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Effects of intramuscular administration of ethanolic extract of *Carica papaya* seeds on semen ejaculate characteristics of goat bucks

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Abstract

This study investigated the effects of intramuscular administration of ethanolic extract of *Carica papaya* seeds (EECPS) on semen ejaculate characteristics of goat bucks. The extract was prepared by cold maceration of dried pulverized seeds of *Carica papaya* in 80% ethanol. Eighteen West African Dwarf goat bucks were used for the study. They were randomly assigned to three groups (A, B and C) of six each. Bucks in Groups A, B and C were given daily intramuscular injections of the EECPS at the doses of 0.1, 0.3 and 0.5 mg/kg body weight, respectively for eight weeks. Semen for laboratory evaluation was collected weekly from bucks in all the groups all through the eight weeks of treatment and additionally for four weeks after treatment stopped (total of 12 weeks). Results showed that the semen colour changed from milky to watery for all the groups across the experimental period. There were no significant variations ($p > 0.05$) in the semen volume across the groups except on week 4 when the semen volume of Group C bucks was significantly ($p < 0.05$) higher than those of other groups. Sperm cell concentration was lower in the Group C bucks when compared with Groups A and B all through the experiment. The percentage motility of sperm cells significantly ($p < 0.05$) varied across the groups with Group C bucks having the lowest all through. The percentage of dead sperm cells was significantly ($p < 0.05$) higher in the Group C bucks when compared with the Groups A and B all through the experimental period. The 12th week results showed gradual recovery from the adverse effects on the semen parameters. It was concluded that treatment of goat bucks with ethanolic extracts of *Carica papaya* seeds as done in this study led to significant dose-dependent adverse effects on their semen ejaculate characteristics.

Keywords: *Carica papaya* seeds; Ethanolic extract; Semen ejaculate characteristics; goat bucks.

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Introduction

Small ruminants are a major component of the livestock sector in most parts of the world, including Nigeria (Odo, 2003). They are primarily kept for meat and milk, and improvement of socio-economic status of individuals and communities (Devandra and Mcleroy, 1982; Payne, 1990; Ajala, 1995). Controlled breeding in small ruminants as in other livestock industries, if not well executed like other aspects of management practices, constitutes an aspect of production constraints. The process of controlled breeding helps the farmer to optimize the use of particular traits selected to achieve the economic objectives and minimize wastes. The success or otherwise of many controlled reproduction techniques is not only a question of influencing the reproductive processes of the females: the outcome also depends on the capability of the males especially in developing worlds like Nigeria, where natural service is the mode of breeding, and unsupervised mating systems are usually common since most farms are small holder schemes. Unsupervised mating is usually characterized by mismating, immature pregnancy with consequent dystocia in females and fighting which results in injuries in males. Various techniques have been devised to control mating of male animals: these include castration, vasectomy, hormonal application and immunization (Weaver, 1986). However, these techniques have their individual disadvantages.

In modern ethno-veterinary practice, the interest to develop breeding control agents from herbs emanates from the latter being quite common. The synthetic breeding control agents such as hormones have some inherent problems especially on long term changes in behavourial patterns of animals. In Pakistan, India, Sri Lanka and other parts of the world, *Carica papaya* has long been used as a folk-remedy for contraception and abortion (Reed, 1976). The seed has been used traditionally for contraception and abortion. The seeds are considered alexentic, abortifacient, counter-irritant, emmenagogue and anthelminthic (Reed, 1976). Research with animals has confirmed the contraceptive and abortifacient capability of *Carica papaya* and found that the seeds have contraceptive effect in male langur monkeys and possibly in adult male humans as well (Lohiya *et al*., 2002).

The efficacy of treatment with *Carica papaya* depends on the quantity of different compounds in the preparation. In trial studies with rats, daily oral doses of benzene and alcohol extracts at 20 mg/kg body weight for 30 days did not affect body weight or reproductive organs weight or adversely affect liver or kidney function (Chinoy and Padman, 1997). Male rats given ethanol seed extracts orally (10 or 50 mg/kg/day) for 30, 60 or 90 days or intramuscularly (0.1 or 1.0 mg/day) for 15 or 30 days had decreased sperm motility (Chinoy *et al*., 1997). Oral dosing with the extract was also reported to lead to decreased testis weight and sperm count (Harsha *et al*., 1996). There is however no reports in available literature on the effects of *Carica papaya* seed extracts on the reproductive indices of goats, even when its fruit bye products including the seeds are commonly fed to goats traditionally. The present study evaluated the effects of intramuscular administration of extract of *Carica papaya* seeds (ECPS) on semen ejaculate characteristics of West African Dwarf (WAD) goat bucks.

