AZADIRACHTA INDICA SEED EXTRACT CAUSED DETRIMENTAL PREGNANCY OUTCOMES AND HAEMATOLOGICAL EFFECTS IN ADULT FEMALE ALBINO RATS

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ABSTRACT

Traditional use of Azadirachta indica in Asian countries suggests that the plant may have antifertility properties but in Nigeria, A. indica is fed to animals and used for traditional treatment of human and animal illnesses. This study therefore determined the reproductive and haematological effects of methanolic seed extract of A. indica (MSEAI), using adult female albino rats (AFAR) as experimental model. Sixty-four AFAR randomly assigned into four treatment groups (A – D) of 16 rats each were used. Group A was the control, while groups B, C and D received 50, 100 and 200 mg/kg doses of the extract daily for 28 consecutive days. Blood samples were collected on days 0 (base line), 14 and 28 to assay for haematological parameters. Thereafter, the rats were bred and fertility tests performed. Results showed that MSEAI significantly decreased (p≤0.05) haemoglobin concentration, packed cell volume and red blood cell count in treated groups on day 28. Total and differential leukocyte counts showed no significant variations (p>0.05). The extract also caused abortion and fetal resorption especially in high dose (100 and 200 mg/kg) treatment groups. However, in rats that had successful parturition, gestation length, litter size, litter weight, number of corpora lutea, number of implantation sites and number of fetuses born alive were not significantly affected (p>0.05). Considering the adverse haematological and reproductive effects, feeding A. indica as forage or the traditional treatment in pregnant/breeding females could be counterproductive. However, the extract could find application as a contraceptive in non-breeding females and in rodent depopulation programme.

Keywords: *Azadirachta indica* seed extract, Forage, Haematological parameters, Reproductive parameters, Female albino rats

INTRODUCTION

Livestock production is a very important source of livelihood and employer of labour in many developing countries. It has contributed immensely to job creation, poverty alleviation, crime reduction as well as the production and supply of animal proteins in Nigeria (Fasoyiro and Taiwo, 2012; Inyeinyang and Ukpong, 2019). In Nigeria there is shortfall in the provision of animal protein due to feed scarcity, endemic animal diseases and the country's huge population in relation to limited number of livestock being produced (Bamaiyi, 2013; Ajibo *et al*., 2020; Nkwocha *et al*., 2020). In rural African settings, ownership of livestock quantifies ones financial and social status, hence it is a form of cash reserve for meeting pressing financial needs (Tirivayi *et al*., 2013). Nevertheless, the sustainability of livestock

production depends greatly on the reproductive performance of the animals, which in turn is largely dependent on availability of quality feed or fodder all year round.

In most developing countries, livestock farmers depend mostly on forage or fodder plants for feeding their animals, as intensification of livestock production particularly ruminants and feeding of concentrates are at the lowest ebbs in most developing countries (Thornton, 2010). Also, these forage or fodder plants are usually very scarce during the dry season. Consequently, *Azadirachta indica* [A. Juss., 1830] also known as neem, Nimtree, "Dogonyaro" (Hausa) and "Akun shorop" (Igbo) is fed to animals especially during times of feed scarcity in most parts of Africa, including Nigeria (Adjorlolo *et al*., 2016). The plant is drought-tolerant, fast-growing and a member of the mahogany tree family called Meliaceace (Ubua *et al*., 2019). The plant is readily available in many parts of Nigeria, especially in the northern part where ruminant production is widely practiced.

Apart from supplementation of feeding during drought, neem is used for traditional treatment of common illnesses due to its anti-helminthic, antimicrobial and other properties (Radhakrishnan *et al*., 2007; Bhowmik *et al*., 2010; Varshney *et al*., 2016). Therefore, neem leaves containing the fruit/seed is intentionally fed to animals due to its acclaimed medicinal values for treatment and prevention of diseases (Subapriya and Nagini, 2005; Alzohairy, 2016; Yadav *et al*., 2016).

