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Comparative Study On The Ameliorative Potential And Synergistic Effect Of Water Melon Rind Species (Charsleton Gray , Jubilee And Dark Green) On Lead Acetate Induced Testicular Toxicity On Semen Parameters And Serum Biochemical Parameters (Ast, Alp And Alt) In Male Albino Rats

Oladiran Samson Sunday

Dr. Inalegwu Bawa

Department of Biochemistry, University of Agriculture,
Makurdi, Benue State, Nigeria

Abstract: ***The present study investigated the comparative ameliorative potential and synergistic effect of watermelon rind species (Charleston gray, Jubilee and Dark green) on lead acetate induced testicular toxicity on semen parameters (sperm count, motility, viability and morphology), and liver enzymes (ALT, AST and ALP) in male albino rats. Fifty adult male albino rats of weights between 150g and 200g, having been subjected to two weeks of acclimatization, were assigned into ten groups of 5 each. Group 1 served as normal control and received normal rat chow and water, while group 2 served as experimental control and received 2.25 mg/kg of lead acetate only. Groups 3,4 and 5 were co- administered with 2.25 mg/kg of lead acetate and 100 mg/kg of WRJA,WRJB and WRJC respectively, while groups 6,7 and 8 were co-administered with 2.25 mg/kg of lead acetate and 200 mg/kg of WRJA, WRJB and WRJC respectively. Finally, group 9 was co-administered with 2.25 mg/kg of lead acetate and 100 mg/kg of mixtures of WRJA, WRJB and WRJC, while group 10 was co-administered with 2.25 mg/kg of lead acetate and 200 mg/kg of mixtures of WRJA, WRJB and WRJC. The lead acetate and the watermelon rind juice were administered orally to the rats for 35 days. On day 36, blood samples were collected from anaesthetized rats by cardiac puncture for determination of some biochemical parameters: ALT, AST and ALP. The testes were also harvested for determination of semen parameters: sperm count, motility, viability and morphology according to standard laboratory procedures. Results obtained showed that, There was a significant ($p \leq 0.05$) reduction in the levels of sperm count, motility and viability in group 2 when groups 3, 4, 6, 7, 9 and 10 were ,in turn, compared with group 2. AST, ALT and ALP activity showed a significant ($p \leq 0.05$) decrease in groups 3, 4, 5 and 9 when in turn compared with group 2. In conclusion, findings from the study suggest that the ameliorative potential of watermelon rind could be dosage dependent which vary with different varieties and could have synergistic effects.***

NB:WRJA: watermelon rind juice from lightgreen specie, WRJB: watermelon rind juice from stripped specie, WRJC: watermelon rind juice from darkgreen specie.

Keywords: watermelon, testicular toxicity, semen parameters, liver enzymes

I. INTRODUCTION

Watermelon (*Citrullus lanatus)* is a tropical plant that belongs to the family of cucumber (*Cucurbitacea*); it grows in almost all part of Africa and South East Asia. It is called Elegede in Yoruba language, Anyu in Igbo, Kankana in Hausa and Pepo in Latin.

The fruit is a berry with a thick smooth exterior rind (exocarp) and a sweet, edible, juicy and fleshy center mesocarp and endocarp. The rind is usually discarded, it

may be applied to feeds or used as fertilizer; but it is also edible and may be used as a vegetable (Pons, 2014). The rinds can also be fermented or blended (Mandel *et al*., 2005). The inner portions of the rind which is usually light green or white contains many hidden nutrients and is also edible; however, most times it is avoided due to its unappealing flavor. It contains mainly citrulline which is a known stimulator of nitricoxide (Rimando and Perkins-Veazie, 2005). The rind has been shown to contain alkaloids, saponin, cardiac glycosides, flavonoids, phenol, moisture, lipid, protein, fiber and carbohydrates (Erukainure *et al*., 2010).

