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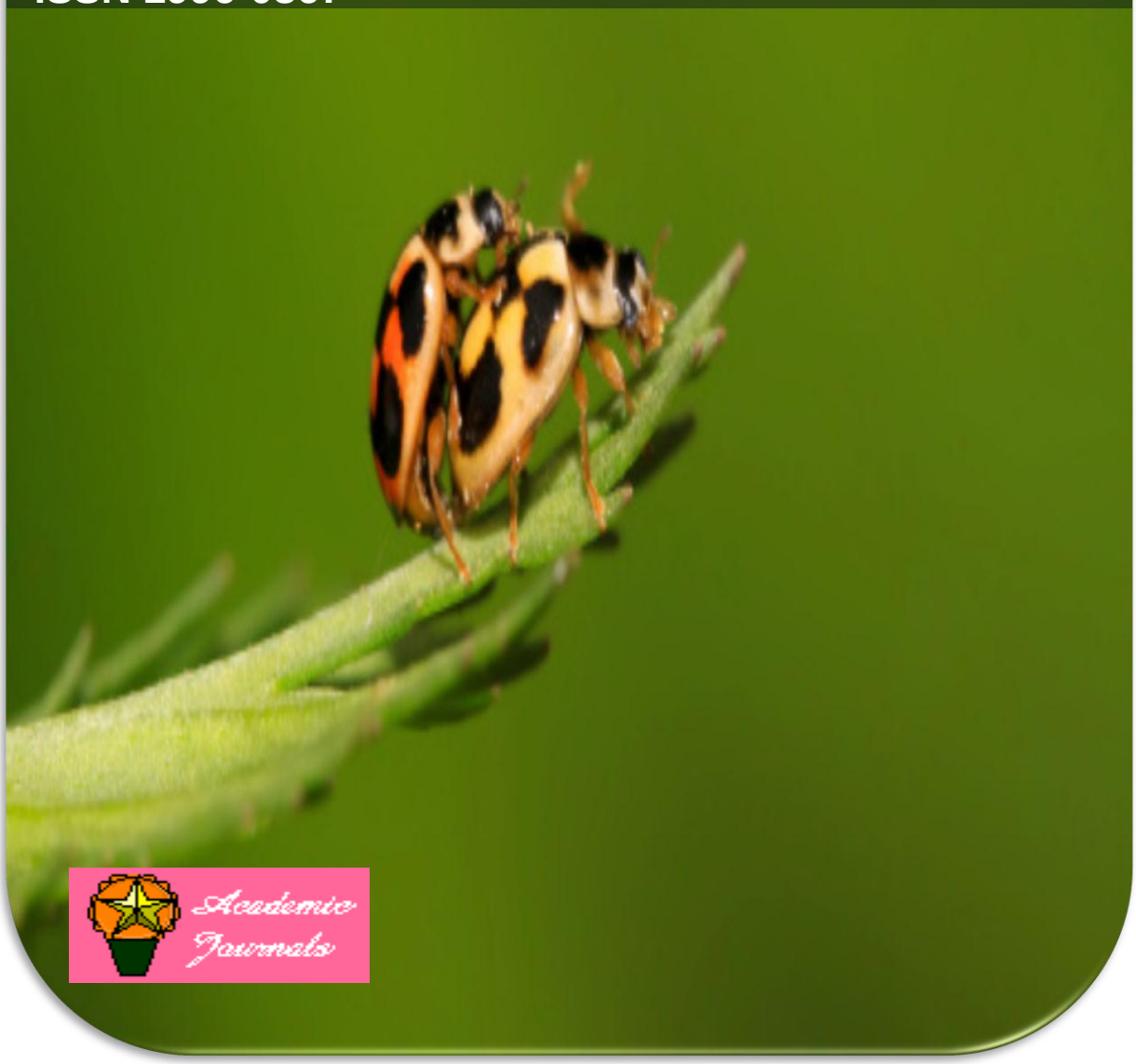

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Sperm characteristics and haemogram of male albino rats (wistar strain) treated with saponin extract from *vernonia amygdalina del. asteraeceae 110192*