These medicinal values are due to the rich phytochemical constituents of *A. indica* which include tannin, steroid, taponin, glycoside, tepernoid, flavonoid, alkaloid, coumarin and anthraquinone (Falana and Nurudeen, 2020). The terpenes account for the anti-inflammatory, antioxidant, anticancer and antiplasmodial properties of *A. indica* (Cox-Georgian *et al*., 2019), Coumarin is responsible for most of the antimicrobial (antibacterial, antifungal, antiviral), anticancer, antihypertensive, anticonvulsant, antihyperglycemic and neuro-protective properties (Pereira *et al*., 2018). Similarly, saponin decrease blood lipids, lower cancer risks, as well as blood glucose level (Shi *et al*., 2004), while alkaloids has analgesics, local anesthetic, neuropharmacologic and antimicrobial effects (Alves de Almeida *et al*., 2017). Ruminants may consume the fruits (containing the seed) while grazing on the leaves. Unfortunately, neem leaf has anti-nutritional bioactive agents, such as azadirachtin and nimbidin (Adjorlolo *et al*., 2016). Traditional use of Nimtree in some places suggested that the seed extract possess contraceptive effect when administered pre or post coitus (Dehghan *et al*., 2005; Bhowmik *et al*., 2010) and antifertility effects in animals (Talwar *et al*., 1997; Mukherjee *et al*., 1999; Chaube *et al*., 2014; Khan *et al*., 2017). Despite these antifertility effects, farmers have continued to feed *A. indica* to their animals because of fodder scarcity and its acclaimed beneficial health effects. The anti-reproductive property of neem makes the plant unfit for use as forage or fodder plant in livestock production. Therefore, the objective of this study was to determine the reproductive and haematological effects of methanolic seed extract of *A. indica* (MSEAI) in adult female albino rats; as reduction in haematological parameters in both pregnant and non-pregnant females could predict adverse reproductive outcomes.

MATERIALS AND METHODS

Neem Seed Extraction: A plant taxonomist from the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, identified (ND, 2020) and authenticated the neem fruit/seed collected from the Botanical Garden, University of Nigeria, Nsukka. The voucher specimen (PSB/2018/4506/22) was deposited at the Departmental Herbarium. The seeds were obtained from the fruits and sundried to constant weight. Three hundred (300) grammes of neem seeds were weighed out and pulverized. The extraction was done by maceration method as described by Attia *et al*. (2018). This method involved soaking the pulverized seeds in one litter of 100% hexane for 72 hours with vigorous intermittent agitation every 3 – 6 hours. The mixture was then sieved with a filter paper into a beaker and the filtrate maintained in standing position for another 72

hours at 37^0C in order to concentrate the filtrate. Using the hexane filtrate, the extraction procedure was repeated with chloroform and then methanol in order to obtain a methanolic fraction of the extract. Thereafter, the crude MSEAI formed was scraped out into a clean container and preserved in a refrigerator until point of use.

Study Design and Experimental Animal: The study adopted a factorial study design which consisted of 4 treatment groups and 4 replicas per group. Sixty four adult female and eight male albino rats with average weight of 183 ± 2.8 g were used for the study. The female rats were randomly assigned into four treatment groups (A, B, C and D) of sixteen rats each. In each group, there were four replicas which consisted of 4 rats each. The 8 males were housed in a separately cage (to be used only during the reproductive study). Group A was the control while groups B, C and D were treated with 50, 100 and 200 mg/kg of the extract respectively daily for 28 consecutive days. The rats were acclimatized for two weeks and were allowed unrestricted access to feed (Top Feed Broiler Finisher with 19 % crude protein and 3300 metabolizable energy) and clean drinking water all through the experiment. Ethical approval for use of the rats (reference number FVM-UNN-IAUCC-2020-0344) was granted by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The experimental animals were handled in compliance with the Guidelines for the Care and Use of Laboratory Animals provided by the National Research Council (NRC, 2011).

