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Efficacy of Withaferin A on lead acetate induced testicular toxicity in Wistar rats - A biochemical analysis

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Abstract

Male infertility has become the major issue in reproductive biology. Almost 15 percent of Indian couples were affected by infertility and almost 40 - 50 percent will be of male infertility. The changing lifestyles, late marriages, improper food habits, reduced sleep, workplace tension, playing a crucial role in infertility. In developing countries toxic pollutants all over the environment in different ways also increase the intake of heavy metals into the body. The most important heavy metal is lead (Pb) due its usage in everyday life and its biological half life (28 to 36 days) in bloodIt is used in ammunition, lead pipes, electric vehicle batteries, used in radiation protecting gears. The widespread disadvantages of lead usage is explored and also there are strict guidelines to reduce its usage. Lead is a strong property of affecting the organism, especially increasing the oxidative stress thereby creating the imbalance in cellular level. The aim of the present research is to find the adverse effects of Lead and its management using the phytocompound Withaferin A, Vitamin A and Selenium.Oxidative stress is created using the lead acetate in groupsII,III & IV animals. The effects of lead toxicity is noted and confirmed in the blood parameters, antioxidant enzymes in testis and oxidative stress markers in testis. Oxidative stress induced animals in groups treated with Withaferin A and Vitamin A + Selenium. Results shown the reversal of the tissue damage in treated animals. Our results concluded the Withaferin A having the property of reversing the tissue damage in testis and its ameliorating effects proven in our present study

Keywords- Lead, Male infertility, Oxidative stress, Reproduction, Antioxidants.

Introduction

Millions of people around the world are affected by reproductive health issues. According to the (WHO) world health organisation data, 48 million couples and 186 million persons were affected. In developing countries like India there is a myth blaming the women for infertility. This creates much social pressure on women and evolves physically and mentally unstable in their marital life. Infertile women are facing the violence double the level of fertile women and data strongly says an increase in the level of violence faced by the women diagnosed with infertility. It is stated that infertility is a disease of the male or female reproductive system that causes failure to attain pregnancy duration after one year or more than one year of sexual intercourse. According to the World Health Organisation, in India primary infertility affects 3.9% to 19.6 %. The prevalence ofFactors of male infertility are (i) Oligospermia / azoospermia, (ii) Asthenospermia and (iii) Teratospermia. The causes for infertility are categorised as (a) Environmental / occupational, (b) Tobacco, marijuana and drugs, (c) Improper diet and weight loss or gain and (d) Ageing.

Sources of industrial chemicals

Lead is present in all over the environment like air, water, and soil. Lead is the most common toxic present in the environment and affects the nervous system and also affects the reproductive system of the organism because of the presence everywhere in the environment. Moreover usage of petroleum as major fuel consumption of mankind [1]. Lead is present in the leaded petroleum products, paints, cosmetics, plumbing materials. Lead usage in the batteries increases the production. Due to the heavy usage of petroleum products as fuels in the automotive vehicles to reduce the pollution, now the leading automobile manufacturers are concentrating on increasing electric vehicles. Due to the large usage of lead batteries due to cheap and efficient electrical surges, lead is the first choice in the production of batteries. General public consumption indirectly through contaminated water, beauty products and metal based medicine in alternate systems of medicine. Smoking cigarettes is another important source of exposure to lead. Single cigarettes consist of 0.6-2.0µg Pb^{2+} and $1/10^{th}$

of lead is inhaled in smoking. Due to the growing concern about presence of lead in day to day usage, The Government of India issued the law and notified the "Regulation on lead content in household and Decorative Paints Rules, 2016." It came into force from November 1, 2017. The law states that lead content in paints should not exceed 90 ppm. Even after government regulation there is no change in the manufacturing of leadbased paints. Toxic studies reflecting the poor implementation of rules. A study conducted in 2018 stating the potential of lead exposure among the children of Aligarh in Uttarpradesh[2].

Biological effects of lead

Industrial chemicals and heavy metals found to have a health hazard on the reproductive system [3]. One of the important heavy metals is lead [4-6]. In the study comparing infertile and fertile men found 12.5 microgram/dl and 6µg/dl respectively and found increased lead level in the blood infertile men [7]. Epidemiological studies also show increased blood lead level from 10 to 40 µg/dl in male workers and shows the increased risk of infertility due to lead toxicity[5,8].Another study of 4000 male workers with increased lead in their blood levels more than 25µg /dl, these workers showed reduction in the numbers of the children compared to control subjects [9].

Animal studies conducted in rats demonstrated lead as an active element in male reproductive imbalances in the parameter[10]. In contradictory studies conducted by Belgium, Finland, Italy and England found no correlation between lead exposure and decreased fertility rates [11]. Study conducted in French lead battery workers did not show any negative impact on the reproductive system [12]. Effects of leads on male reproductive system at low levels of exposure are not adequately studied. Recent studies show 15 percent of couples met failure in the pregnancy[12,13]. Studies also show there is male factors are more responsible for infertility, comes around half of cases related to the male infertility factors [14]the most important cause for the male related infertility due to the environmental exposure especially due to the occupational exposure in the developing countries and also because of the lack of knowledge of safety precautions while working in the hazardous environment

Genetic Polymorphism and male infertility

There is a strong relationship between genetic determinants and male infertility. One of the causes is gonadotropin releasing hormone (GnRH) deficiency. Y deletions are associated with non obstructive azoospermia[15].HLA-A28 and Bw40 (Human leukocyte antigen) expression with azoospermia is found but otherside studies supporting genetic response to environmental toxicity are scarcely documented. When compared to male infertility with female infertility with environmental toxicity,females are least affected in the work related exposures. Male are more sensitive to the toxic metals but the reason is not understood.

Spermatogenesis

Exposed individuals' sperm count and sperm concentration is markedly reduced in the studies [16,17]. A decreased volume of ejaculaton was found[17]. Animal studies clearly show abnormality in testicular tissue and functions [18-20]. Lead acetate administered mice showed reduction in the number of spermatozoa and in rats entire spermatogenesis is stopped[21]. Some researchers failed to show the relation of abnormal sperm morphology and lead exposure in rabbits [22]. Gross changes in the organs were found in exposed laboratory animals such as decreased weight of the testes, seminal vesicles, epididymis and prostate were found in the laboratory experiments[23,24]. There was a decrease in the germ cell layer population in the reproductive system [25-27]. Seminiferous tubule degeneration in

mice was explored in the studies[28]. Analysis using electron microscopy revealed that testicular alterations in monkeys and this alteration persist in their remaining life [29]. The functional alterations such as sperm Motility affected lead exposed workers [17]. Lead is having a negative impact in motility and viability at the level of ≤10µg/dl of lead in blood[30]. There is also a strong reduction in the functional maturity of sperm at the mean blood lead level of 45µg/dl [31]. In a study conducted in rats and mice dose dependent decrease in the number of sperm attaching to ova [32], penetration power also reduced and high level of loss of embryos in the post implantation [33].