The different varieties of watermelon include: little baby flower, yellow doll, crimson sweet, golden midget, moon and stars, starbrite, stars 'n' stripe, charsleton gray , jubilee, sugar baby, empire no.2, china baby, green mountain, new dragon, minipol, mielhart pilimore, golden crown, eve, liliput, extazy, densuke watermelon, melitopolski, cream of saskatchewan, the moon and stars, orangeglo, yellow crimson watermelon, carolina cross, diana, hybrid blackita f1, hybrid marita f1and hybrid early mara f1.

Some health benefits of watermelon include: Cardiovascular and bone health, reduces body fat, anti-inflammatory and antioxidant support, diuretic and kidney support, muscle and nerve support, alkaline-forming, improves eye health, immune support, wound healing and prevents cell damage (Sikka and Wang, 2008).

Several environmental factors have been shown to have adverse effects on male reproductive function (Plas *et al*., 2000). Perhaps the progressive decline in male reproductive health and fertility over the past 30 years may be linked to these environmental toxicants and xenobiotic agents (Sikka and Wang, 2008). One of these toxicants, reported the adverse effects on male reproductive function in lead (Chowdhury, 2009). Lead is an abundant heavy toxic metal which is known to induce a broad range of physiological and behavioral dysfunction in humans Lead poisoning still remains an important health problem associated with several clinical symptoms with limited molecular mechanism underlying the toxicity (Falana and Oyeyipo, 2012). Recent studies suggests that oxidative stress is a potential contributor to lead toxicity and that lead directly and or indirectly changes the pro- oxidant and antioxidant balance in biological tissues and all toxic metals have in common the ability to cause oxidative damage .Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage (Ercal *et al.*,2001).

Therefore, there is a need to identify naturally occurring nutritional agents that could possibly help ameliorate the toxic effects of lead on the reproductive system. Anecdotal reports from our environment suggests that consumption of watermelon fruits possibly enhances sexual activity. The present study therefore attempts to explore the possible ameliorative effects of consumption of the rind of watermelon on lead acetate induced toxicity on the reproductive system using male albino Wistar rats as models.

II. MATERIALS AND METHODS

A. CHEMICAL REAGENT

Lead acetate, Picric acid solution, Mayer's reagent , Ferric chloride solution ,Conc. H_2S04, Acetic anhydrides ,1% aqueous HCl, Chloroform, Glacial acetie acid , eosin solution, normal saline, 10% formalin, ethanol, xylene , paraffin wax, hematoxylin, Conc. Ammonia solution, distilled water, methanol, folincio caltean, 20% aqueous ethanol, normal butanol, 5% aqueous sodium chloride, saturated sodium carbonate solution, ethyl acetate, amyl alcohol.

B. EQUIPMENT

Test tubes, Racks, Beakers and water bath, Round bottom flasks ,Blender, glass slide, light microscope, Neubauer haematocytometer, hormonal kits, digital weighing balance, mortal and pestle, whatman filter paper, spatula, micro pipette, water bath, crucible, conical flask, plastic cages, stirrer, Buchner funnel, hand gloves, mortal and pestle, oven, desicator, spectrophotometer, evaporating dish.

C. COLLECTION OF PLANTS AND RATS (EXPERIMENTAL ANIMALS)

a. WATERMELON FRUITS COLLECTION

Three varieties of fresh fruits of watermelon were obtained from Lafia main market, Lafia Nasarawa state, Nigeria. These are: Charleston gray (light green specie), Jubilee (stripped green specie) and Sugar baby (dark green specie).

b. EXPERIMENTAL RATS COLLECTION

Fifty male albino rats of weight between 150 and 200g were used for this study. They were purchased from Biochemistry Division animal house of National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. They were housed in metal cages and fed with pelletized rat feed and water. The rats were acclimatized for two weeks.