Toxicity Test: Ten adult rats comprising of five males and five females were used for the toxicity test to determine the lethal dose (LD_{50}) of the extract as described by Lorke (1983). The rats were dosed at 7500 mg/kg each by oral gavage and observed for three consecutive days for signs of toxicity and mortality.

Haematological Studies: Blood samples were collected from four randomly selected rats from each treatment group on day 0, for determination of baseline haematological parameters. The samples were collected via the medial canthus of the eyes using a heparinized capillary tube into 10 ml test tubes containing ethylenediaminetetraacetic acid (EDTA). Daily dosing of the animals with the extract commenced the same day after the baseline sample collection. On treatment days 14 and 28, samples were equally collected again for haematological profiling. All the rats sampled were randomly selected without replacement. Haematological parameters such as haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, total white blood cell (WBC) count and differential WBC were ascertained. All the blood parameters were determined according to the methods described by Cheesbrough (2010).

Reproductive Studies: At the end of the haematological studies, two male rats were assigned to the remaining four females in each group, for mating. Evidence of mating (yellowish protein coagulates - a remnant of the copulatory plug) was determined as described by Ochiogu *et al*. (2006). Body weights of the female rats were recorded 48 hourly till the end of the experiment in order to detect rapid weight gain suggestive of pregnancy. For female rats that conceived and had successful parturition, the gestational length (number of days between conception and parturition), litter size (number of fetuses born), litter weight (overall weight of the fetuses at birth) and the number of fetuses born alive were counted. Thereafter, the dam was humanely sacrificed, dissected to expose the viscera and the number of resorption sites, fetal attachment sites, implantation sites and corpora lutea were manually counted.

Statistical Analysis: Mixed ANOVA (analysis of variance) with repeated measures via a general linear model was used for the data analysis. Treatment doses, treatment duration and the interaction between treatment and time were compared for each of the studied parameters. Where ANOVA showed a significant difference, differences between means were compared using Fishers' Least Significant

Difference (LSD) post hoc multiple comparisons. Reproductive and fertility parameters were analyzed using One-way analysis of variance and Tukey's honestly significant difference (HSD) post hoc multiple comparison. In both analyses, statistical significance was accepted at $p \leq 0.05$ but the actual p-values were also provided to indicate the level of significance. All the analyses were performed using IBM® SPSS statistical package version 16 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Extraction and Toxicity Test: The total crude extract yield was 33.79 % after extraction from 300 g of dried *A. indica* seeds. Hexane fraction yielded 18.37 %, chloroform 4.85 % and methanol 10.57 % of the crude extracts respectively. There was no mortality and the rats showed no observable signs of toxicity effect even after 72 hours post-administration of the extract at 7500 mg/kg; which indicated that the MSEAI is relatively safe.

Effects of MSEAI on Haematological Parameters

Haemoglobin concentration, packed cell volume and red blood cell count: The extract caused both dose and time-dependent decrease on haematological parameters in rat as haemoglobin concentration, packed cell volume and red blood cell count were significantly decreased in the high dose (100 and 200 mg/kg) treatment groups on day 28 (Table 1).

Statistically, simple main effect for group showed a significant effect for group on Hb [$F (3, 12) = 3.416$, $p = 0.050$, partial $n^2 = 0.461$]. Pairwise comparisons for group showed a significant decrease in Hb levels in the 100 and 200 mg/kg groups when compared to control on day 28 at $p = 0.016$ and 0.003 respectively. Post hoc test showed a significant decrease ($p<0.05$) in Hb levels in group D when compared with groups A and B. For the mean RBC counts, simple main effect analysis showed a significant effect for group [$F (3, 12) = 1.877$, $p = 0.029$, partial $n^2 = 0.319$]. Similarly, pairwise comparisons for group showed a significant decrease ($p<0.05$) in RBC levels in Groups B, C and D when compared to Group A on day 28 at $p = 0.018$, 0.004 and 0.003 respectively. Furthermore, mean PCV concentration also showed a significant main effect for Group [$F (3, 12) = 1.583$, $p = 0.050$, partial $n^2 = 0.284$]. Similarly, pairwise comparisons for group showed a significant decrease ($p<0.05$) in PCV concentrations in Group D when compared to control at $p = 0.036$.