Effect of lead in hormones

Lead exposed workers facing problems in hypothalamo pituitary – testosterone axis [27,34] and damaged hypothalamus and pituitary gland. There was also found hyper responsiveness to stimulation with gonadotropin and luteinizing hormones in lead toxicity.

Lead (Pb)

It is a wellknown heavy metal with toxic properties used worldwide in various forms, especially in developing countries. In India it is used extensively in various products such as leaded petrol, leaded paints, batteries and the list is going on. Especially childrens are especially a more vulnerable group due to the usage in the paints. Habit of licking doors in childhood and ingestion of particles of lead results in lead poisoning. Still is a vague line in defining the safe limit of lead exposure. But due to the devastating effects of lead in human beings, developed countries came forward to stop the lead contents in the paint. Lead is naturally occurring metal in the soil and its chemical formula is Pb. It means Plumbum derived from the Latin word for water work. It extensive use in the making pipes for the water works

Lead was added in the gasoline in the year 1920 in order to reduce the wear and tear and engine knocking. After the exposure of lead in industry workers makes them sick. This became an eye opening to limit the lead exposure but still the lead continued in the industries for its anticorrosive property, ductility. All these advantages over lead results in continuation of its usage. After 1970 leaded gasoline was taken out from the market and finally banned in 1996. In India ``Regulation on lead content in Household and decorative Paints Rules 2016". Even Though the implementation of law. Abolishing lead addition in the paints is not fully possible. According to the rule laid by the government, it should not exceed 90 ppm with effect from November 1, 2017. According to Quan Lu, an associate professor of environmental genetics and pathophysiology at Harvard T.H. Chan School of Public Health, investigating the harmful effects of lead on stem cells and development of the nervous system.

Effect of lead on reproduction

Air borne particles within the respirable range can easily enter into metabolism and can cause serious effects [35]. Most common airborne component lead and cadmium is a very important source of airborne heavy metals in the industrialised areas.Lead and cadmium accumulate in the male reproductive system [36]. Based upon the studies reported, it was found men exposed to industrial aerosol of lead results in the decreased family size, delay in conception and sperm count reduction. Lead concentration both in seminal plasma and blood plasma are increased in the lead exposed workers [37]. Concentration of lead in blood plasma and seminal plasma shows positive correlation. Workplace exposure of lead causes low infertility in the factory workers because of the increased duration of the lead exposure.

WITHAFERIN A

The terminology withanolides is the composition of the "withan" from the genus Withania, and "olide" a chemical terminology for the lactone moiety [38]. Withaferin A was the first withanolide isolated by Lavie in 1965 [39,40] from Withaniasomnifera. Since then, more than 400 withanolides have been isolated from 58 solanaceous species belonging to 22 genera [41-44]. Biological properties of *Withaniasomnifera* is due to the presence of secondary metabolites, called withanolides produced through mevalonic and non-mevalonate pathways with a contribution of 3:1[45]. The important chemical constituents of *WithaniaSomnifera* are withaferin A, withasomniferin A, 5-dihydroxy withanolide, withanolides I-VII, withanone, withanolide A, withanolide D, oxygenated and sulfatedwithanolide, somniferin, somniferinin, tropine and pseudotropine [46,47].The active principle of *Withaniasomnifera* is glycowithanolides (WSG), consisting of sitoindosides VII to X and withaferin [48]. Withanolides are a group of naturally available chemical compounds consisting of a steroidal backbone attached to a lactone or one of its derivatives [49].*Withaniasomnifera* has a various class of biologically active compounds and one of the biological active components is withaferin A.

VITAMIN E

In 1922 Evans and Bishop discovered vitamin E and it was initially noted as an antisterility factor called X [50].Vitamin E is a powerful supplement to increase the reproductive rate [51]. Further research found its ability to scavenge the reactive oxygen species (ROS) [52,53].

SELENIUM

Selenium is a good antioxidant able to reduce the effect of antioxidants and can balance the homeostasis of the system. Jacob Berzelius, Swedish chemist in 1817 found selenium and has been considered as an essential trace element for organisms [28]. Selenocysteine, an important component in proteins encoded by the TGA codon. Selenium is one of the important micronutrients for human beings[54]. It plays an important role in the antioxidant defence system. Previous studies suggest that the deficiency of selenium leads to various diseases [55]. It is commonly considered very important for human health as it protects the cells from harmful effects of free radicals [34]. Selenium compounds convert the free radicals into stable compounds [56]. Selenium is required for the function of the number of enzymes that depend on selenium. They maximise the activity of selenoenzymes such as selenium-containing glutathione peroxidases which reduce the damaging reaction oxygen species [56,57]. Selenium has beneficial effects against cadmium, induced hepatotoxicity [58], chromium induced hepatotoxicity [59] and CCl_4 induced hepatotoxicity [,60] and methotrexate induced hepatotoxicity [61].

MATERIALS & METHODS

Analytical grade lead acetate was purchased from Sigma Chemical company (St Louis Missouri, USA, Vitamin E and Selenium was also purchased from Sigma chemical company. Withaferin A, an active compound present in the Ashwagandha or also called *Withaniasomnifera* was also purchased from Cayman Chemicals, USA.

Animal maintenance

Male wistar Albino rats with a body weight of 180 to 200 grams were obtained from Mass Biotech, Chengalpattu, India. After procuring the animals, they were kept in a Polypropylene

cage containing sterile paddy husk as bedding. Animals were maintained in lighting and temperaturecontrolled laboratory conditions (22–25degree Celsius). Animals were maintained in a 12:12 hour light dark cycle. Rats were feeded with a standard pellet diet and tap water ad libitum. As per guidelines provided by the committee for the purpose of control and supervision on experiments on Animals, government of India (CPCSEA, 2003). Institutional animal ethical committee reviewed and approved the study (BRULAC/SIMATS/IAEC/12-2019/044).

Animal grouping

Animals were randomly segregated into 5 groups. Each group consists of 6 animals. Group – I: Control, Group – II: Lead acetate, Group – III: Lead acetate + Withaferin A, Group – IV: Lead Acetate + Vitamin E & Selenium, Group – V: Withaferin A.

Preparation of Lead Acetate solution

Lead acetate is purchased from the Sigma Chemical company USA. 0.15% solution of lead acetate is prepared using distilled water. Water with Lead acetate is kept as replacement for the regular water feed for the 3 groups of animals (group 2, 3 & 4). Water feeding with lead is continued for 8 weeks/60 day. Animals were monitored closely and morphological changes are monitored and documented.At the end of the experiment, animals were weighed, blood samples, tissue samples collected

Sample collection: Orbital technique used for blood sampling. Blood was collected from the retro-bulbar plexus of the medial canthus of the eye of the rat. The blood was kept at room temperature for 30 minutes to clot. Afterwards, the test tube containing the clotted blood sample was centrifuged at 3,000 revolutions per minute for 10 minutes.The clear serum supernatant was then carefully aspirated with syringe and needle and stored in a clean sample bottle at -20 degree Celsius until use for biochemical assay. Testis tissue homogenization prepared to find antioxidant estimation. The levels of creatinine, uric acid, urea, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (SGOT), alanine transferase (SGPT), low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides and cholesterol were analysed. Testicular Antioxidant enzymes superoxide dismutases (SOD), catalase (CAT), glutathione peroxidase (GPX) and oxidative stress markers lipid peroxidation(LPO), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) were also estimated using the standard procedure.