c. SAMPLE PREPARATION

Three varieties of fresh fruits of watermelon were obtained from Lafia main market, Lafia Nasarawa State, Nigeria. These are: Charleston gray, Jubilee and Sugar baby. The fruits were washed by tap water and peeled. Rind was separated from flesh and blended by household electrical blender. Watermelon Rind Juice was filtered through a filter paper. The filtered juice was placed in a glass bottles and pasteurized in a covered water bath at a temperature of 72oC for 15s. The juice was then kept in a refrigerator (4oC) until use.The fruits were divested of the red pulp and peeled. The green part between the real pulp and the peel was blended using an industrial blender. Water was added to the blended pulp and filtered through a muslin cloth. The filtrate was further filtered using a cotton wool and the filtrate poured into

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a stainless steel trays and dried in the ovum at 50oC to obtain the extract.

d. RECONSTITUTION OF EXTRACT FOR ADMINISTRATION

The extracts were reconstituted in distilled water before administration to the rats. The concentration of the extracts administered were prepared using the formula below:

$$\text{Concentration (mg/ml)} = \frac{\text{Dosage (mg/kg x weight of rats (kg)}}{\text{Volume (ml)}}$$

The maximum convenient volume for rats 20ml/kg as prescribed by the OECD, (2001) was used.

Quantities of extract per ml differed according to the dosages administered to the groups of rats.

The volume that each rat took was calculated as follows

$$\text{Volume (ml)} = \frac{\text{Dosage (mg/kg) x weight of rats (kg)}}{\text{Concentration (mg/ml)}}$$

D. EXPERIMENTAL DESIGN

Fifty male albino wister rats weighing between 150 and 200g were used for this study. They were purchased from National Veterinary Research Institute (NVRI) Vom, Jos, Plateau state, Nigeria. The animals were housed in cages for two weeks and allowed to acclimatize and fed with standard diet and water ad-libitum.They were divided into ten groups (Groups 1 to 10) of 5 rats each. Rats in each group were numbered 1 to 5 and placed in separate cages in the Animal House of Federal University of Agriculture, Makurdi, Nigeria under natural day and night cycles. The rats were allowed free access to normal rat chow and tap water *ad libitum*. They were allowed two weeks of acclimatization to their environment and subsequently treated for five weeks as stated below. The work lasted for a period of seven weeks (two weeks of acclimatization plus five weeks of treatments).

a. EXPERIMENTAL ANIMAL GROUPING

Group 1: Normal control group fed normal rat chow and water.

Group 2: Experimental control group received 2.25 mg/kg Lead acetate only.

Group 3: Received 2.25 mg/kg of Lead-acetate + 100 mg/kg (Low dose) of juice from *C. lanatus* rind (WRJA)

Group 4: Received 2.25 mg/kg of Lead-acetate + 100 mg/kg (low dose) of juice from *C. lanatus* rind (WRJB).

Group 5: Received 2.25 mg/kg of Lead-acetate + 100 mg/kg (low dose) of juice from *C. lanatus* rind (WRJC).

Group 6: Received 2.25 mg/kg of Lead-acetate + 200 mg/kg (High dose) of juice from *C. lanatus* rind (WRJA).

Group 7: Received 2.25 mg/kg of Lead-acetate + 200 mg/kg (High dose) of juice from *C. lanatus* rind (WRJB).

Group 8: Received 2.25 mg/kg of Lead-acetate + 200 mg/kg (High dose) of juice from *C. lanatus* rind (WRJC).

Group 9: Received 2.25 mg/kg of Lead-acetate + 100 mg/kg (low dose) of juice from *C. lanatus* rind (WRJA + WRJB+WRJC)

Group 10: Received 2.25 mg/kg of Lead-acetate + 200 mg/kg (High dose) of juice from *C. lanatus* rind (WRJA + WRJB+WRJC)

NB: WRJA: watermelon rind juice from lightgreen specie, WRJB: watermelon rind juice from stripped specie, WRJC: watermelon rind juice from dark-green specie.

b. INDUCTION

Testicular toxicity was induced in the experimental albino rats by oral gavages administration of lead acetate ($C_4H6O_4Pb.H_20$) at the dosage of 2.25mg/kg b.w. (Megalli *et al*., 2005).

c. TERMINATION, COLLECTION AND PREPARATION OF SERA SAMPLES

After 35 days of treatment, the animals were fasted for 24 hours prior to sacrifice. The animals were anaesthetized using chloroform and then sacrificed on the 36th day, blood samples were collected from the anesthetized rats through cardiac puncture. The blood samples were placed in heparinized sample bottles and subsequently centrifuged at 1500 rpm for 5 minutes and plasma obtained for assay of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone.