Total and differential leukocyte counts: The extract slightly increased the mean total WBC across the groups and treatment duration but the marginal increases were not statistically significant ($p>0.05$) (Table 2). On differential WBC counts, mean lymphocyte count slightly increased from 73.5 ± 4.0 in the control to 77.0 ± 4.0 on day 28 of treatment in the 200 mg/kg treatment group. On the contrary, mean neutrophil count decreased from 25.0 ± 3.8 in the control group to 22.3 ± 3.6 on day 28 post treatment in the 200 mg/kg group. Generally, there were no significant variations ($p>0.05$) in the mean total and mean differential WBC counts in the groups and across the treatment groups (Table 2).

Effects on MSEAI on Reproductive and Fertility Parameters: Twenty-five percent and 100 % of pregnant does in groups C and D respectively had abortion post confirmation of mating. However, all the fetuses were born alive in the control, 50 and 100 mg/kg groups. The litter weight decreased from 52.9 ± 5.0 g in the control to 46.6 ± 15.6 g in the 100 mg/kg treatment group. The number of resorption sites increased from zero in the control to 0.3 ± 0.3 in the 50 mg/kg group. Details on the effects of the extract on reproductive parameters of female albino rats are shown in Table 3.

Statistical analysis showed that gestational length, litter weight, litter size, number of fetal attachment sites, number of corpora lutea and number of implantation sites were not statistically significant ($p>0.05$) across the experimental groups.

Table 1: Haemoglobin, red blood cell and packed cell volume values of female albino rats exposed to different doses of methanolic seed extract of *Azadirachta indica*

Haematological parameters	Treatment days	Treatment groups			
		Control	50 mg/kg	100 mg/kg	200 mg/kg
Haemoglobin concentration (g/dl)	Day 0	13.7 ± 0.3	14.0 ± 0.8	13.9 ± 0.6	12.9 ± 1.2
	Day 14	15.0 ± 1.0	13.8 ± 0.4	15.0 ± 1.4	13.8 ± 0.5
	Day 28	16.3 ± 0.9^{a}	16.2 ± 0.5^{a}	14.3 ± 0.3^{b}	13.7 ± 2.4^{b}
Red blood cell counts (10^6/µl)	Day 0	4.1 ± 6.3	4.8 ± 0.3	4.1 ± 0.1	3.6 ± 0.5
	Day 14	4.6 ± 0.7	4.9 ± 0.6	5.2 ± 0.2	4.5 ± 0.2
	Day 28	5.7 ± 0.2^{a}	4.7 ± 0.3^{b}	4.4 ± 0.3^{b}	4.3 ± 0.3^{b}
Packed cell volume (%)	Day 0	41.3 ± 1.1	44.3 ± 1.7	41.8 ± 1.9	38.0 ± 4.9
	Day 14	41.3 ± 1.1	44.3 ± 1.7	41.8 ± 1.9	38.0 ± 4.9
	Day 28	47.0 ± 2.0^{a}	45.8 ± 1.2^{ab}	43.5 ± 1.9^{ab}	41.8 ± 0.8^{b}

Results are presented as means ± SE, n = 16, Means with different superscript on the same row are significantly different (p<0.05), while means with similar superscript are not significantly different (p>0.05)

Table 2: Total and differential leukocyte counts of female albino rats exposed to different doses of methanolic seed extract of *Azadirachta indica*

