extract

Phytochemical screening of the extracts was carried out according to the methods of Trease[16] and Evans (1989) to discover the presence or absence of phytoconstituents such as carbohydrates, protein, flavonoids, alkaloids, tannins, steroids, terpenoids, saponins and quinones.

Dose Selection of the *C. pepo* Seed Extract

The dosage of *C. pepo* seed extract was fixed based on the literature available. Acute toxicity study conducted by Malgwi[17] *et al*., 2014 stated that aqueous *C. pepo* L. seed extract was safe up to 5000 mg/kg body weight. Hence the dosages 100 and 1000 mg/kg were selected for the current study.

Chemicals

Lead acetate trihydrate was obtained from Oxford Lab. Co., India. Lead acetate was dissolved in distilled water at concentration of 30 mg/kg body weight of 1% solution.

Animal Procurement

30 adult male wistar rats with mean weight of 120 ± 35g were procured from Biogen Animal Facility, Bangalore (971/PO/RcBiBt/S/06/CPCSEA).The animals were kept

for one week acclimatization in the experimental room having the temperature 28 ± 2°C, tolerable humidity conditions with 12:12 light and dark photoperiod. Animals were housed in clean cages bedded with sterile paddy husk. They had access to standard rat pellet diet and water *ad libitum*.[18] The procedures were carried out after receiving approval from the Institutional Animal Ethical Committee of the Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore (Ethical Approval No: AIW:IAEC.2020:FSN:02).

Experimental Design

After one week of acclimatization period, 30 male wistar rats were randomly allocated into 5 groups containing 6 rats each.

Group 1: Control (Normal saline)
Group 2: Lead acetate (LA) -30 mg/kg
Group 3: *Pepo* seed extract high dosage (PSE HD) -1000 mg/kg
Group 4: LA + *Pepo* seed extract low dosage (LA+PSE LD) -100 mg/kg
Group 5: LA + PSE HD -1000 mg/kg

Rats were treated by oral gavage once daily for 45 days excluding the acclimatization period. After giving last dosage, the animals were sacrificed within 24 hours by the mode of decapitation.

Serum Preparation

After the treatment period, the rats were anaesthetized using light ether anaesthesia in lethal chamber. Blood samples were collected from each rat through retro orbital sinus puncture. Sample was allowed to clot at 37°C and centrifuged at 3000 rpm for 15 minutes. The serum (supernatant) was collected and stored at −20°C for antioxidant analyses.[19] The serum concentration of enzymatic antioxidants such as Superoxide dismutases (SOD) and glutathione peroxidase (GPx) was measured by standard biochemical methods.

Preparation of the Tissue Homogenate

The organ associated with reproduction which includes testes was excised out and cleaned thoroughly in phosphate buffer. Specimens from testis were homogenized in 1.17% potassium chloride (KCl) included ice cold phosphate buffer with molarity 0.1 and pH 7.4 was used in the preparation of 10% homogenate solution of each tissue sample. For estimating the lipid peroxidation levels, a part of this homogenate was used and the remaining solution was centrifuged at 10000 rpm for 10 minutes at a temperature of 4°C.[19] The supernatant was utilized for carrying out the antioxidant enzymatic assays consisting of SOD, catalase (CAT), GPx and lipid peroxidation (MDA) assays.

Determination of (SOD)

Testis homogenate (0.5 mL) was diluted with equal deionized water. 0.25 mL ethanol and 0.15 mL of chilled chloroform were added followed by shaking this mixture for 1-minute and centrifuging at 2000 rpm. The SOD enzyme in the supernatant was estimated. 1.5 mL of buffer was added to 0.5 mL of the supernatant. The reaction was initiated by adding 0.4 mL epinephrine and change in optical density per minute was detected at 480 nm using a double beam UV-vis spectrophotometer (UV 1700, Szhimadzhu). SOD activity was expressed as U/mg. Change in optical density per minute at 50% inhibition to adrenochrome transition by the enzyme was considered as one enzyme unit.[20]