Statistical Analysis: Results were expressed as mean ± standard deviation, SD. Statistical analysis was carried out with a oneway analysis of variance (ANOVA) followed by Tukey's test. Values of $p < 0.0001$ were considered to be significant

RESULTS

Biochemical assays

The change in the biochemical parameter caused by the Withaferin A on lead induced animals, control group animals treated with lead acetate, group of animals treated with vitamin A and selenium were in the Tables. Renal function test tabulated in the Table 1. Liver enzymes tabulated in Table 2. Lipid profile tabulated in the Table 3. Testicular antioxidant level tabulated in the Table 4. Oxidative stress markers tabulated in the Table 5.Renal function test showed that there is a significant increase in the creatinine, uric acid and urea level in Lead group

Table 1: Effect of Withaferin A on creatinine, uric acid & urea parameters of animals induced with lead acetate. (Kidney function parameters)

Treatment	Control	Lead	Lead + Withaferin A	Lead + Vitamin A & Selenium	Withaferin A
Creatinine	0.75±0.07	1.17±0.06^{a****}	0.81±0.15^{b***}	0.87±0.11^{b***}	0.74±0.7^{b****}
Uric acid	1.1±0.01	3.09±0.4^{a****}	1.33±0.25^{b****}	1.27±0.05^{b****}	1.74±0.31^{b****}
Urea	18.88±0.93	28.04±0.6^{a****}	18.93±0.22^{b****}	22.96±0.55^{b****}	20.24±0.13^{b****}

Data were expressed as mean ± SD. a: significantly different when compared to the control group ($p < 0.05$); b: significantly different when compared to the lead acetate treated group ($p < 0.05$) (n = 6), (P value <0.0001is denoted as ****).

Table 2 Effect of Withaferin A on ALP, SGOT & SGPT parameters of animals induced with lead acetate.

Treatment	Control	Lead	Lead + Withaferin A	Lead + Vitamin A & Selenium	Withaferin A
ALP	109.58±1	135.86±2^{a****}	68.28±0.88^{b****}	72.39±1.22^{b****}	54.17±0.8^{b****}
SGOT	86.44±0.84	120.45±13.63^{a****}	41.73±0.71^{b****}	3.33±0.34^{b****}	6.67±0.79^{b****}
SGPT	13.89±0.35	22.32±0.13^{a****}	17.5±0.35^{b****}	18.64±0.3^{b****}	20.83±0.45^{b}

Data were expressed as mean ± SD. a: significantly different when compared to the control group ($p < 0.05$); b: significantly different when compared to the lead acetate treated group ($p < 0.05$) (n = 6), (P value <0.0001is denoted as ****).

Table 3: Effect of Withaferin A on LDL, HDL, Cholesterol & Triglycerides parameters of animals induced with lead acetate.

Treatment	Control	Lead	Lead +	Lead +	Withaferin A

			Withaferin A	Vitamin A & Selenium	
LDL	37.85±0.5	58.88±0.33^{a****}	36.61±0.45^{b****}	28.11±0.71^{b****}	29.34±0.6^{b****}
HDL	55.65±0.46	32.76±0.46^{a****}	38.17±0.39^{b****}	39.79±1.02^{b****}	54.85±0.51^{b****}
Cholesterol	32.26±0.31	53.29±0.96^{a****}	35.15±0.35^{b****}	38.03±0.57^{b****}	35.69±0.96^{b****}
Triglycerides	42.37±8,88	66.74±0.43^{a****}	44.62±2.26^{b****}	52.83±0.08^{b****}	41.65±0.47^{b****}

Data were expressed as mean ± SD. a: significantly different when compared to the control group ($p < 0.05$); b: significantly different when compared to the lead acetate treated group ($p < 0.05$) ($n = 6$), (P value <0.0001is denoted as ****).

Table 4 Effect of Withaferin A on antioxidant enzymes (SOD, CAT, GPx,) of animals induced with lead acetate.

Treatment	Control	Lead	Lead + Withaferin A	Lead + Vitamin A & Selenium	Withaferin A
SOD	19.9±0.25	12.75±0.88^{a****}	17.96±1.56^{b****}	21.99±0.77^{b****}	21.26±0.63^{b****}
CAT	5.27±0.91	2.43±0.3^{a**}	4.2±0.38$^{\mathbf{b**}}$	4.25±1.34^{b} (ns)	4.06±1.54^{b} (ns)
GPX	3.79±0.2	1.21±0.37^{a****}	2.62±0.14^{b****}	2.76±0.33^{b****}	3.35±0.42^{b****}

Data were expressed as mean ± SD. a: significantly different when compared to the control group ($p < 0.05$); b: significantly different when compared to the lead acetate treated group ($p < 0.05$) ($n = 6$)(P value <0.0001is denoted as ****)

Table 5 Effect of Withaferin A on oxidative stress markers (LPO, OH radical, H2O2) of animals induced with lead acetate.

Treatment	Control	Lead	Lead + Withaferin A	Lead + Vitamin A & Selenium	Withaferin A
LPO	74.34±10.68	41.62±1.13^{a****}	68.45±3.42^{b****}	76.09±0.98^{b****}	80±4.6^{b****}
OH RADICAL	20.89±4.09	40.73±0.55^{a****}	26.97±0.88^{b****}	29.9±0.75^{b****}	32.64±0.56^{b****}
H2O2	32.13±0.19	57.84±2.93^{a****}	24.46±0.31^{b***}	26.43±1.09^{b****}	33.37±5.53^{b****}

Data were expressed as mean ± SD. a: significantly different when compared to the control group ($p < 0.05$); b: significantly different when compared to the lead acetate treated group ($p < 0.05$) ($n = 6$). (P value <0.0001is denoted as ****)

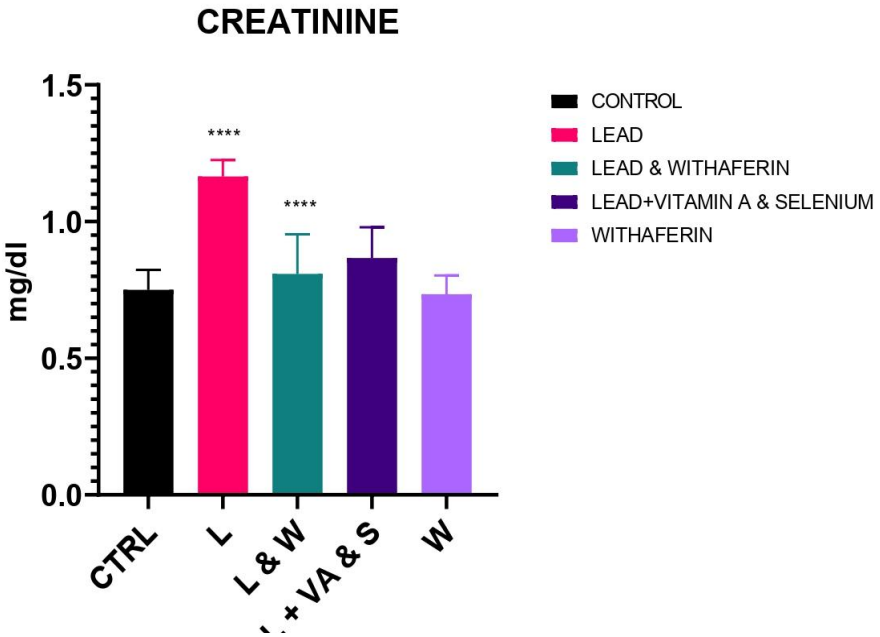

Figure 1: Bar graph shows the level of creatinine in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the Creatinine level in mg/dl. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