Materials and Methods

The *Carica papaya* fruits from which the seeds used for the study were collected were harvested from *Carica papaya* trees at the Botanical Garden of the University of Nigeria, Nsukka, Nigeria. Nsukka is located at latitude 6.85783 N and longitude 7.39577 E. The *Carica papaya* plant and fruits were authenticated by a Botany Technologist at the Biodiversity and Conservation Project Nsukka, Nigeria. The *C.*

papaya fruits were harvested unripe, then cut open and the seeds collected. The seeds were air-dried, and the dried seeds were ground into coarse form using a Laboratory Miller. Five hundred gramme of the coarse powder was dispensed into a 2.5 litre capacity Winchester bottle and soaked in one litre of 80% ethanol. The Winchester bottle containing the mixture was subjected to intermittent vigorous shaking for 48 hours after which it was filtered into a beaker using a funnel and Whatmann filter paper. The extract was concentrated in a hot air oven set at 40^{o}C for 72 hours to remove the ethanol. The extract was weighed and the yield of the extraction was calculated. The extract was stored at 4^{o}C until when it was used for the study.

Experimental Animals: Eighteen West African dwarf bucks were used for this study. The mean weight of the goats was 7.0 ± 1.68 kg, while their ages ranged from 5 – 12 months. They were sourced from the local goat markets of Orie Orba, Afor Opi and Nkwo Ibagwa, Enugu State, Nigeria. Only apparently healthy bucks were purchased. They were housed in a clean and disinfected pen at room temperature, fed on grasses and pelleted commercial feed supplement, and clean drinking water was given *ad libitum*. The goats were acclimatized for a period of one month during which they were dewormed with Ivermectin (Pantex, Holland) and treated with broad spectrum antibiotics Penstrep® (Veyogpharma, China) to obviate any infection.

Handling of experimental animals during the study: The guidelines set out by the University of Nigeria, Nsukka Animal Research Ethics Committee for medical and scientific research with animals, which among others include good, clean, and hygienic housing, provision of adequate feed and clean drinking water and humane handling of animals was strictly followed in the handling the animals throughout the experiment. These guidelines were in conformity with the guiding principles for biomedical research involving animals as issued by the Council for International Organisations of Medical Sciences (CIOMS). Valid approvals and ethical clearance were obtained from the University of Nigeria, Nsukka Animal Research Ethics Committee before the commencement of the experiment.

Experimental Design: The 18 goats were randomly assigned to three groups of six each (Groups A, B and C). Semen ejaculate samples were collected from each of the goats to serve as the baseline values, and afterwards each of the goats in the specific groups were treated as follows: Groups A – 0.1 mg/kg extract, Group B – 0.3 mg/kg extract; Group C – 0.5 mg/kg extract. The choice of these doses were made based on a previous study by Chinoy et al, (1997) who dosed male rats at 0.1 mg/kg/day and reported decreased sperm cell motility. The treatments were given daily by intramuscular injection for 8 weeks. Semen ejaculates were collected weekly from the bucks for laboratory evaluation, all though the 8 weeks of treatment and for four more weeks post-treatment to make up a total of 12 weeks of the experimental period.

Semen collection and analysis: Semen ejaculate samples were collected using an electro-ejaculator by passing varied degrees of electrical stimulation to the lumber sympathetic nerves through the anus to promote semen emission and to the pelvic splanchnic and internal pudendal nerves to promote ejaculation and erection of penis. Semen samples were collected from each buck using petri dishes. The collected semen were transferred to a sample bottle and immersed in a water bath with set temperature at 37^{o}C. Semen volume was estimated using tuberculin syringe to aspirate the ejaculate and the volume read off in ml. Semen colour was visually evaluated.

A drop of the semen was mixed with two drops of eosin-nigrosin stain and smeared on a clean glass slide and examined under the light

microscope. The dead sperm cells took up the pinkish stain while those alive remained whitish in colour. A count of 100 cells was made in each case and the number of dead sperm cells expressed as percentage (Bjorndahl *et al*., 2003). From the stained smear of the semen sample, 200 cells were counted and the abnormal cells expressed as percentage (Graham, 2001) Sperm cell concentration was estimated using a haemocytometer and light microscope with a semen diluting fluid (dilution at 1 in 200) as described by Patrick (2012).

Data analysis: Data collected were subjected to one way analysis of variance (ANOVA), and variant means were separated post-hoc using the least significant difference method. All the analysis was done using IBM SPSS software, version 20. Significance was accepted at probability less than 0.05%. Results were presented as line graphs of means with standard deviation.

Results

The percentage yield of the seed extract was 5.45% weight per weight dry matter.

There were no significant variations ($p > 0.05$) in the semen volume of all the groups from week 0 (baseline) till week 9 of the experiment, but on week 10 and 11, the semen volume of the Group B bucks was significantly ($p < 0.05$) higher than those of Groups A and C bucks (Figure 1). The semen colour changed from milky to watery, in all the groups as the experiment progressed.