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The reproductive effects of saponin extract from *Vernonia amygdalina* Del. *Asteraeceae 110192* leaves on 14 adult male Wistar rats was studied. The rats were divided into four groups (A, B, C and D) treated with graded doses (100, 200, 400 and 0 mg/kg respectively) of saponin extract of *V. amygdalina* over a period of 14 days. After 14 days oral administration of the saponin extract, the rats were sacrificed and their testicles removed through scrotal incision. Blood samples were collected periocularly into ethylene diamenetetraacetic acid (EDTA) sample bottles to prevent blood clotting. The result shows an enhancing effect at a higher dose with respect to sperm cell motility and concentration. However, the number of morphologically abnormal sperm cells was within the normal range of 10%. The packed cell volume (PCV) was slightly reduced in group C that had the highest dose (42.67±0.68%) of the saponin than for group A that had the lowest dose (47.0 ±0.24%) and (47.33 ± 0.47%) for the control group. For the morphological characteristics, there were dose dependent decrease in rudimentary tail, bent tail, curved mid-piece and bent mid-piece. In conclusion, the saponin component of *V. amygdalina* did not produce adverse effects on the reproductive potentials of the rats and can therefore be used to boost reproduction in male wistar rats.

Key words: Sperm characteristics, saponin, *Vernonia amygdalina,* rats, testicles, spermatozoon, Haematology.

INTRODUCTION

Vernonia amygdalina is a multipurpose plant that contains some bioactive compounds that have been identified following various studies done on the extracts. These compounds include saponins, tannins, vernodaline and vernomyadine (Akindahunsi and Salawu, 2005). These compounds are reported to be responsible for the various physical properties of the leaf such as bitterness and formation of stable foams. Minerals found in sundried leaves of *V. amygdalina* are calcium, phosphorus, sodium, potassium, iron, zinc and magnesium (Akindahunsi and Salawu, 2005).

V. amygdalina has many medicinal uses. Its stem and

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leaf have been used to cure stomach ache and treat malaria (Philipson et al., 1996). Obute (2005) also reported that bitter leaf sap has been used as antifungal agent in the south-eastern part of Nigeria. Apart from the beneficial medicinal uses of bitter leaf, its methanolic extract has been reported to exhibit haemolytic activities (Price et al., 1987; Oboh, 2001). Saalu et al. (2013) reported that at higher doses (300 and 600 mg/kg) of *V. amygdalina* leaves resulted in testicular toxicity in rats, while lower doses (100mg/kg) had no adverse effect on the testis.

Saponins are a group of triterpenoid or steroid linked to one or more sugar groups (Das and Mahato, 1983). Saponins are surface-active glycosides and they are found naturally in many plant species, including wild plants and cultivated crops (Francis et al., 2002). There are several reviews on the biological actions of saponins (Yoshiki et al., 1998; Francis et al., 2002; Thakur et al., 2011; Begum et al., 2014; Soetan et al., 2014). Jisaka et al. (1993) reported that *Vernonia amygdalina* contain stigmasiranerype saponins such as vernonioside A, B1, 42, A3, 82, D3, A4 and C.

Several studies has been done and reported on the chemotherapeutic effects of *V. amygdalina* in the treatment of diseases but information is scarce on the reproductive effects of its saponin component.

The aim of the study was to determine the effect of saponins in *V. amygdalina* on the haemogram and to determine their effects on the semen characteristics and morphology.

MATERIALS AND METHODS

Fourteen adult male Wistar rats with an average weight of 260 g ± 5 aged between 13 to 18 weeks were used for this study. They were housed in the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The animals were divided into Groups A and B (n=4 rats each in each group) and groups C and D (n = 3 rats each group). All the experimental rats were fed with grower's pelleted feed for rodents made by Bendel feeds®.

The rats were given clean water *ad-libitum.* The rats were fed the grower's feed for 6 weeks to allow them acclimatize with the feed and environmental conditions.

Plant materials

The plant material used for this study was *V. amygdalina Del. Asteraeceae 110192* (bitter leaf) obtained within the University of Ibadan, Ibadan premises. The identification and voucher specimen number was done at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State.