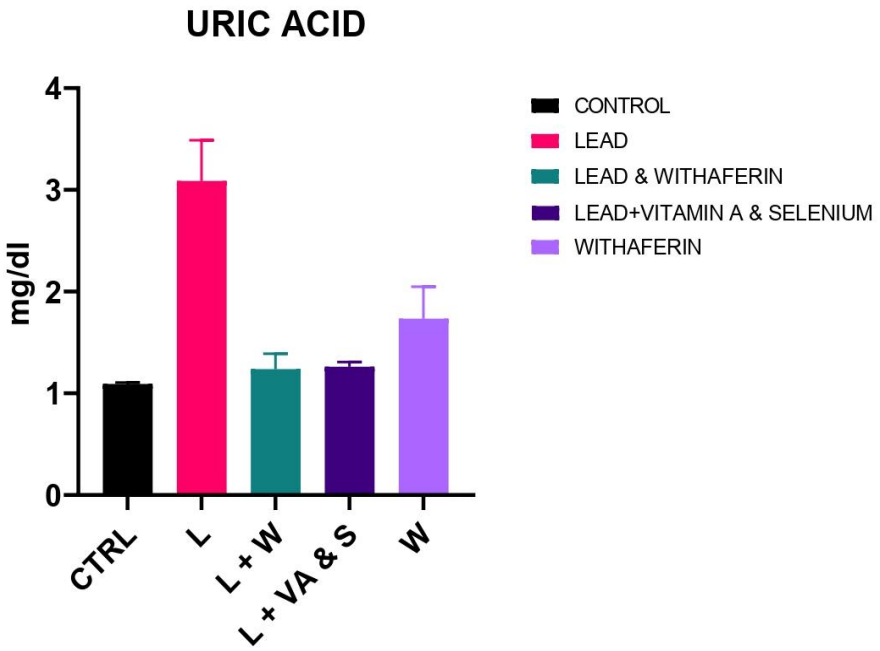

Figure 2: Bar graph shows the level of uric acid in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the uric acid level in mg/dl. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

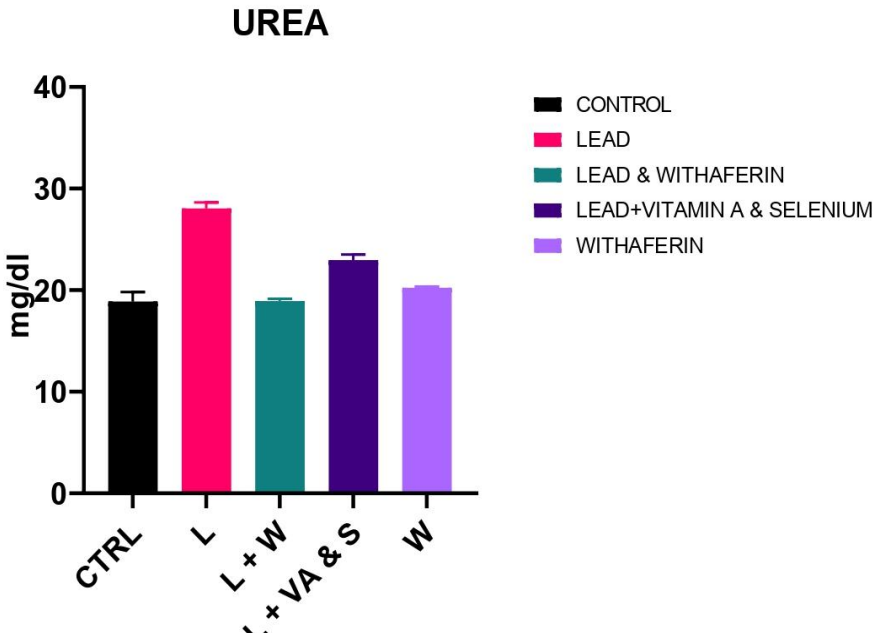

Figure 3: Bar graph shows the level of urea in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in mg/dl. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

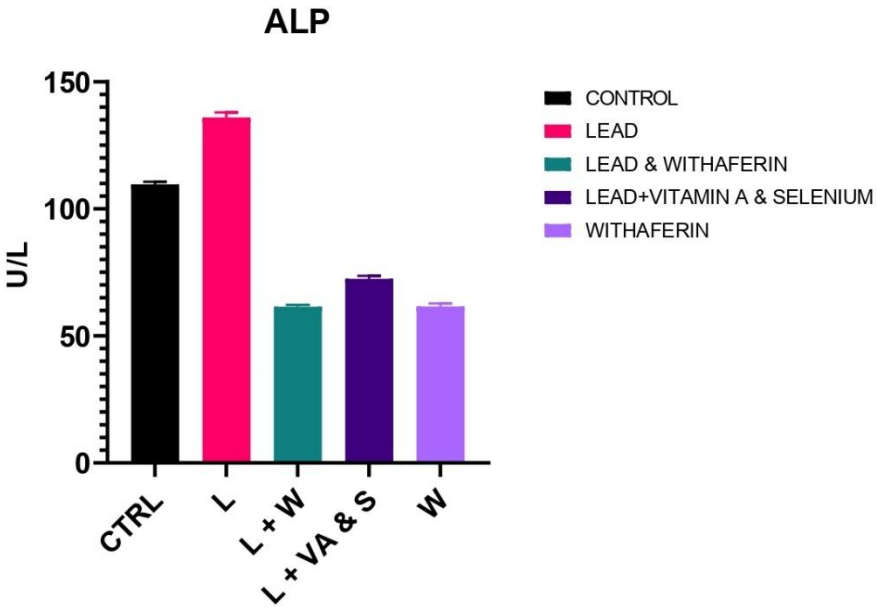

Figure 3: Bar graph shows the level of ALP in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in U/L. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