E. DETERMINATION OF BIOCHEMICAL PARAMETERS

a. DETERMINATION OF SEMEN PARAMETERS

b. MOTILITY

As described by Kaur and Bansa, (2004), the caudal epididymis was identified and its content squeezed into 1ml of normal saline at room temperature. One drop of the semen suspension was discharged into a Makler counting chamber and the number of motile and non-motile spermatocytes counted in ten random fields. The number of motile spermatocytes was then expressed as a percentage of the total number of the counted spermatocytes (Mahaneem *et al*., 2011).

c. SPERM MORPHOLOGY

This was determined by smearing a drop of the stained semen suspension obtained during determination of sperm count on a glass slide; the smear was allowed to dry and subsequently examined under the light microscope at X 400 magnification. For each sample, spermatocytes were carefully observed and the percentage of total abnormalities of the spermatocyte head and total abnormalities of the spermatocyte tails were determined as described by (Narayana *et al*., 2005).

d. SPERM VIABILITY

Fluid from the caudal epididymis was carefully dropped on a slide and mixed with a drop of 0.5 % eosin

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solution. After 2 minutes, the slide was examined under a light microscope at X 40 magnification. The percentage of viable (unstained) and non-viable spermatocytes (stained red) were determined as described by (Cheesbrough, 2006).

e. SPERM COUNT

This was determined as described earlier by (Narayana *et al.*, 2005. Briefly, the caudal epididymis was carefully separated from the testis and minced in 2ml of normal saline followed by filtration through a nylon mesh. The suspension was then stained with 2% eosin in normal saline. The spermatocytes heads were counted using a Neubauer haematocytometer. Chamber counts for the sperm head in eight chambers (except the central chamber) were averaged and expressed as the number of sperm per caudal epididymis (Mahaneem *et al*., 2011).

F. DETERMINATION OF LIVER FUNCTION TESTS

a. DETERMINATION OF ACTIVITY OF ALKALINE PHOSPHATASE (ALP) IN SERUM

Principle:

p-Nitrophenyl phosphate is converted to p-Nitrophenol and phosphate by alkaline phophatase.The rate of formation of p-Nitrophenol is measured as an increase in absorbance which is proportional to the ALP activity in the sample.

$$\text{p-Nitrophenyl phosphate} \xrightarrow{\text{AL P}} \text{p-Nitrophenol} + \text{phosphate}$$

This was determined as described by Youn, (1995). Two test tubes were labeled sample and control. 20µl of the sample and 1000µl of reagent were pipetted into the two test tubes respectively. The working reagent was pre warmed at 37ºC for two minutes prior to addition of sample. The mixture was thoroughly mixed and incubated at 37ºC for 60 seconds. The change of absorbance per minute (ΔA/minute) during 180 seconds was taken.

Calculation:

The absorbance was taken at 405nm wavelength

Activity of sample (IU/L) = (ΔA/min) × 2712.

b. DETERMINATION OF ACTIVITY OF ALANINE AMINOTRANSFERASE (ALT) IN SERUM

Principle:

Kinetic determination of ALT activity.

ALT catalyzes the transfer of amino group of L-Alanine to α-ketoglutarate to give L-glutamate.

$$\text{L-Alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + \text{NAD}^+$$

This was determined as described earlier by Youn, (1995). Two test tubes were labeled sample and control. 100µl of the sample and 1000µl of reagent were pipetted into the two test tubes respectively. The working reagent was pre warmed at 37ºC for two minutes prior to addition of sample. The mixture was thoroughly mixed and incubated at 37ºC for 60 seconds. The change of absorbance per minute (ΔA/minute) during 180 seconds was taken.