Determination of CAT

To testis homogenate (0.1 mL), 1.0 mL of phosphate buffer and hydrogen peroxide were added. The reaction was arrested by adding 0.2 mL dichromate acetic acid reagent. Standard hydrogen peroxide between the ranges of 4 to 20 µL was treated in similar way. The tubes were kept in a boiling water bath for 10 minutes. The developed green colour was read at 570 nm in a double beam UV-vis spectrophotometer. Catalase activity was expressed as U/mg.[21]

Determination of GPx

The glutathione peroxidase activity was calculated according to the method given by Rotruck[22] *et al*., 1973. EDTA (0.2 mL each), sodium azide, reduced glutathione, H_2O_2; 0.4 mL of buffer and 0.1 mL of enzyme (testis homogenate) were mixed and incubated at 37°C for 10 minutes. The reaction was arrested by adding 0.5 mL of TCA and the tubes were centrifuged. To 0.5 mL of supernatant, 3 mL of sodium hydrogen phosphate and 1-mL of DTNB were added and the developed color was read at 412 nm immediately in a double beam UV-vis spectrophotometer. Peroxidase activity in serum was expressed as µg/mg.

Determination of Lipid Peroxidation

In 1 mL of testis homogenate was mixed with 0.2 mL 4% (w/v) sodium dodecyl sulfate, 1.5 mL 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 15 mL of 0.8% thiobarbituric acid (TBA, pH 7.4). The mixture was kept in a hot water bath at 85°C for 1-hour. The developed pink colour was read against a reagent blank at 532 nm following centrifugation at 1200 g for 10 minutes. The concentration was expressed as n moles of malondialdehyde (MDA) per mg of protein using 1,1,3,3,-tetra-ethoxypropane as the standard.[23]

Statistical Analysis

Statistical analysis was carried out using one-way Analysis of Variance (ANOVA) followed by Dunnett's test for comparison between groups. *p*-values of <0.05, <0.01 and <0.001 were considered to be significant, very significant, and highly significant, respectively.

Results

Table 1 illustrates the preliminary phytochemical screening of *C. pepo* L. seeds aqueous extract which revealed appreciable levels of alkaloids and tannins, moderate levels of carbohydrates and steroids and remaining compounds in trace levels.

Fig 1 and 2 show the effect of *C. pepo* seeds on serum concentration of SOD and GPx respectively. The LA group caused a significant ($p<0.001$) drop in SOD and GPx levels in comparison with the control group whereas LA+ PSE LD group had a slight improvement when compared to LA group. From the graphical representation, it is clear that co-administration of *C. pepo* seed extract at high dose with lead acetate (LA+PSE HD) had a considerable significant improvement in SOD and GPx in comparison with LA and LA+ PSE LD group. Surprisingly, PSE HD group was found to have both SOD and GPx levels almost comparable to that of control. PSE HD group was found to have a highly significant improvement when compared to other treated groups indicating potent antioxidant capacity.

Table 2 reveals the effect of *C. pepo* seeds extract on testis concentration of SOD, CAT, GPx and MDA in experimental rats. LA group was found to have a highly significant decrease in SOD (0.525 ± 0.0086 to 0.4 ± 0.0115 U/mg protein), catalase (28.52 ± 0.987 to 19.30 ± 0.216 µmole/mg protein), considerably significant ($p<0.01$) decrease in GPx (4.605 ± 0.164 to 3.505 ± 0.1588 µg/mg protein) and a highly significant ($p<0.001$) increase in lipid peroxidation marked by high MDA level (13.35 ± 0.214 to 20.18 ± 0.736) when compared to control group. Even though LA+ PSE LD group exhibited a considerable significant decrease in SOD, CAT and GPx when compared to control, there was a slight improvement even at low dosage in comparison with LA group. Also, LA + PSE LD exhibited a significant decrease in MDA compared to LA group. Compared to other lead acetate treated groups, LA+ PSE HD showed highly significant improvement in SOD, CAT and GPx and also exhibited a highly significant decrease in MDA. In conclusion, treatment with *C. pepo* seed extract at 1000 mg/kg body weight (high dose) but not at 100mg/kg dose (low dose) markedly improved the antioxidant status and also reduced testis MDA which was significantly different from the lead acetate group (LA). Interestingly, PSE HD disclosed a notable enhancement in SOD than control itself and a highly significant improvement in CAT and GPx when compared to other treated groups. In addition, PSE HD group was found to have a remarkable reduction in lipid peroxidation marked by decreased MDA level compared to other treated groups.