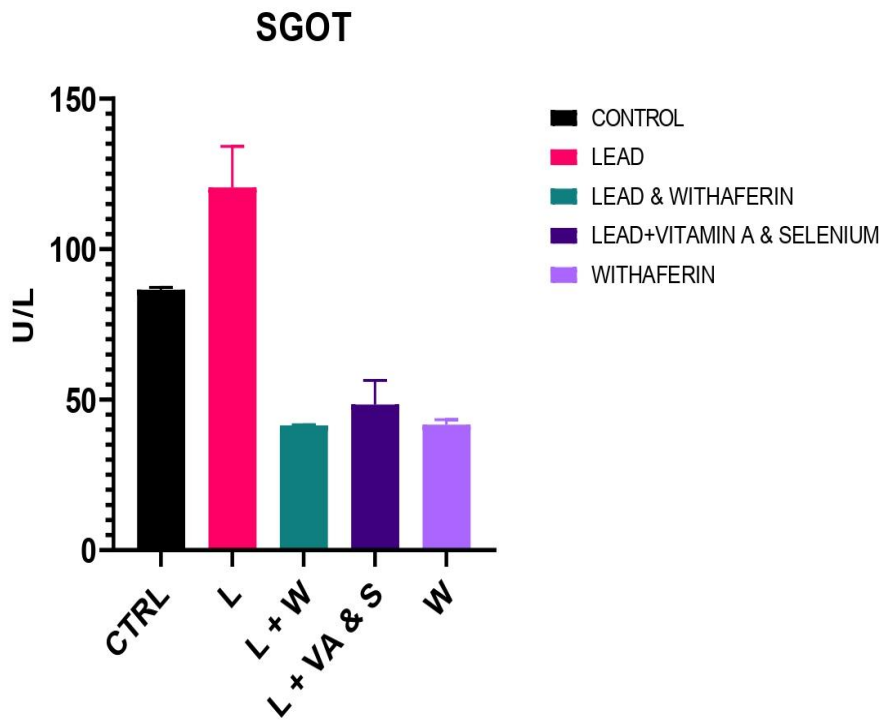

Figure 5: Bar graph shows the level of SGOT in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in U/L. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

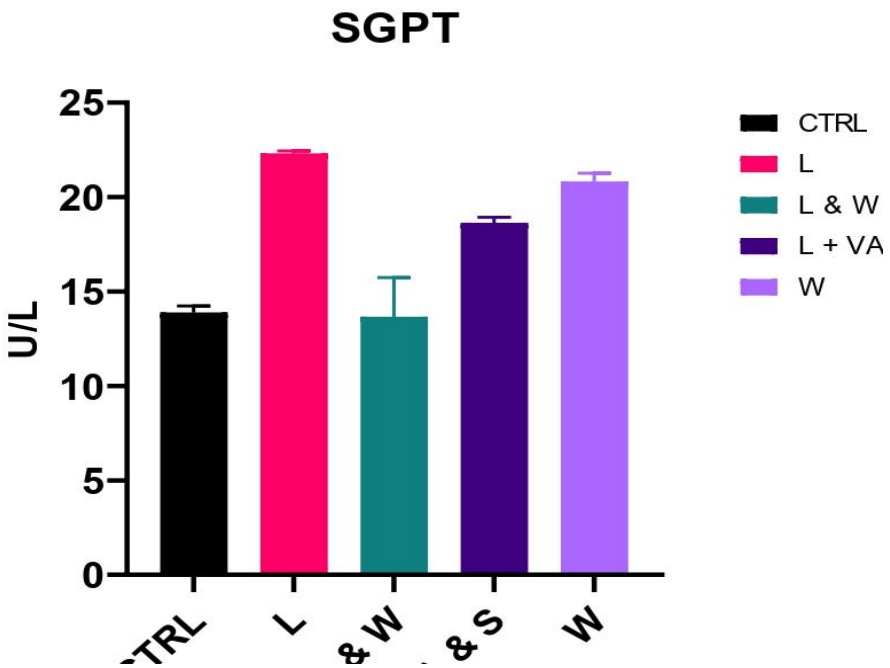

Figure 6: Bar graph shows the level of SGPT in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in U/L. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

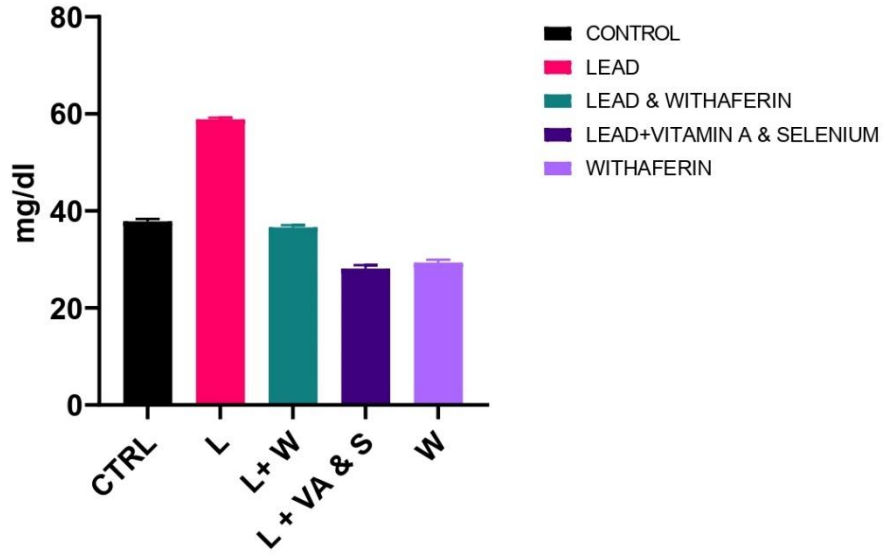

Figure 7: Bar graph shows the level of LDL in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in mg/dl. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

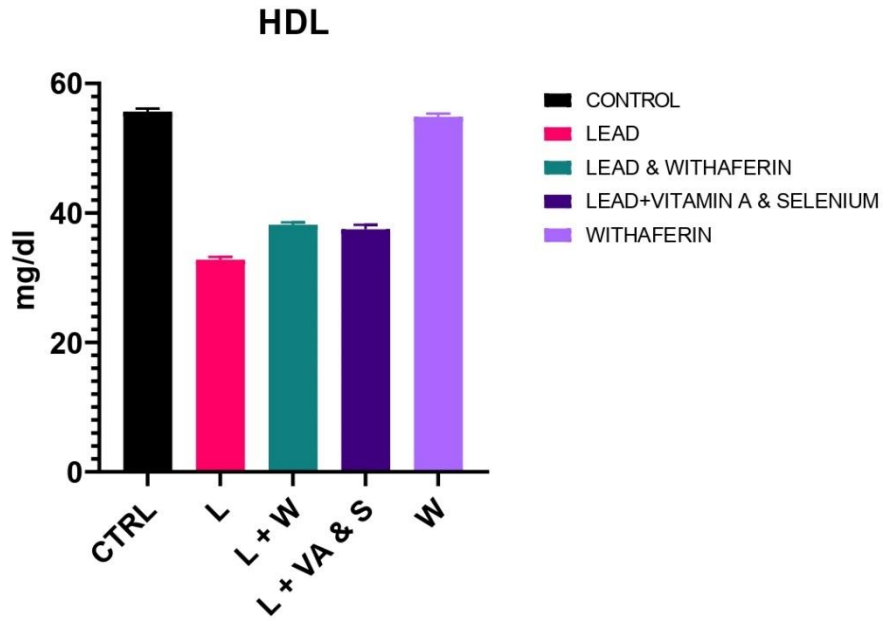

Figure 8: Bar graph shows the level of HDL in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in mg/dl. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