Extraction of saponin

The *V. amygdalina* leaves were sun dried. The dried leaves were ground using mortar and pestle. The ground leaves were exhaustively separated for 10 h in a Soxhlet extractor using hexane (boiling range 68-69°C). This removed the lipids and other pigments (Fenwick et al., 1992). The solvent was changed to methanol (boiling range 64-65.5°C) and the extraction was continued for the next 12 h. This removed the saponins, together with low molecular weight substances such as sugars, the phenolic compounds, oligosaccharides and flavonoids (Fenwick et al., 1992). The resulting solutions were evaporated to dryness to yield the methanolic extracts. The presence of saponins in the methanolic extract was detected by the characteristic frothing test (O'Dell et al., 1959). The saponin extract was kept in a screw capped bottle and stored in a refrigerator at 4°C until use.

Administration of the saponin extract

The animals were divided into Groups A and B (n=4 rats each in each group) and groups C and D (n = 3 rats each group). The rats in groups A, B and C were given oral administration of the saponins at graded doses of 100, 200 and 400 mg/kg respectively. The doses were chosen based on the report of Adedapo et al. (2007). The rats in group D which served as the control group were given distilled water. The administration of the extract lasted for 14 days. After the 14 days of administration, the rats were sacrificed by putting them in a glass jar containing a piece of cotton wool soaked in chloroform, which caused loss of consciousness in the rats. Blood samples were collected intra-occularly into sample bottles containing ethylene ditetra-acetic acid (EDTA) to prevent clotting of the blood samples.

The testicles of the rats were removed through a lower abdominal incision. The right and left epididymis were trimmed off the body of the testes and semen samples were collected from the tail of the epididymis through an incision by means of a clean scalpel blade. A Pasteur pipette was used to suck out the semen and stored in an insulated collection tube.

Semen and blood analysis

The semen was analyzed for mass activity, motility, live/dead ratio (percentage livability) and morphological studies according to the method of Zemjanis (1977) and Oyeyemi et al. (1996). The blood samples were analyzed for packed cell volume (PCV), red blood cell count (RBC), leucocyte count and differential white blood cell count (WBC) according to the method of Jain, (1986).

Our study is consistent with the standard of the use of laboratory animals reported by World Medical Association and American Physiological Society report of (2002).

Statistical analysis

The statistical analysis of the data for analysis of variance (ANOVA), multiple comparisons and homogeneity of variance was done using the SPSS computer software package. Values of $p<0.05$ were considered significant.

RESULTS

Results of spermiogram

Motility

The results of the effect of saponin extract on semen characteristics are presented in Table 1. The percentage motility obtained in group A treated with 100 mg of

Table 1. Effects of Saponin extract of *Vernonia amygdalina* on rats spermatozoa characteristics.

Identification	Motility (%) ± SEM	Percentage liveability (%) ± SEM	Cell count (x10^6 cells/ml) ± SEM
Group A	52.5±1.44^b	92.0±1.16	54.5±1.07^b
Group B	50.0±4.08^b	93.3±1.14	51.75±0.80^b
Group C	88.3±3.12^a	97.0±0.71	70.0±0.82^a
Group D	46.67±6.24^b	92.0±4.30	50.67±1.03^b

SEM, Standard error of mean. ^{ab}Means along the same column with different superscripts are significantly (P < 0.05) different.

Table 2. Effects of saponin extract of *Vernonia amygdalina* on haematology of rats.

Identification	Packed cell volume (%)	Haemoglobin (g %)	Red blood cells (x10^{12}/1)	White blood cells (x10^9/1)	Lymphocytes %	Neutrophils%
Group A	47.0±0.24	15.38±0.42	12.24±0.17	14.10±0.54	39.75±3.39	60.25±3.39
Group B	46.0±0.47	14.63±0.26	11.15±0.24	17.10±0.75	43.5±3.07	56.5±2.93
Group C	42.67±0.68	14.03±0.02	10.83±0.078	10.83±0.078	41.0±1.08	59±1.08
Group D	47.33±0.47	15.33±0.47	12.12±0.44	15.78±1.02	54.67±3.68	44.67±3.70

No significant difference was observed in the values of the various blood composition at (p>0.05).

saponin per kg of body weight was 52.5±1.44 compared with 88.3±3.12 obtained for group C given 400 mg of saponin per kg body weight. The difference in the values was significant (p<0.05). The value obtained for percentage motility in group B treated with 200 mg of saponin per kg body weight was 50.0±4.08 while the value obtained for group C was 88.3±3.1. The difference between groups B and C was significant at p<0.05.