Leukocytes counts	Treatment days	Experimental groups			
		Control	50 mg/kg	100 mg/kg	200 mg/kg
Total count	Day 0	5.7 ± 0.8	7.2 ± 0.9	6.4 ± 0.6	5.6 ± 0.9
	Day 14	5.3 ± 0.5	5.7 ± 0.2	6.4 ± 0.4	6.2 ± 0.5
	Day 28	5.2 ± 0.4	5.4 ± 0.4	6.0 ± 0.7	5.8 ± 0.3
Lymphocyte	Day 0	73.5 ± 2.7	72.8 ± 2.6	74.6 ± 2.4	73.3 ± 4.0
	Day 14	72.3 ± 3.3	74.0 ± 3.2	74.5 ± 3.7	72.0 ± 3.2
	Day 28	73.5 ± 4.0	73.0 ± 3.9	73 ± 2.8	77.0 ± 4.0
Neutrophil	Day 0	25.3 ± 3.1	26.0 ± 3.0	24.0 ± 2.7	25.5 ± 4.1
	Day 14	26.5 ± 3.1	26.0 ± 3.0	24.0 ± 3.7	25.5 ± 3.5
	Day 28	25.0 ± 3.8	25.8 ± 3.4	25.8 ± 3.3	22.3 ± 3.6
Monocyte	Day 0	0.8 ± 0.5	0.8 ± 0.5	1.0 ± 0.7	0.8 ± 0.5
	Day 14	0.8 ± 0.5	0.8 ± 0.5	1.0 ± 0.4	1.0 ± 0.4
	Day 28	0.8 ± 0.3	1.0 ± 0.6	1.0 ± 0.7	0.8 ± 0.5
Basophil	Day 0	0.0 ± 0.0	0.3 ± 0.3	0.3 ± 0.3	0.0 ± 0.0
	Day 14	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3
	Day 28	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Eosinophil	Day 0	0.5 ± 0.3	0.3 ± 0.3	0.5 ± 0.3	0.5 ± 0.3
	Day 14	0.5 ± 0.5	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3
	Day 28	0.5 ± 0.5	0.3 ± 0.3	0.3 ± 0.3	0.0 ± 0.3

The values are presented as means ± SE, n = 16, p = 0.05

However, there was a significant difference in the number of resorption sites seen between the groups at p = 0.008. Furthermore, Post Hoc multiple comparisons showed that significant increase (p≤0.05) existed in the number of resorption sites in the 50 and 100 mg/kg groups in comparison to the control.

DISCUSSION

In this study, reproductive and haematological effects of *A. indica* were determined in female albino rats to ascertain the safety of *A. indica* when fed as forage or used for traditional treatment of infection in pregnant or breeding female animals.

Table 3: Reproductive parameters of female albino rats fed methanolic seed extract of *Azadirachta indica*

Groups	Experimental Groups		
	Control	50 mg/kg	100 mg/kg
Gestation length (GL) in days	18.5 ± 0.3	22.3 ± 0.8	17.3 ± 5.8
Litter weight (LW) in gramme	52.9 ± 5.0	44.5 ± 8.3	46.6 ± 15.6
Litter size (LS)	8.3 ± 0.9	6.8 ± 1.3	7.8 ± 2.6
Corpus luteum (CL)	9.0 ± 0.7	11.8 ± 0.5	8.8 ± 2.9
Foetal attachment sites (FAS)	8.3 ± 0.9	6.8 ± 1.3	7.8 ± 2.6
Resorption sites (RS)	0.0 ± 0.0^{a}	2.8 ± 0.9^{b}	0.3 ± 0.3^{a}
Implantation sites (IS)	8.3 ± 0.0^{a}	9.5 ± 1.6^{b}	8.0 ± 2.7^{a}

Results are presented as means ± SE, n = 16, Means with different superscript on the same row are significantly different (p<0.05), while means with similar superscript are not significantly different (p>0.05)

Unavailability of forage plants during the dry season usually compels small-scale farmers to incorporate available green plants like *A. Indica* in their livestock rations; as substitute to conventional fodder plants in Sub-Saharan Africa (Adjorlolo *et al.*, 2016). Our finding showed that MSEAI at high doses (≥100 mg/kg body weight) had negative effects on the haematological parameters of female albino rats; which implied that the extract could adversely impact reproductive and fertility parameters in female rats.