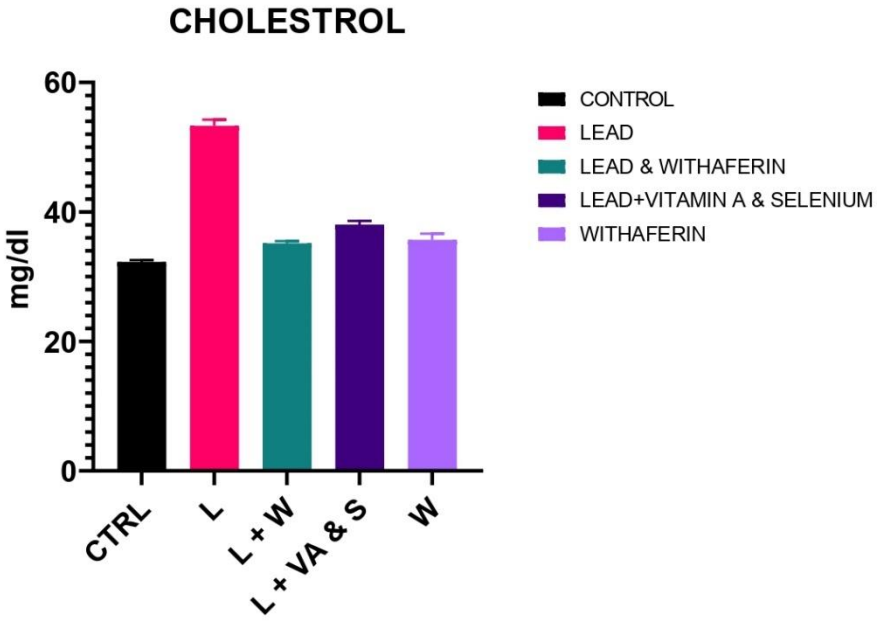

Figure 9: Bar graph shows the level of cholesterol in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in mg/dl. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

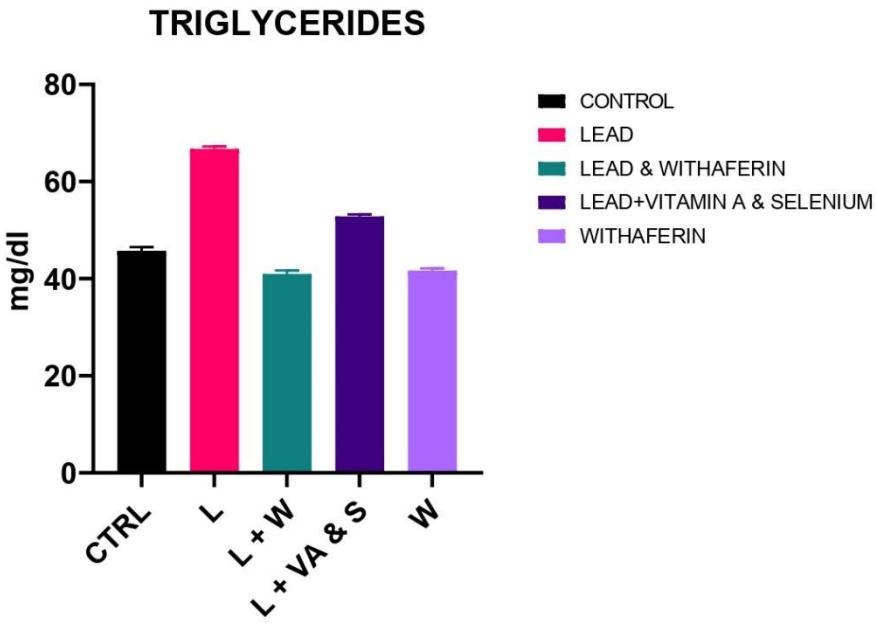

Figure 10: Bar graph shows the level of triglycerides in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in mg/dl. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I

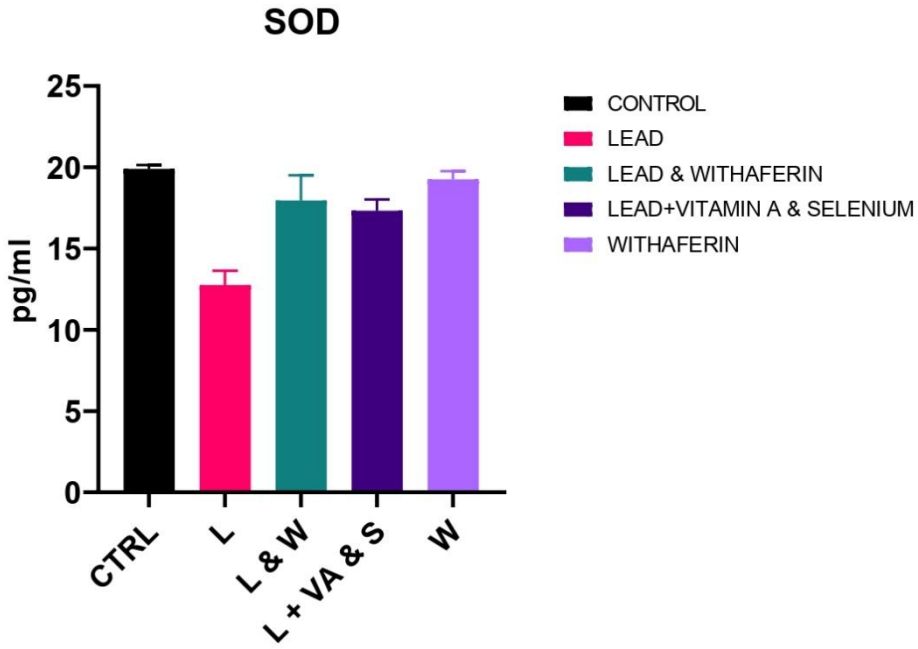

Figure 11: Bar graph shows the level of SOD in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in pg/ml. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

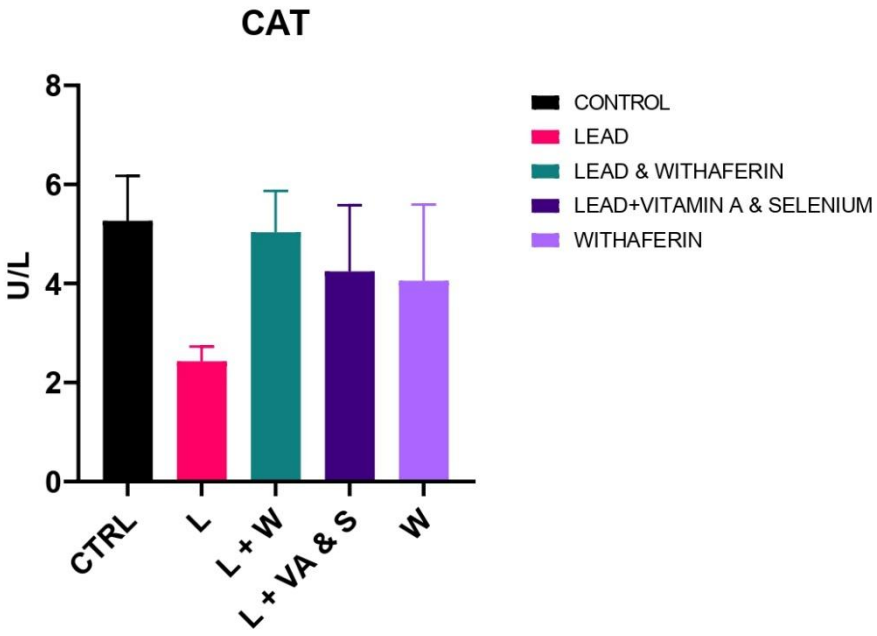

Figure 12: Bar graph shows the level of CAT in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in U/L. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