Percentage livability

The percentages of spermlivability in the various groups A, B, C and D were 92.0±1.16, 93.3±1.14, 97.0±0.71 and 92.0±4.30, respectively. Comparing the values obtained for the various groups, there was no significant difference between them (p>0.05).

Sperm count

The concentration of sperm cells in group C was 70.0±0.82 x 10^6 spermatozoa/ml while the values for groups A, B and D were 54.5±1.07 x 10^6, 51.75±0.80 x 10^6 and 50.67±1.03 x 10^6 spermatozoa/ml, respectively. The value obtained for concentration of sperm cells in group C when compared to that of groups A,B and C was significantly higher (p<0.05).

Result of haemogram

The values obtained for the various blood compositions are presented in Table 2. No significant difference was observed in the values of the various blood composition at p>0.05.

In the data, the PCV (42.67±0.68%) in group C given the highest dose of saponin 400 mg/kg body weight was considerably lower compared to that of the control group D (47.33±0.47) and group A (47.0±0.24) and group B (46.0±0.47). However, the PCV values obtained were within the normal range given by Harkness and Wagner, (1989).

Sperm morphological abnormalities

The morphological abnormalities of the spermatozoa in the semen of the experimental and control in groups A to D are presented in Table 3. Spermatozoa abnormalities commonly observed were normal head without tail (tailless head), normal tail without head (headless tail), rudimentary tail, bent tail, curved tail and curved midpiece.

Headless tail (normal tail without head)

The value obtained for headless tail in group A is 13 (0.90%), group B is 17(1.16%) while control groups C and D are 11 (0.96%) and 14(1.33%) respectively. There was a significant difference in the values obtained when groups A and B were compared as well as when other groups were compared with each other (P<0.05).

Rudimentary tail

Rudimentary tail abnormalities for group C was 2 (0.17%) while 10 (0.69%), 9 (0.61%) and 9 (0.86%) were obtained for A, B and D, respectively. Comparing the value for

Table 3. Morphological abnormalities of spermatozoa in the semen of the experimental rats.

Identification	Group A	Group B	Group C	Group D
Headless tail	13 (0.90%)[a]	17 (1.16%)	11 (0.96%)[b]	14 (1.33%)
Rudimentary tail	10 (0.69%)	9 (0.61%)	2 (0.17%)[a]	9 (0.86%)
Bent tail	31 (2.14%)	28 (1.90%)	17 (1.48%)[a]	24 (2.29%)
Curved tail	30 (2.69%)	30 (2.04%)	18 (1.57%)[a]	24 (2.29%)
Curved mid-piece	28 (1.93%)	27 (1.84%)	16 (1.39%)	27 (2.57%)[a]
Bent mid-piece	31 (2.14%)	30 (2.04%)	16 (1.39%)	25 (2.38%)
Total abnormal cell	159 (10.97%)	164 (11.16%)	95 (8.26%)[a]	135 (12.86%)

[ab]Means along the same row with different superscripts are significantly (P < 0.05) different, parenthesis, % difference.

group C with the other groups these values differ significantly (p<0.05).

Bent tail

The bent tail abnormalities in group A was 31 (2.14%), B was 28 (1.90%), C was 17 (1.48%) and D was 24 (2.29%). There were significant differences (p<0.05) in the values obtained for groups A and D when compared with group C.

Curved tail

The values obtained for curved tail abnormality in groups A, B, C and D are 30 (2.69%), 30 (2.04%), 18 (1.57%) and 24 (2.29%) respectively. The value obtained for curved tail morphology in group C was significantly lower than that obtained for group D (p<0.05).

