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Original Research Article

Effects of Methanol Seed Extract of *Citrullus Lanatus*on Haematology, Serum Biochemistry And Semen Quality In Rabbit Bucks

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ABSTRACT

The present study was aimed at determining the effect of watermelon seeds on semen quality and blood profile in rabbit buck. A total of 60 adult rabbits (30 males for semen quality assessment and 30 females for fertility trials) were included in the study. Eighteen male rabbits were randomly assigned to three groups: A, B, and C. Group A served as the control, while groups B and C received 250 mg/kg and 500 mg/kg of methanolic seed extract of *Citrullus lanatus* respectively, administered orally for six weeks. Semen was collected from the 18 males (6 per group) for six consecutive weeks, and semen quality parameters such as ejaculate volume, sperm motility, forward progressive motility, and sperm viability were evaluated. At the conclusion of the experiment, 30 females (10 per group) were mated with the treated males. The results indicated a significant improvement in semen quality parameters across all treatment groups in a dose-dependent manner. Additionally, hematological and serum biochemical parameters were positively improved. However, the reproductive performance of the females mated with the treated males did not significantly differ from the control group. This study highlights the beneficial effects of methanolic seed extract of *Citrullus lanatus* on semen production and quality, suggesting the need for further research on its impact on reproductive hormones.

Key Words: Watermelon seeds, semen quality, haematology, serum biochemistry, Rabbit bucks, Rabbit does.

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Introduction

Citrullus lanatus, water melon, a tropical fruit is an herbaceous creeping plant belonging to the family cucurbitaceae. It grows in Africa, Asia, India, and USA with a lot of ethnomedicinal significance attached to its various parts, such as the fruit pulp, juice, rind, seeds and leaves of the plant.[1] *Citrullus lanatus* fruit contain many smooth compressed seeds that thickened at the margin of black or yellow-white colour.[2] *Citrullus lanatus* seeds are source of food particularly protein and oil. *Citrullus lanatus* seeds were reported to contain about 50% fat and 35% protein.[3]Watermelon seeds are highly nutritious and are utilized for human consumption as snacks after salting and roasting in Arabian and Asian regions.[4]Watermelon seed oil is light in appearance, and is valued as a potential source of essential fatty acid (linoleic acid) with health benefits.[5]

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Watermelon seeds are a source of protein, B vitamins, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others.[6]Dried watermelon seeds contains appreciable amount of crude protein content (30.9 ± 0.9%) making it to be a good source of supplementary protein for man and livestock.[7]
Watermelon with red flesh is exceptionally rich in carotenoids such as lycopene which has lots of nutritional and health benefits. Lycopene has also been found to be protective against a growing list of cancer.[8]
Watermelon has strong antioxidant activity and has been recognized to be of immense health benefit. Pharmacological studies conducted on *Citrullu slanatus* depicted antioxidant, antimicrobial, antigiardial, hepatoprotective, anti-diabetic, laxative, antisecretory effects, anti-prostatic hyperplasia, analgesic, antifungal and anti-inflammatory effects in animal models.[9]In addition preliminary studies have shown that lycopene supplementation in drinking water improved semen quality (volume &total number of spermatozoa, viability, sperm kinetic, and storage life).[10] Dietary consumption of *C. lanatus* can ameliorate infertility potential of *Carica papaya* seeds extract in rat.[11]
Rabbits are mammals belonging to the family leporidae and order lagomorpha. They have been classified according to weight, size and pelt type by The United States Department for Agriculture (USDA). Small rabbits weigh about 1.4 – 2kg at maturity, medium breeds 4 – 5.4kg, and large breeds 6.4 – 7.3kg.[12]
Animal protein is very vital as it contains all the essential amino acids needed for growth, development and maintenance of human and animal life. A shortage of this protein is prevalent in most part of Africa where it is estimated that on the average, 10g of animal protein is consumed per day as compared to what was recommended to be taken daily (35g).[13] Rabbit production is veritable way of alleviating animal protein

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deficiency in Nigeria.[14] Rabbit meat is ranked sixth after beef, fish, mutton, goat meat and bush meat or game animals in the parametric assessment of meat animal production and consumption in Nigeria.[15] Rabbit farming in Nigeria is faced with myriad of problems, which have resulted in a gross shortage of meat to meet the population challenge in our country.[16] White meat of rabbit is very nutritious, easily digestible and extremely low in cholesterol and sodium level.[17]

Therefore, a re-introduction of rabbit production for meat is a feasible approach to solving the problem of food insecurity because unlike societies where people are used to monotonous food culture where acceptability of rabbit meat as food may be difficult, because rabbit is highly acceptable as meat in developing countries.[18]In less income (developing) countries where the population is predominantly poor and located at the rural areas, production and consumption of rabbits has been proven to contribute to economic safety net and social security for the poor farmers. According to reports[19], rabbit production in rural communities of Bangladesh by poor farmers is affordable cost-wise, intensive production is feasible, and a contributor to household food consumption and regarded as an important micro livestock component that can produce meat for meeting up extra demand of the country.[18]