Calculation:

The absorbance was taken at 340nm wavelength

Activity of sample (IU/L) = (ΔA/min) × 1746.

c. DETERMINATION OF ACTIVITY OF ASPARTATE AMINOTRANSFERASE (AST) IN SERUM

Principle:

Kinetic determination of AST activity.

AST catalyzes the transfer of the amino group of L-Aspartate to α-ketoglutarate to give L-glutamate

$$\text{L-Aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L-Malate} + \text{NAD}^+$$

This was determined as described earlier by Youn, (1995). Two test tubes were labeled sample and control .100µl of the sample and 1000µl of reagent were pipetted into the two test tubes respectively. The working reagent was pre-warmed at 37ºC for two minutes prior to addition of sample. The mixture was thoroughly mixed and incubated at 37ºC for 60 seconds. The change of absorbance per minute (ΔA/minute) during 180 seconds was taken.

Calculation:

The absorbance was taken at 340nm wavelength

Activity of sample (IU/L) = (ΔA/min) × 1746.

G. STATISTICAL ANALYSIS

Statistical analysis was done using SPSS 21.0 version. Significant differences were determined using the one way analysis of variance (ANOVA). The Duncan Multiple Range tests was used as post hoc test. Data were considered significant at $p \leq 0.05$

III. RESULTS

A. EFFECTS OF *CITRULUS LANATUS* RIND JUICE (LOW DOSE: 100MG/KG) ON SEMEN PARAMETERS OF MALE ALBINO RATS FOLLOWING EXPOSURE TO LEAD ACETATE

Table 1 shows the effects of low dose (100 mg/kg) of different species of *Citrullus lanatus rind juice* on some semen parameters of male albino rats when co-administered in turn with lead acetate. Values of semen parameters (sperm count, viability and motility) were significantly different ($p \leq 0.05$) when groups 3, 4 and 9 were, in turn, compared with group 2. There was a significant reduction ($p \leq 0.05$) in the levels of sperm count, motility and viability in group 2 when groups 3,4and 9were, in turn, compared with group 2.However,the values of sperm count, motility and viability significantly increased in groups 3,4 and 9 when compared in turn, with group 2.

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Groups and treatments	Sperm Count ($x10^6$/ml)	Sperm cell Characteristics Viability (%)	Abnormalities (%)	Motality (%)
Group 1 (Normal Control group)	41.6 ± 0.32^a	78.9 ± 4.10^a	20.8 ± 0.81^a	80.20 ± 4.10^a
Group 2 (Lead acetate only)	9.5 ± 0.71^b	31.6 ± 1.20^b	57.6 ± 0.50^b	26.5 ± 1.20^b
Group 3 (Leadacetate + WRJA (100mg/kg)	22.9 ± 0.25^c	49.1 ± 2.54^c	33.7 ± 0.62^c	55.3 ± 2.54^c
Group 4 (Leadacetate + WRJB (100mg/kg)	26.4 ± 0.53^d	42.1 ± 0.22^c	38.4 ± 1.21^c	$68.5.1 \pm 0.22^a$
Group 5 (Leadacetate +WRJC (100mg/kg)	16.4 ± 0.37^e	35.3 ± 0.61^b	37.2 ± 1.57^c	48.3 ± 0.61^c
Group 9 (Leadactate + WRJA (100mg/kg) + WRJB (100mg/kg) + WRJC (100mg/kg)	31.4 ± 0.72^f	65.2 ± 0.28^d	21.0 ± 0.20^a	73.5 ± 0.28^a

All values are expressed as mean ± SEM; n=5.Values with different superscript down the column are considered statistically significant at $p \leq 0.05$. WRJA:watermelon rind juice from lightgreen specie, WRJB: watermelon rind juice from stripped specie,WRJC: watermelon rind juice from darkgreen specie.