The significant decrease ($p<0.05$) in the mean haematological parameters (Hb, RBC and PCV) of the treated female rats on day 28 highlights the potential harmful haematological effect of neem on female animals following prolonged ingestions. The result was in contrast with the findings of Ndodo *et al.*, (2013) and Iyare and Obaji (2014) who reported increase in Hb, RBC and PCV values of rats treated with neem leaf extract. The dichotomy in the findings could be due to the part of neem tree used and the duration of the treatment. It appears that while shot-time consumption of *A. indica* leaf enhances haematological values (this probably explains its traditional use in malaria treatment), prolong ingestion of the fruit/seed (as evidenced in this study) causes detrimental haematological effects.

In addition, it is most probable that a component of the MSEAI called praneem (containing azadirachtin) was responsible for the detrimental reproductive (contraceptive and anti-fertility) effects (Talwar *et al.*, 1997). Praneem, a purified neem seed extract, caused abrogation of pregnancy in rodents by marked reduction of serum progesterone level is early pregnancy (Mukherjee and Talwar, 1996). Preneem also caused increased cytokine (TNF-alpha and gamma-interferon) and immune cell (CD4 and CD8) levels in rats treated with neem seed extract and these resulted in abortions and fetal resorption (Talwar *et al.*, 1997). In addition, spermicidal and contraceptive effects of praneem post coitus have been reported (Raghuvanshi *et al.*, 2001).

Since animals may consume neem fruit/seed alongside the leaf while grazing on the plant, it is important therefore not to feed *A. indica* to livestock (particularly to pregnant or breeding females) in view of the negative effects. If the plant must be fed, especially during drought to salvage starving livestock, it may be worthwhile to painstakingly remove the fruits/seeds to avoid the negative health and reproductive consequences.

Although, Vyas and Purohit (2018) found no significant variation in the haematological parameters of female rabbits treated with neem seed oil, the MSEAI used in this study seems to have dose-dependent effect, which increased with treatment duration (tends towards chronicity). The discrepancies in the findings may be attributed to differences in the animal models used, type and dose of extract administered and the duration of the treatment. This dose/time-dependent effect of MSEAI may explain the significant mean decrease in all the haematological values on day 28 in the treatment groups and not in the

control group. These findings therefore portend that prolonged exposure to MSEAI, may hamper reproducibility in female animals since reduced haematological parameters are inimical to reproduction in both humans and animals.

Since there was no significant difference in the gestational length between the treated and control groups, it appears that the extract had no major effect on initiation of labour and fetal expulsion (Upadhyay, *et al*., 1990). The fetal expulsion is dependent on contraction of uterine and abdominal muscles through cascades of biochemical processes involving Ca^{2+} (Garfield and Maner, 2007), hence the normal gestation length observed. Additionally, MSEAI may have inhibited implantation by creating hostile uterine environment that makes attachment and survival of developing zygotes in the endometrium difficult (Timeva *et al*., 2014); however, the number of corpora lutea, litter size and litter weight in fetuses that survived were not affected. This agrees with the findings of Upadhyay, *et al*. (1990) who reported that neem seed extract had anti-implantation effect but no significant effect on the number of corpora lutea, litter size and litter weight in surviving fetuses. The hostile endometrial environment may have led to fetal resorption and hence the significant difference in the number of resorption sites found between the treated and control groups.