Table 1: Phytochemical screening of *C. pepo* seed aqueous extract

Sl. no	*Phytochemicals*	*Inference*
1	Carbohydrates	++
2	Protein	+
3	Flavonoids	+
4	Alkaloids	+++
5	Tannins	+++
6	Steroids	++
7	Terpenoids	+
8	Saponins	+
9	Quinones	+

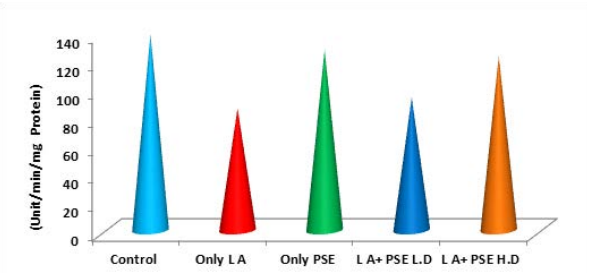

Fig. 1: Serum Concentration of SOD

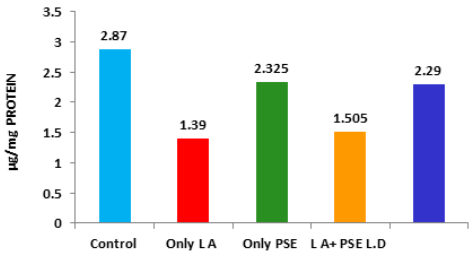

Fig. 2: Serum Concentration of Glutathione Peroxidase (GPx)

Discussions

Many studies have reported that the type of solvent used has a key role in determining the activity of the extract.[24] This is due to the disparity in the relative solubility of different phytochemicals in the solvents with different polarities. The systemic phytochemical analysis of plant extracts is a significant strategy to discover the new phytocompounds of therapeutic value.[25] The preliminary phytochemical screening of the aqueous extract of *C. pepo* seeds was carried out. Phytochemical analysis revealed that (Table 1) the *C. pepo* seeds had appreciable levels of alkaloids and tannins and moderate levels of carbohydrates and steroids whereas the other phytocompounds were present in trace amounts. The result of the current investigation was in accordance with the findings of Malgwi[17] *et al.*, (2014) and Rawat[26] and Garg (2021) who reported that the above phytochemicals were present in aqueous extract of the *C. pepo* seeds.

Lead acetate may induce oxidative stress characterized by excessive free radical generation which leads to modification in enzymatic antioxidant system and disruption of membrane structure.[27] Significant antioxidant enzymes together form a mutually beneficial defence system against free radicals. Therefore,

Table 2: Enzymatic Antioxidant Concentration in Testis

Parameter	*Control*	*Only LA*	*Only PSE H.D*	*LA+ PSE L.D*	*LA+ PSE H.D*
SOD (unit/min/mg protein)	0.525 ± 0.0086	0.400 ± 0.0115***	0.545 ± 0.0086^{ns}	0.45 ± 0.0058**	0.515 ± 0.0144^{ns}
CAT (µmole of H_2O_2 consumed/ Min/mg protein)	28.52 ± 0.987	19.30 ± 0.216***	23.92 ± 0.56**	19.85 ± 0.548***	21.11 ± 0.456***
GPx (µg/mg protein)	4.605 ± 0.164	3.505 ± 0.1588**	4.35 ± 0.133^{ns}	3.59 ± 0.173**	4.27 ± 0.167^{ns}
LPO (nmol of MDA/mg protein)	13.35 ± 0.214	20.18 ± 0.736***	12.52 ± 0.424^{ns}	18.62 ± 0.416***	13.17 ± 0.225^{ns}

*Statistical comparison: (****P<0.001, **P<0.01, *P<0.05) Control group was compared with standard group-2. Treated groups 3, 4 and 5 were compared with group 1.*

oxidative stress has been assessed using these enzymatic antioxidants activities.[28-30] In the study undertaken, protective role of *C. pepo* seed extract on the lead induced effects in the serum and testicular oxidative status has been investigated through *in-vivo* method. The biochemical pathways underlying the lead toxicity were investigated by monitoring the levels of MDA and the activity of primary antioxidant enzymes such SOD, CAT, and GPx.