Materials and Method

Study Area

The study was carried out at the college of Veterinary Medicine, Federal University of Agriculture Zuru, Kebbi State, Nigeria. Zuru occupies about 9000sq Km, between longitude 5ºand 14' 5'' E and latitude 11º 26' 6'' N, with average annual rainfall of 300mm – 1200mm and an average annual temperature of 40ºC.[20]

Seed collection, identification and extraction

Matured watermelon pods were obtained from a local market in Zuru, Kebbi State, Nigeria (December. 2022). It was authenticated by the herbarium unit of the department of biology, (*C. lanatus* 2/019). The seeds were removed from the pods. The seeds collected were thoroughly washed, shade dried and were taken to Department of crop sciences, college of Agriculture, FUAZ, for Identification. The dried seed samples were processed into powder using mortar and pestle. The shade dried powdered seed material was subjected to proximate and phytochemical analysis. The powder (20 g) was macerated in 20ml ethanol (70%) or 20ml distilled water for 48h and allowed to extract for 2h using Soxhlet device, then it lyophilized, weighted and preserved in freezer until usage.[21]

Ethical clearance

Ethical approval for the study was sought from the animal ethics committee, College of Veterinary Medicine, Federal University of Agriculture Zuru (Fuaz/AEC/2024/08)

Experimental Animals

Thirty (60) adult rabbit (30 male, 30 females) aged 36 weeks old at the time of the experiment with an average body weight of 750-800 g were used for the study. The rabbits were sourced from local breeder in Zuru, kebbi state and maintained at the animal pen, College of Veterinary Medicine Federal University of Agriculture Zuru throughout the study. The rabbits were housed in cages and allowed to acclimatize for 14 days before the commencement of the experiment. They were fed Pelleted feed (Chikunfeed® growers mash) and water *ad libitum* throughout the study.

Determination of Median Lethal Dose (LD_{50})

The median lethal dose (LD_{50}) was determined through a two phase approach using 12 rabbit bucks as described by[22]. An LD_{50} of 5000mg/kg was obtained. Therefore the doses used were 5% and 10% of 5000mg/kg which is equal to 250 mg/kg and 500mg/kg, respectively.

Experimental Design

On commencement of the experiment, the Rabbit bucks were divided into 3 groups of 6 bucks each (A, B and C).Group A served as control while groups B and C received 250 mg/kg and 500mg/kg of the seed extract. Semen was collected once a week for evaluation for 6 weeks. For haematological (serum biochemistry), blood was collected once weekly.

Semen Collection and evaluation

Bucks were trained for semen collection and the semen was collected using an improvised artificial vagina made of a plastic cylinder with a latex liner secured around the rim, warm water was placed in-between the latex liner and the cylinder. The artificial vagina was pre-warmed in water at 50ºC to 55°C, ensuring a temperature of 40ºC to 42°C at the time of collection As described by.[23] The semen was collected in the morning weekly for 6 consecutive weeks. Semen quality parameters were measured in each ejaculate immediately after collection.

Ejaculate volume and color

Ejaculate volume was measured immediately after collection using a 1ml graduated test tube. Physically with the eyes, semen colour was determined and recorded as milky, creamy or watery and was designated 1 (creamy), 2 (milky) or 3 (watery) immediately after collection.

Mass motility

Determination of mass motility was carried out using microscope. A drop of undiluted (raw semen) was placed on a pre-warmed slide and covered with a cover-slipped, viewed using a microscope at x40 objectives and the wave pattern examined for the assessment of gross sperm motility. Individual motility was determined by placing about four drops of diluents (saline of sodium citrate) on a warm glass slide and a drop of the semen placed into the saline and a warm cover slip was placed on the slide and examined using a field microscope at x40 magnification using a scoring pattern as described by.[24]

Sperm concentration

Concentration of spermatozoa was determined using Neubaeuhaemocytometer, 0.5 mark of the micropipette was filled up with semen sample by aspiration and diluted with normal saline by filling the 1.0 mark of the pipette. The pipette was cleaned to remove any adhering semen then gently mixed. A cover slip was placed on the haemocytometer and two drops of diluted semen were then added under the cover slip on each side of the haemocytometer. The haemocytometer was placed in a pre-wetted chamber and lid was closed and left for 5min. Sperm cells were counted from four corner and center squares (5 large squares or a total of 80 small squares) at x40 magnification under the microscope.[25]

Percentage live sperm cells

Percentage live sperm cells were determined as described by.[26] A thin smear of the sperm was made on a clean grease free slide and stained with eosin-nigrosin stain. One hundred sperm cells were counted using light microscopy (Olympus CH Japan) at x40 magnification. Dead spermatozoa were stained pink or reddish while live spermatozoa remained colourless.