Even in the male, care should be taken in administration of MSEAI as spermicidal and other repro-toxicity effects have been reported (Mishra and Singh 2005; Oguejiofor *et al*., 2020). Neem leaf extract interfered adversely with spermatogenesis by reducing the amount, motility and morphology of spermatozoa; and also affected functionality of the testes which caused the decreased semen quality (D'Cruz *et al*., 2010; Daniyal and Akram, 2015). However, Mishra *et al*. (2018) found that the suppression of spermatogenesis and fertility in mice treated with aqueous extract of *A. indica* could be reversible as signs of toxicity were not detected in the treated animals.

Since decreased Hb, RBC and PCV values can adversely affect homeostasis and other physiological processes in the body (Kuhn *et al*., 2017), it is possible that the fetal resorption observed in the 50 mg/kg treatment group and abortion in the 100 and 200 mg/kg treatment groups were due to the decreased haematological parameters. Anaemia during early pregnancy causes fetal resorption, abortion, preterm birth and low birth weight in humans and animals (Upadhyay and Upadhyay 2017; Guo *et al*., 2019). During pregnancy, the body's nutritional and physiological demands increases due to the developing fetus (Upadhyay and Upadhyay, 2017). Therefore, decrease in haematological values such as Hb, RBC and PCV may lead to conception failure or abrogation of pregnancy, evidenced by fetal resorption, abortion or preterm birth, as the anemic dam may not be able to cope with her physiological needs and that of the developing fetus (Sifakis and Pharmakides, 2000). Besides these, other fetal and reproductive abnormalities may ensue following marked reduction of haematological parameters in pregnant females (Means, 2020).

The susceptibility of pregnant animals to haematological problems following consumption of *A. indica* underscores the need to specially care for their nutritional needs; through the provision of hay and concentrates to discourage them from grazing on neem or other harmful plants particularly during the dry season. This can be achieved through mass cultivation of fodder crops all year round for livestock production in order to avoid undesirable pregnancy outcomes associated with ingestion of neem.

Furthermore, neem plant has been reported to contain anti-nutritional factors such as azadirachtin and nimbidin (Ubua *et al*., 2019). These factors may have played crucial role (haemolysis) in decreased haematological parameters found especially in the high dose treatment groups. Furthermore, it is possible that the anti-nutritional factors directly or indirectly inhibited absorption of Fe^{2+}, folic acid, cyanocobalamin and other essential nutrients requisite for haeme synthesis and erythropoiesis (Koury and Ponka, 2004) hence the reduction in haematological values being reported in the high dose groups.

The mean total and differential WBC counts obtained in this study showed no

significant variations between treated and untreated groups. This was in tandem with the findings of Ndodo *et al*. (2013), who found no significant increase in total WBC count in rats treated with MSEAI. This suggested that the MSEAI might have no stimulatory effects on the immune system hence no significant increase in mean total and differential leukocyte counts. In view of the fore going, it is improbable that the mechanism of pregnancy termination, exerted by the extract is immuno-modulatory since no significant difference existed in the WBC counts. Our findings therefore implied that the termination of pregnancy, evidenced by abortion or resorption in 100, 200 and 50 mg/kg groups, might have been caused by decreased blood volume and or Hb concentration, rather than the activation of immune cells to fight off the pregnancy as reported by Upadhyay *et al*. (1992). Hence we hypothesize that the extracts depleted the red blood cells, packed cell volume and haemoglobin concentrations of female albino rats necessary for development and maintenance of pregnancy.

Conclusion: The MSEAI caused detrimental reproductive and haematological effects (marked reduction in Hb concentration, RBC count and PCV value) in female albino rats. These effects were more in the 200 and 100 mg/kg groups and increased with duration of treatment. Therefore feeding *A. indica* as forage to animals on the grounds of its medicinal benefits or fodder scarcity could be counterproductive. However, the antifertility and anti-reproductive properties of the plant could be exploited in the control of rodent population in livestock/poultry farms. The contraceptive properties give credence to the possible use of MSEAI to achieve contraception in non-breeding female animals, especially bitches kept for security purposes. The MSEAI can also find application in the control of stray dog population known to be reservoirs of rabies virus in Nigeria.

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