Curved mid-piece

The value obtained for the control group D was 27 (2.57%),while the values for groups A, B and C were 28 (1.93%), 27 (1.84%), and 16 (1.39%) respectively. These values differed significantly (p<0.05) to each other.

Bent mid-piece

The values obtained for group A was 31 (2.14%), B was 30(2.04%), C was 16 (1.39%) and D was 25 (2.38%). The values for groups A, B and C were not significantly different at p>0.05 when compared with that of the control group D.

Total abnormal cell

The value obtained for total abnormal cell in group A was 159 (10.97%), B was 164 (11.16%), C was 95 (8.26%) and D was 135 (12.86%). A marked difference was observed at p<0.05 when the value for group C was compared with the other three groups.

DISCUSSION

The effects of saponin extract of *V. amygdalina* on rats spermatozoa characteristics is shown in Table 1 while the effects of saponin extract of *V. amygdalina* on haematology of rats is shown in Tables 2 and the morphological abnormalities of spermatozoa in the semen of the experimental rats is shown in Table 3.

The observed morphological abnormalities of the sperm cells were within the proposed percentage range (8-10%) reported by Reece (1997). The 14 days administration of the saponin extract of V*. amygdalina* may not be unconnected to the very mild effect observed in the sperm morphology. However, fertility may not be affected by the saponin component of the *V. amygdalina* administered. Increase in motility was observed with increasing dose of the saponin extract with the highest percentage of 88.3±31 in group C that was given the highest dose of 400 mg/kg body weight and the lowest percentage of 46.7±6.2 for group D; the control group which received distil water. Higher doses of saponin extract were observed to enhance sperm motility. This therefore indicates that the administration of saponin extract of *V. amygdalina* to Albino rats may boost the fertilizing capacity of the spermatozoa with respect to fertilization (Hafez, 1993).This agrees with the report of Francis et al. (2013) that motility increased with increasing inclusion levels of *V. amygdalina* in the diet of the giant African Catfish (*Heterobranchus bidorsalis*) brood stock.

The percentage livability of the sperm cells had no correlation to the amount of the saponin administered since there was no significance different (P>0.05) in the value obtained all groups when compared. However, overdosing the rats may produce remarkable sign on the livability of the sperm cells. For the sperm cell concentra-

tion, in group D, the value of $50.67 \pm 1.03 \times 10^6$ spermatozoa/ml of semen was significantly lower ($p>0.05$) than the group C value of $70.0 \pm 2.82 \times 10^6$ spermatozoa per ml of semen. This indicates that the saponin component of *V. amygdalina*is highly androgenic and enhances spermatogenesis and hence increase sperm count in male Wistar rat. This is contrary to the report of Orlu and Ogbalu, (2011) in which a lower sperm counts was observed in *V. amygdalina* treated groups.

Blood composition was observed to be unaffected by the saponin administration except for the packed cell volume (PCV). The values obtained were within the normal physiological range proposed by Harkness and Wagner, (1989).

The PCV value obtained in group C that had the highest dosage of 400 mg/kg body weight was slightly lower when compared with the control group (group D) and group A with the lowest dose of saponin extract ($P>0.05$). The reduced PCV in group C may be associated with haemolysis. This is in agreement with the report of Price et al. (1987) and Oboh (2001) that methanolic extract of *V. amygdalina* causes haemolysis (Price *et al*., 1987; Oboh, 2001).

Conclusion

The saponin extract of *V. amygdalina* administered to male rats in this study showed potential to increase male fertility. As there may need to improve male fertility in terms of motility and concentration of sperm cells in an infertile male rat, male rats to be used either for artificial insemination (AI) or natural breeding programmes could be given saponin extract of bitter leaf (*V. amygdalina*) alongside adequate feeding to enhance their reproductive potential. The study concluded that saponin extract of *V. amygdalina* improved fertility of male rats.

More studies are needed on the effects of saponin extract of *V. amygdalina* on the reproductive capability of male of domestic animals species.

Conflict of interests

The authors did not declare any conflict of interest.

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