Spermatozoa morphology

Spermatozoa morphology was determined as described by.[26] In percentage live sperm but in this case normal spermatozoa and those with morphological defects of acrosomes, head, mid piece and tail were counted and recorded as described by.[27]

Blood collection and evaluation

Blood sample was collected weekly during the experiments, Sample were collected after proper restrain using 5ml syringe, about 2mls of blood was collected 1mls of the blood was transferred to EDTA bottle. Haematological parameters such as Red Blood Cells (RBC), White

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Blood Cell (WBC) were determined using the Neubauerhaemocytometer, Packed Cell Volume (PCV) were determined using microhaematocrit method, Haemoglobin (Hb) concentration also was determined. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were determined as described by [28]. Sample was collected from all the bucks through the ear venipuncture using 25gauge hypodermic needle.

Fertility trial

At the end of the semen evaluation period, 30 multiparous rabbit does (10 females/group) were housed in cages and fed a commercial diet *ad libitum*. The rabbit does were monitored for regular oestrus cycle using cytology before introducing rabbit bucks for mating. Fertility rate (number of kindlings/number of insemination×100), total newborn, live born and litter weight at kindling were recorded.

Data analysis

Data obtained were expressed as means and their standard errors and presented in tables and charts. One-way Analysis of Variance (ANOVA) was used to compare group means and Tukey's post-hoc test was used to test for significance. Values with $P \leq 0.05$ were regarded as significant.

Results and Discussion

Rabbit bucks treated with methanolic seed extract of *Citrillus lanatus* significantly ($P<0.05$) increased hemoglobin (Hb) concentration, hematocrit value, count of RBCs and platelets, while significantly ($P<0.05$) decreased count of WBCs as compared to control group, but dosage variation did not affects the values significantly as shown in Table 3. In agreement with the present findings.[29] reported enhanced haematological values as a result of lycopene administration. Similarly, exposure of diabetic rats to lycopene significantly ($P<0.05$) increased Hb concentration and RBCs count.[30]The observed improvement in hematological parameters as a result of methanolic seed extract of *Citrillus lanatus* could be attributed to antioxidant properties of watermelon. Unchecked accumulation of reactive oxygen species ROS in haemopoietic cells tends to have severe consequences.[31] Thus, reactive oxygen species scavengers properties of watermelon could possibly be the reason why improved haematologiacal values were recorded.

Table 1: Proximate analysis of watermelon seed

Serial No.	Parameter	Value (%)
1	Moisture	5.99
2	Ash	3.55
3	Lipid	28.60
4	Protein	15.00
5	Fibre	37.65
6	Cho	46.86

Table 2: Phytochemical Analysis of Watermelon Seeds

Serial No.	Parameter (mg/100g)	Value
1	Saponin	29.30
2	Tannin	0.90
3	Phytate	0.21
4	Oxalate	0.56

Treatment of rabbit bucks with Methanolic seed extract of *Citrillus lanatus* significantly ($P<0.05$) increased biochemical blood parameters (total proteins, albumin, globulin, glucose and HDL concentrations) but significantly ($P<0.05$) decreased concentration of urea, creatinine, total lipids, triglycerides, total cholesterol and LDL in blood serum of rabbit bucks as compared to control bucks as shown in Table 4. These change were seen in dose dependent manner.

These findings are in accordance with those of[32] in rabbit as a result of tomato powder supplementation. These findings could be as result of beneficial effects of both watermelon as antioxidants on protein metabolism.

Table 3: Effect of Methanolic Seeds Extract of *Citrullus Lanatus* on haematology In Rabbit Bucks (n=6)

Parameter	Control group	250mg	500mg
Hemoglobin (mg/dl)	9.26^{c}	11.29^{a}	11.05^{b}
Hematocrit (%)	41.50^{b}	47.75^{a}	44.50^{ab}
RBCs (x 106 /mm3)	4.72^{b}	5.88^{a}	5.73^{a}
WBCs (x 103 /mm3)	7.47^{a}	6.20^{b}	6.29^{b}
Platelets(x 103 /mm3)	206.00^{c}	254.75^{a}	240.00^{b}

Rows with different superscript are significantly ($p < 0.05$) different

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Table (4): Effect of Methanolic Seeds Extract of *Citrullus lanatus* on serum biochemistry In Rabbit Bucks (n=6)