Determination of serum levels of SOD and GPx indicated that lead acetate treated group had a highly significant reduction in both the enzymatic antioxidants when compared to that of control. This finding is in agreement with the results of El-Sherbini[31] *et al*., 2017 who reported that the lead acetate-induced depletion of antioxidants is confirmed by the observed decrease in serum total antioxidants and circulating antioxidants. Lead acetate with seed extract at low dosage was found to have a considerable increase in SOD and GPx compared to lead acetate treated group whereas lead acetate with seed extract at high dosage had a highly significant improvement. *C. pepo* seed extract at high dosage when treated alone indicated SOD level almost same as control. This group also indicated a highly significant increase in SOD and GPx levels in comparison with other treated groups. The antioxidant activity exhibited by *C. pepo* seed extract in the reversal effect on the lead induced damage couldn't be overlooked. Hence, it can be deduced that *C. pepo* seed can exert potent antioxidant activity at higher doses and can reverse the lead induced oxidative damage.

Testis concentrations of SOD, CAT, GPx and MDA were estimated. The results revealed that lead acetate treated alone had a highly significant decrease in SOD, catalase and GPx and a highly significant increase in lipid peroxidation marked by high MDA level when compared to control group. A similar result was reported by Rania[32] and Heba, 2014 whose findings confirmed that SOD and CAT activities were significantly reduced (p< 0.001) in lead acetate treated rats. Rats exposed to lead experienced a substantial decrease in all antioxidant enzymes, including SOD and CAT, in the mitochondrial and post-mitochondrial fraction of the testis.[33] In the testes of lead-exposed rats, SOD and CAT activity levels were found to be significantly decreased.[34] Even though lead acetate with seed extract at low dosage had a highly significant reduction in the testis SOD, CAT and GPx when compared to that of control, MDA was significantly lower than that of lead acetate treated group. Co-administration of *C. pepo* seed extract and lead acetate led to an increase in the level of SOD, CAT and GPx when compared to its level in rats treated only with lead acetate. The rats treated with *C. pepo* seed extract high dosage alone had a remarkable increase in all the antioxidant enzyme concentrations when compared to other treated groups.

The present investigation revealed that MDA levels in the testis of the rats treated with lead acetate were substantially higher than the control group. This indicates that lipid peroxidation increased oxidative stress in the lead acetate-treated rats. It is understood that two processes, including enhanced ROS production and direct depletion of antioxidant reserves, might result in lead acetate-induced oxidative stress and tissue damage.[35] Lead exposure may generate intense lipid peroxidation that may disrupt the mitochondrial and cytoplasmic membranes, leading to more severe oxidative damage in the tissues and, as a result, releasing lipid hydroperoxides into circulation that represent the induction of oxidative stress.[30] At least in part, lead-induced testicular injury has been linked to toxicant-induced oxidative stress. A prolonged lead exposure results in increased lipid peroxidation and inhibited SOD function, and leads to testicular oxidative damage.[36, 37] In two distinct ways, lead acetate toxicity causes free radical damage, including the direct depletion of antioxidant reserves and the production of hydroperoxides, singlet oxygen, and hydrogen peroxides, which are measured by MDA levels as the end products of lipid peroxidation.[38] Rats treated with *C. pepo* seed extract at a dose of 1000 mg/kg body weight did not experience a rise in MDA when exposed to lead acetate. This indicates that the antioxidant activity of *C. pepo* seeds could minimalize the toxicity induced by lead acetate. Inhibiting lipid peroxidation as indicated by MDA levels, the antioxidant protection mechanism reduces oxidative stress and scavenges the free radical responsible for the testis injury. The significantly lower levels of MDA found in the tissues of the groups treated with *C. pepo* seed extract demonstrate that lipid peroxidation has been attenuated in comparison with the lead acetate group. It was confirmed that, *C. pepo* seeds work as a potent

antioxidant and free radical scavenger to lower the MDA level perturbed by lead acetate.