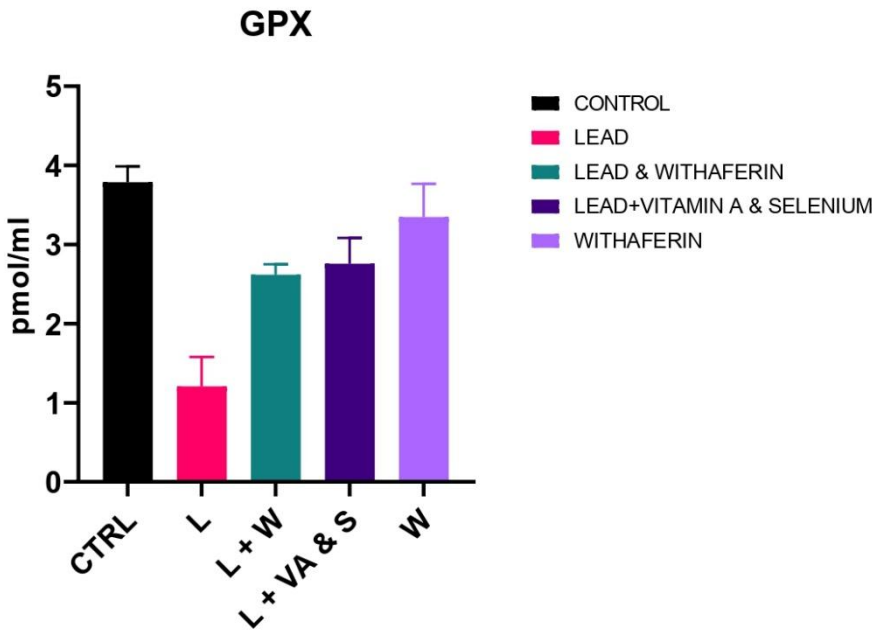

Figure 13: Bar graph shows the level of GPx in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in pmol/ml/dl. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

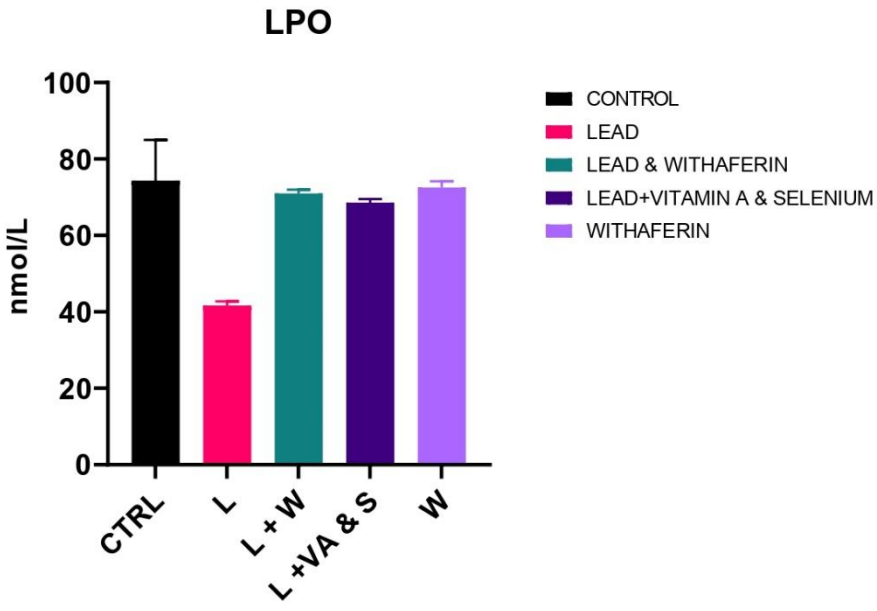

Figure 14: Bar graph shows the level of LPO in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in nmol/L. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I

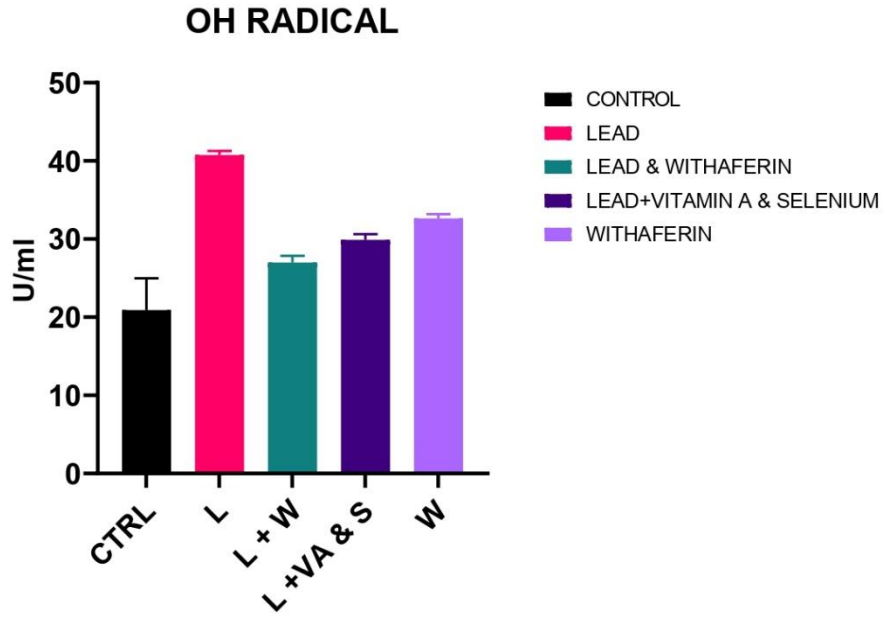

Figure 15: Bar graph shows the level of OH radical in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level in u/ml. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I