Parameter	Control	250mg	500mg
Total proteien	6.02[a]	6.94[b]	6.84[b]
Albumin(g/dl)	3.29[a]	3.53[a]	3.42[a]
Globulin (g/dl)	2.84[a]	3.41[b]	3.34[b]
Glucose (mg/dl)	99.82[a]	106.25[b]	107.30[b]
Urea (mg/dl)	32.51[a]	28.87[b]	29.63[c]
Creatinine (mg/dl)	1.03[a]	0.91[b]	0.82[b]
Total lipids (mg/dl)	216.00[a]	175.51[b]	142.45[c]
Triglycerides (mg/dl)	65.74[a]	54.75[b]	51.12[c]
Total cholesterol (mg/dl)	87.50[a]	73.75[b]	79.50a[b]
Low density lipoproteins	109.45[a]	82.95[b]	83.54[b]
High density lipoproteins	61.00[a]	71.75[b]	66.50[c]

Rows with different superscript are significantly (p < 0.05) different

Decrease urea and creatinine indicates an improved kidney function. Our results, are in agreement with[33] who reported positive effects in blood of heat stressed rabbit bucks treated with royal jelly.

In the present study, semen characteristics are presented in Table 5. Methanolic seed extract of *Citrillus lanatus* resulted in significant increase in ejaculate volume (P<0.05) compared to control group. In dose dependent manner. Our findings are agreement with those of[10] in rabbit buck as result of lycopene exposure. This could be as result of strong antioxidant effect of watermelon.

Sperm concentration in the present study was also found to be positively affected as result of Methanolic seed extract of *Citrillus lanatus,* this is in accordance with the findings previous findings in male rabbits exposed to vitamin E.[34] It is also in agreement with the findings of[35] In present study, Methanolic seed extract of *Citrullus lanatus* positively affects sperm motility.[36] Reported improved semen characteristics in male domestic fowl following antioxidant supplementation in drinking water. [37]Also reported that a higher antioxidant intake was associated with a greater motility and sperm numbers in man. Higher sperm motility was also found after caffeine addition (10 mM/L) in rabbit semen stored for 24 h at 18°C.[38] No severe sperm morphological anomalies were noted in all the treatment groups or the control. The improved semen characteristics recorded in our study as results of Methanolic seed extract of *Citrillus lanatus* are in accordance with several studies reported in the literature on the antioxidant property of watermelon and its potential effects on reproductive quality in animals and humans.[39] reported that lycopene, which is found in watermelon, significantly increased sperm concentration and motility in rats. This could be as result of the antioxidant effect of watermelon to prevent structural and functional damages.[40]

In the present study, reproductive performance of rabbit doe is shown on Table 6. Methanolic seed extract of *Citrillus lanatus* did not significantly affect reproductive performance of multiparous rabbit does mated naturally. Similar results were reported in laying hens inseminated with fresh semen from cockerels that received lycopene in drinking water[10] and in rabbit inseminated with fresh and stored semen from rabbits fed alfa-tocopheryl acetate.[41] In accordance with our findings,[42] reported that reproductive performance of female breeder turkeys were not affected after artificial insemination of semen enriched in vitamin E.

Table 5: Effect of Methanolic Seeds Extract of *Citrullus lanatus* On Spermiogram of Rabbit Bucks (N=6)

Parameter	Control	250mg	500mg
Ejaculate volume (ml)	0.61[a]	0.78[b]	0.86[c]
Semen pH value	7.10[a]	7.01[a]	7.03[a]
Mass motility (Score 1-5)	2.20[a]	3.10[b]	3.30[b]
Individual sperm motility (%)	58.60[a]	71.50[b]	72.10[b]
Dead sperm (%)	28.90[a]	17.60[b]	14.50[c]
Sperm abnormality (%)	17.40[a]	13.10[b]	9.72[c]
Acrosomal damage (%)	31.10[a]	18.50[b]	12.30[c]
Sperm cell concentration (x106/ml)	198.60[a]	242.30[b]	294.51[c]
Total sperm output (x106/ejaculate)	275.32[a]	342.61[b]	385.10[c]

Rows with different superscript are significantly (p < 0.05) different

Table 6: Pregnancy Outcomes of Rabbit Does Mated With Bucks Exposed to Methanolic Seeds Extract of *Citrullus lanatus* (n=10)

Parameter	Control	250mg	500mg
Conception rate	50[a]	70[b]	80[c]
Kidding rate	30[a]	50[b]	70[c]
Litter size at birth/doe	5.4[a]	6.2[b]	6.4[b]
Percentage birth weight (g)	11.22 ± 0.24[a]	12.12 ± 0.44[a]	12.25 ± 0.23[a]
Crown-rump length(inches)	7.08± 0.23[a]	7.18± 0.14[a]	7.14± 0.42[a]

Rows with different superscript are significantly ($p < 0.05$) different

Conclusion

Methanolic seed extract of *Citrillus lanatus* at 250 mg/kg and 500 mg/kg improves semen quality parameters and haematologial and biochemical indices in rabbit buck.

Conflict of Interest Statement

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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