A similar finding has shown that, in rats with lead acetate-induced testis injury, vitamin C and E improved the antioxidant status and reduced lipid peroxidation. These results suggest that the antioxidant activities of vitamin C and E are primarily focused on the lipid component of cells. In numerous biological systems, antioxidants like vitamins C and E have been shown to prevent the production of free radicals and to reduce lipid peroxidation.[39, 40]

Compared to untreated control rats, we noticed an increase in oxidative stress in lead administered rats evidenced by the markedly decreased antioxidant enzymes levels in both the serum and testis tissue. The oxidative injury besides any defects in the components of the free radical antioxidant enzyme defense system may serve as an upstream pathway of amplified oxidative stress,[41] leading to high inclination of lipid to peroxidation. An antioxidant works by delaying and hindering the process of oxidation by free radicals. Increased levels of antioxidant enzymes clearly forecasted the antioxidant potential of *C. pepo* seed. As the first line of defence against reactive oxygen species (ROS), SOD is a crucial endogenous antioxidant enzyme that converts superoxide radicals to H^2O^2 and protects against the harmful effects of radicals.[42] Hydroxyl radicals are created as a result of this interaction, which can also be harmful. These enzymes work as antioxidants by virtue of scavenging these hydroxyl radicals.[43] *C. pepo* seed extract at these doses (100 and 1000 mg/kg) brought about dose dependent changes on these antioxidant parameters. Co-administration of *C. pepo* at high dosage (1000 mg/kg) along with lead acetate resulted in the significant reversal of the effect of the lead acetate. The activities of antioxidant enzymes under study were restored to their normal level by *C. pepo* administration to lead acetate treated rats. This investigation reveals a ROS scavenging activity of *C. pepo* seeds.

The observed ameliorative effect could be owing to the numerous phytochemicals in the *C. pepo* seed extract as they are reported to possess certain antioxidant activity. Among these, flavonoids have frequently been linked to the ability of any plant extract to act as an antioxidant. According to the theory put forth by Ye[44] *et al., 2012,* flavonoids have a very strong efficiency to expel free radicals from blood and promote the activity of antioxidant enzymes including SOD and CAT. These actions of flavonoids are also dose-dependent. This background explains why there was an increase in the serum levels of antioxidant enzymes in our study. Apart from these, *C. pepo* seeds have been shown to contain substances including polyphenols, anthocyanins, tannins, saponins, alkaloids, glycosides, steroids, iron, and vitamins A, C, and E, all of which have been linked to their antioxidant capacity by numerous researchers. The potential antioxidant properties of tannins were previously suggested by Tsumbu[45] *et al.,* 2011. Ghani,[46] 1990 proposed that alkaloids and glycosides have antioxidant properties. Therefore, the presence of these antioxidant chemical components, which are known to lower oxidative stress by a variety of mechanisms, can be used to explain the antioxidant activity of *C. pepo* seed extract.

The study could infer that lead acetate treatment could significantly decrease the antioxidant enzymes and augment lipid peroxidation. The enzymatic antioxidant inhibition amplifies free radicals in testicular tissues and thereby affects male reproductive ability. Co-administration of *C. pepo* seeds with lead acetate exhibited defensive effects against lead toxicity induced oxidative stress. The enzymatic antioxidants were notably improved and lipid peroxidation was decreased after *C. pepo* seed extract administration. These activities could be attributed to the numerous phytoconstituents of *C. pepo* seeds as found in this study as well as previously reported by other researchers. The current research could pave a way to prove scientifically that *C. pepo* seeds can enhance enzymatic antioxidants; thereby counteract the deleterious effects of oxidative stress. Hence this study strongly recommends *C. pepo* seeds as a potent natural antioxidant source promising active protection against lead toxicity induced oxidative stress which could be pharmacologically explored for drug synthesis. Further researches are required to decode the probable mechanism through which *C. pepo* seeds exert their antioxidant potential.

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