Table 1: Effects of Citrulus lanatus rind juice (Low Dose: 100mg/kg) on semen parameters of male albino rats following exposure to lead acetate

B. EFFECTS OF *CITRULUS LANATUS* RIND JUICE (HIGH DOSE: 200MG/KG) ON SOME SEMEN PARAMETERS OF MALE ALBINO WISTAR RATS FOLLOWING EXPOSURE TO LEAD ACETATE

Table 2 shows the effects of high dose of different species of *Citrullus lanatus rind juice* on some semen parameters of male albino Wistar rats when co- administered in turn with lead acetate. Values of semen parameters (sperm count, viability and motility) were significant different ($p \leq 0.05$) when groups 6, 7 and 10 were, in turn, compared with group 2. There was a significant reduction ($p \leq 0.05$) in the levels of sperm count, motility and viability in group 2 when groups 6,7 and 10 were ,in turn, compared with group 2.However,the values of sperm count, motility and viability significantly increased in groups 6,7 and 10 when Compared in turn, with group 2.

Groups and treatments Sperm Count$X10^6$/ml)	Sperm cell characteristic Viability (%)	Abnormalities	Motility (%)
Group 1 (Normal Control group)	41.6 ± 0.32^a	78.9 ± 4.13^a	20.8 ± 0.85^a	80.20 ± 4.10^a
Group 2 (Lead acetate only)	9.5 ± 0.76^b	29.6 ± 1.29^b	57.6 ± 0.53^b	26.5 ± 1.20^b
Group 6 (Lead acetate + WRJA (200 mg/kg)	28.0 ± 0.33^c	55.9 ± 0.23^c	26.5 ± 3.28^a	59.3 ± 2.54^c
Group 7 (Lead acetate + WRJB (200 mg/kg)	34.2 ± 0.51^d	61.3 ± 3.27^c	16.0 ± 2.53^a	$75.7.1 \pm 0.22^a$
Group 8 (Lead acetate + WRJC (200 mg/kg)	21.6 ± 1.26^e	35.8 ± 0.65^b	39.3 ± 0.92^c	52.3 ± 0.61^c
Group 10 (Lead acetate + WRJA (200 mg/kg) + WRJB (200 mg/kg) + WRJC (200 mg/kg)	45.5 ± 3.02^f	69.0 ± 1.83^a	16.6 ± 1.77^d	82.5 ± 0.28^a

All values are expressed as mean ± SEM; n=5.Values with different superscript down the column are considered statistically significant at $p \leq 0.05$.WRJA: watermelon rind juice from light green specie, WRJB: watermelon rind juice from stripped specie WRJC: watermelon rind juice from dark green specie.

Table 2: Effects of Citrulus lanatus rind juice (High Dose: 200mg/kg) on some semen parameters of male albino Wistar rats following exposure to lead acetate

C. EFFECTS OF *CITRULUS LANATUS* RIND JUICE ON SOME SERUM BIOCHEMICAL PARAMETERS: ASPARTATE AMINO TRANSFERASE (AST), ALKALINE PHOSPHATASE (ALP) AND ALANINE AMINOTRANSFERASE (ALT) AT 100 MG/KG B.W

Table 3 shows the effects of different species of *Citrulus lanatus* rind juice on serum biochemical parameters (Aspartate amino transferase (AST), alkalin phosphatase (ALP) and alanine aminotransferase (ALT) at 100mg/kg b.w. There was a significant increase ($p \leq 0.05$) in the serum activities of AST, ALP and ALT in the lead acetate group 2 when groups 3,4,5 and 9 were, in turn ,compared with group 2. However, a significant reduction ($p \leq 0.05$) in serum AST, ALP and ALT activity were observed in groups 3, 4, 5 and 9 when compared to lead acetate group2.