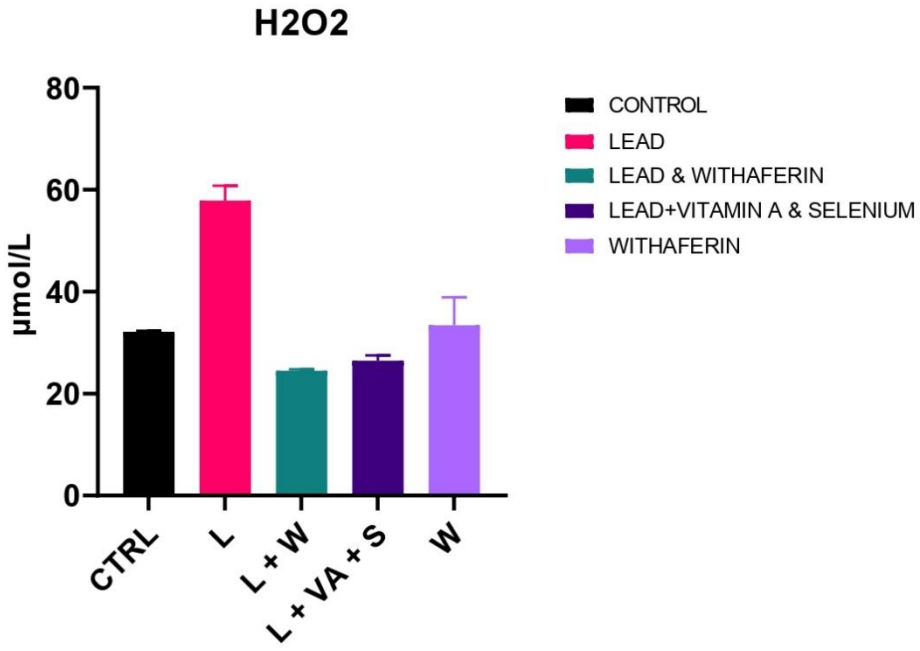

Figure 16: Bar graph shows the level of H2O2 in Control and experimental groups. The x-axis represents the grouping and Y-axis represents the urea level inumol/L. All the values are expressed as Mean ± Standard Deviation. On comparing between the groups, Group-II showed (P<0.0001) indicating statistically significant increase from Group-I.

Discussion

Oxidative stress is the important mechanism in all the diseases and also it is playing a characteristic feature in the male infertility [52]. Lead toxicity causes major damage to sperm quality by disturbing the antioxidant and reactive oxygen species (ROS) balance and finally leads to abnormalities of spermatogenesis and male infertility [56]. In the present study the effect of lead toxicity on male fertility is investigated in terms of biochemical analysis. The observations revealed that the lead exposure showed detrimental impact on male reproductive system especially on testis leading to impaired spermatogenesis and testicular dysfunction.

Renal function tests showed that there is a significant increase($P< 0.05$) in the serum creatinine, uric acid and urea level in Lead group than Control group. And it was much decreased in Lead with Withaferin A treated group and Lead with Vitamin A and Selenium treated group. Within this treatment group, Lead with Withaferin A treated group showed better reduction and restoration of creatinine, uric acid and urea level within the limit nearer to Control. This could be due to the free radical quenching property of Withaferin A under oxidative stress condition inflicted by lead toxicity on major functioning organs such as kidney. This damage to various system leads to organ dysfunction reflecting on elevated renal enzyme levels [62].

Some reports documented that lead toxicity results in healthrelated impairments characterized by marked antioxidant suppression together with lipid peroxide elevation leading to oxidative imbalance[63].

Liver function tests revealed that there is a significant increase($P< 0.05$) in the serum ALP, SGOT and SGPT level in Lead group than Control group. Moreover it was very much decreased in Lead with Withaferin A treated and Lead with Vitamin A and Selenium treated group. Within this treatment group, Lead with Withaferin A treated group showed better reduction and reestablishment of ALP, SGOT and SGPTlevel nearer to Control group. This could be because of the hepatoprotective and liver enzyme modulating effect of Withaferin A on lead acetate induced toxicity[64].

Yangliu Xia et al., 2022 reported that Withaferin A is used in the treatment of several liver diseases such as hepatic injury, hepatic cancer and systemic inflammation. The therapeutic property of this isolated individual active component Withaferin Afrom *Withaniasomnifera* was well explored and scientifically validated very recently by animal experimentation[65].

LDL, cholesterol andtriglyceride levels were elevated significantly (($p < 0.05$) to 2 fold in Lead group than Control group. Moreover it was decreased in Lead with Withaferin A treated and Lead with Vitamin A and Selenium treated group. Among the treatment group, Lead with Withaferin A treated group showed comparatively remarkable improvement in restoring the lipid levels. Whereas the HDL level was significantly decreased in Lead group than Control group and increased in both the treatment groups. Our present result depicts an association with lipid peroxidation process and cellular oxidative stress phenomenon which lead to the increase in serum parameters depicting the cytotoxicity of long term lead exposure.

Studies reported that Withaferin A has many pharmacologically wellestablishedproperties such as anti-inflammatory, anti-stress, antitumor, antioxidant, hepatoprotective,immune-modulatory, antiproliferative, cardioprotective, hypoglycemic, diuretic, and hypercholesterolemic properties.

In our study, superoxide dismutase is showing significant difference between the lead induced animals versus withaferin A treated animals (P value <0.0001) and also significant difference between lead induced animals versus vitamin E & Selenium treated animals. Catalase shows the significant difference among the lead induced animals versus withaferin A treated animals(p value <0.0026) but there is no significant change compared to lead induced animal versus vitamin E & Selenium group animals. Glutathione peroxidase shows the significant difference among the lead induced animal versus withaferin A treated animals (p value <0.0001) and also significant changes with vitamin E and selenium.

Study conducted by Mabrouk and Ben Cheikh shows no significant difference with lead induced animals with thymoquinone treated animals [66]. Thymoquinone is showing potent antioxidant property but it does not produce any significant changes alone. Antioxidant property of zinc against testicular oxidative is only because of the competition between zinc and lead binding factors. Our study also showed the lipid peroxidation markers between lead induced animals versus withaferin A treated animals (p value <0.0001) and also with Vitamin E and Selenium. Hydroxyl radical markers show significance between the lead induced animals versus Withaferin A treated animals (p value <0.0001) and also with lead induced versus vitamin E and selenium. Vitamin E and selenium show the effectiveness but it is not acting standalone in treating the oxidative stress.

In our study there is significant change observed when compared to lead induced animals versus vitamin E & selenium treated animals. Withaferin A is a standalone choice of substance in managing oxidative stress significantly showing positive results. Further studies should be conducted for better understanding of withaferin A as a potent antioxidant. Our study concludes that the activities of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase(GPX), and oxidative stress markers such as lipid peroxidase (LPO), H2O2 and OH radical in testis were restored to normal level by withaferin A administration to lead acetate treated rats. The spermatozoa membranes are predominantly were polyunsaturated fatty acids hence are susceptible to ROS attack and lipid peroxidation.

Conclusion

The inability of the body to readily detoxify the reactive intermediates and impairment to repair the resulting damage occurs as a consequence of imbalanced antioxidant system upon lead induced oxidative stress. So the availability of substances with antioxidant properties plays a great role in the body's defence system. Our finding gives a better result in reversing the tissue damage in the organs that we can infer from the parameters analysed and also through the antioxidant markers showing the promising results. Hence, it might be postulated that the protective effect of withaferin A could be attributed to its antioxidant principles and ROS scavenging effect which highlights its therapeutic role upon testicular toxicity upon lead exposure.

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