Groups	AST (IU/L)	ALP (IU/L)	ALT(IU/L)
Group 1 (Control group)	116.06 ± 0.04^a	37.13 ± 3.05^a	12.60 ± 5.05^a
Group 2 (Lead acetate group)	170.23 ± 1.05^b	86.54 ± 4.05^b	78.10 ± 4.05^b
Group 3 (Lead acetate + WRJA (100 mg/kg)	102.34 ± 3.00^a	36.06 ± 5.02^a	16.70 ± 3.07^a
Group 4 (Lead acetate + WRJB (100 mg/kg)	81.40 ± 5.04^{ac}	39.05 ± 3.05^a	18.70 ± 2.06^a
Group 5 (Lead acetate + WRJC (100 mg/kg)	97.10 ± 2.33^a	43.00 ± 2.04^a	30.10 ± 6.04^a
Group 9 (Lead acetate + WRJA (100 mg/kg)+ WRJB (100 mg/kg)+ WRJC (100 mg/kg)	77.23 ± 7.01^{ac}	31.21 ± 6.05^a	15.32 ± 5.02^a

All values are expressed as mean ± SEM; n=5.Values with different superscript down the column are considered statistically significant at $p \leq 0.05$. WRJA: watermelon rind juice from lightgreen specie, WRJB: watermelon rind juice from stripped specie, WRJC: watermelon rind juice from darkgreen specie.

Table 3: Effects of Citrulus lanatus rind juice on some serum biochemical parameters: Aspartate amino transferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) at 100 mg/kg b.w

D. EFFECTS OF *CITRULUS LANATUS* RIND JUICE ON SERUM BIOCHEMICAL PARAMETERS: ASPARTATE AMINO TRANSFERASE (AST), ALKALINE PHOSPHATASE (ALP) AND ALANINE AMINOTRANSFERASE (ALT) AT 200 MG/KG B.W

Table 4 shows the effects of different species of Citrulus lanatus rind juice on serum biochemical parameters (Aspartate amino transferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) at 200mg/kg b.w. The serum AST, ALP and ALT activities significantly increased in lead acetate group 2 when groups 6,7,8 and 10 were, in turn, compared with group 2. However, a significant reduction ($p \leq 0.05$) in serum activity of AST, ALP and ALT were observed

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in groups 6, 7, 8 and 10 when compared to lead acetate group 2.

Groups	AST (IU/L)	ALP (IU/L)	ALT(IU/L)
Group 1 (Control group)	116.06± 0.04[a]	37.13± 3.05[a]	12.60± 5.05[a]
Group 2 (Lead acetate group)	170.23± 1.05[b]	86.54± 4.05[b]	78.10± 4.05[b]
Group 6 (Lead acetate + WRJA (200 mg/kg)	90.06± 5.03[c]	31.34± 3.13[a]	16.24± 6.02[a]
Group 7 (Lead acetate + WRJB (200 mg/kg)	65.05± 4.02[d]	34.65± 2.25[a]	15.00± 3.55[a]
Group8 (Lead acetate + WRJC (200 mg/kg)	82.33± 5.05[c]	37.26± 3.05[a]	27.45± 3.05[a]
Group 10 (Lead acetate + WRJA (200 mg/kg) WRJB(200 mg/kg)+ WRJC(200 mg/kg)	59.18± 2.04	28.08± 0.03[a]	12.00± 0.07[a]

All values are expressed as mean ± SEM; n=5.Values with different superscript down the column are considered statistically significant at $p \leq 0.05$. WRJA: watermelon rind juice from lightgreen specie, WRJB: watermelon rind juice from stripped specie, WRJC: watermelon rind juice from darkgreen specie.

Table 4: Effects of Citrulus lanatus rind juice on serum biochemical parameters: Aspartate amino ransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) at 200 mg/kg b.w.

IV. DISCUSSION

The present study describes the possible ameliorative potential and synergistic effect of the co-administration of different species of *Citrulus lanatus* rind juice with lead acetate on semen parameters and liver enzymes of male albino rats. The reduction in semen parameters following lead acetate administration, seen in the present study, is in agreement with previous reports of possible impairment of reproductive capacity induced by lead (Benoff *et al*., 2003). The sperm parameters which include sperm count, motility and morphology are vital indices of male fertility; as they are markers of spermatogenesis and epididymal maturation of spermatocytes (Morakinyo *et al*., 2010). These parameters along with sperm viability were all found to be negatively affected by lead acetate administration in the present study.

The observed increase in the serum activity of Aspartate amino transferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) in group 2 could be due to damage to the liver cells by lead intoxication. These observations are in agreement with previous study which reported that lead has hepatotoxic effect (Abdou *et al*., 2007). Several mechanisms have been proposed to account for the deleterious effect of lead on male reproductive function: Reduction of plasma testosterone concentration, Exerting a direct cytotoxic effect on maturing or matured spermatocytes in the epididymis (Chinoy, *et al*., 1985), and by Increasing the production of reactive oxygen species and also increasing the generation of testicular hydrogen peroxide and hydroxyl radicals in experimental rats (Aruldhas *et al*., 2005).

Many studies have demonstrated the reproductive toxicity of lead (Adhikari *et al.*, 2001). During Lead exposure, it accumulates in the testes tissue in a dose dependent manner (Adhikari *et al.*, 2001). Lead toxicity induces a significant increase in apoptotic cell death in the seminiferous tubule of young growing rat (Adhikari *et al*., 2001) it is also associated with disruption of spermatogenesis and histoarchitecture and lowered enzyme activities in the testes.

Co-treatment of lead acetate with different species of *Citrulus lanatus* rind juice ameliorated the effect of lead acetate on most of the parameters under investigation as seen in Groups 3 to 10 rats; with the return of the sperm count, motility, morphology towards fairly normal values comparable to values seen among Group 1 control rats. The plasma activity of liver enzymes (ALT, AST and ALP) however, became reduced. Similarly, co- administration of lead acetate with mixtures of different species of *Citrulus lanatus* rind juice produced significant increases in semen parameters (sperm count, motility, viability and morphology in groups 9 and 10. This possible ameliorative effect of the C*itrulus lanatus* rind juice maybe attributed to its contents of phenols and flavonoids (Erukainure *et al*., 2010), ascorbic acid content (Edwards *et al*., 2003), carotenoids such as lycopene, lutein and β carotene (Chandrika *et al.*, 2009) , vitamin C, thiamine and riboflavin which contains a high level of polyphenolic compounds ,all of which have antioxidant properties and the ability to remove free radicals presumably generated by lead acetate which may otherwise cause oxidative stress (Dietrich *et al*., 2003). The flavones and catechins are perhaps the most powerful flavonoids for protecting the body against damage by reactive oxygen species (Sodipo *et al*., 2000).

Several studies show that alkaloids and terpenes are widely spread in the genus *Citrulus* (Ali and Pandey, 2007). These secondary metabolites are responsible for the pharmacological activities such as antiulcer, antimicrobial, antioxidant, analgesic, aphrodisiac and many other ethnomedicinal uses (Khan *et al*., 2011).

The mechanisms by which *C. lanatus* rind juice protects against experimentally induced testicular toxicity may be as a result of the rich source of vitamin C, thiamine and including riboflavin which contains a high level of polyphenolic compounds present in the plant. High concentration of vitamin C in *C. lanatus* rind provides highly effective anti-oxidants, reversing the negative effect caused by the lead-acetate following the administration of the extracts to the experimental animal as seen in the groups 3 to 10 of the different parameters when compared to group2. This effect may be influenced by the presence of flavonoids in the juice which contains antioxidants.

V. CONCLUSION AND RECOMMENDATIONS.

A. CONCLUSION

Findings from the study suggest that watermelon rind juice exerts a possible beneficial effect on male reproductive parameters and validates previous reports of the beneficial effects of watermelon consumption from our environment. The study also suggests that the ameliorative potential of watermelon rind could be dosage dependent, vary with different varieties and could be synergistic.

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B. RECOMMENDATIONS

Further studies are recommended on the dosage and varieties dependency as well as the synergistic effect of watermelon varieties.

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