The mean sperm cell counts of the semen ejaculates decreased progressively in all groups following treatment with the extract, and there were no significant ($p > 0.05$) variations among the groups all through the experiment except on week 3 when the mean sperm cell count of the ejaculates of the Group C bucks was significantly ($p < 0.05$) lower than those of Groups A and B (Figure 2). The mean sperm cell motility of the Group C bucks was significantly ($p < 0.05$) lower than those of Groups A and B from week 1 to 5 of the experiment, and again from week 9 to week 12 of the experiment, though from week 10 upwards (two weeks after treatment was stopped), there was evidence of recovery (increase in mean sperm cell motility) in all groups (Figure 3). In contrast to the sperm cell counts and motility, the mean percentage of dead sperm cells increased in all groups following treatment, and was significantly ($p < 0.05$) higher in the Group C bucks treated with the highest dose of 0.5 mg/kg body weight of the extract, when compared with other groups (Figure 4). At weeks 5 and 7 of the experiment, diarrhoea was observed in the Group C bucks.

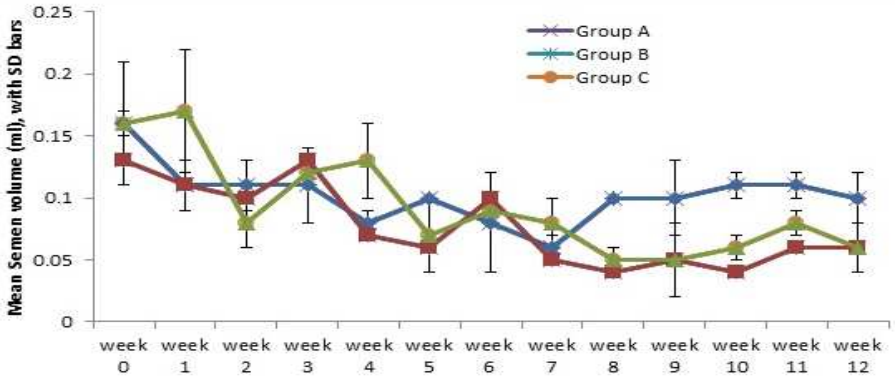

Figure 1. Semen ejaculate volume of goat buck groups treated with varied doses of ethanolic extract of *Carica papaya* seed for eight weeks.

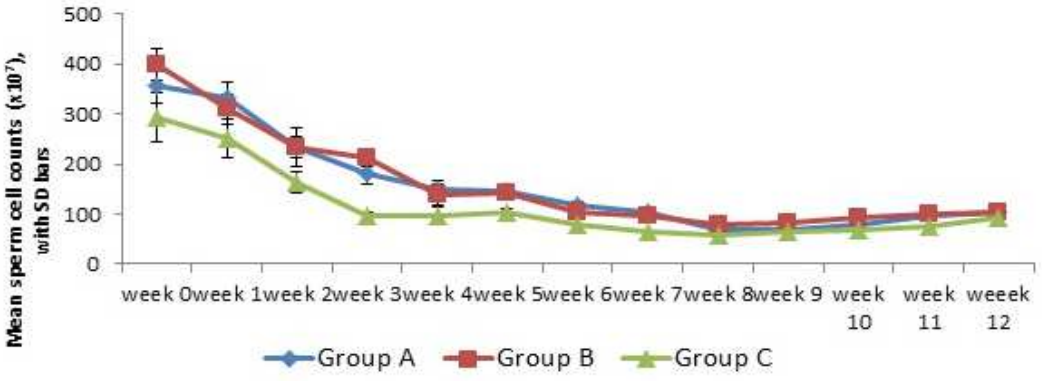

Figure 2. Sperm cell concentration in semen ejaculates of goat buck groups treated with varied doses of ethanolic extract of *Carica papaya* seed for eight weeks.

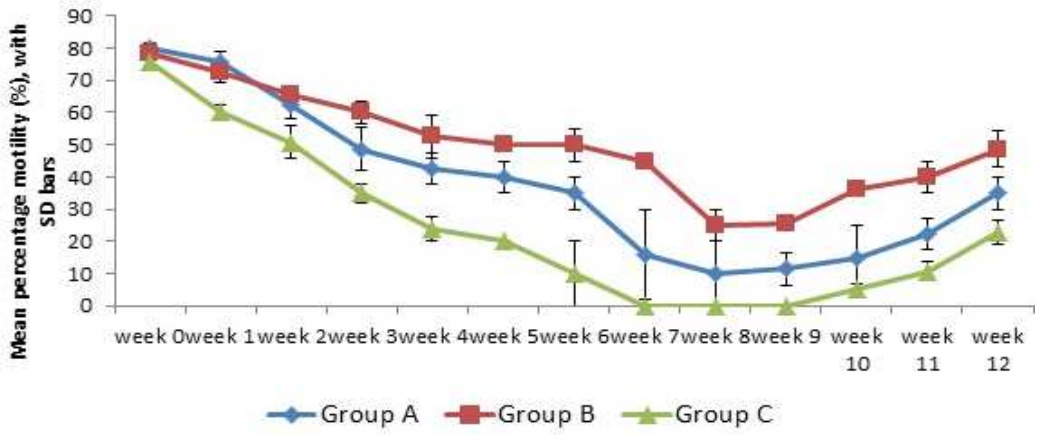

Figure 3. Sperm cell motility in semen ejaculates of goat buck groups treated with varied doses of ethanolic extract of *Carica papaya* seed for eight weeks.

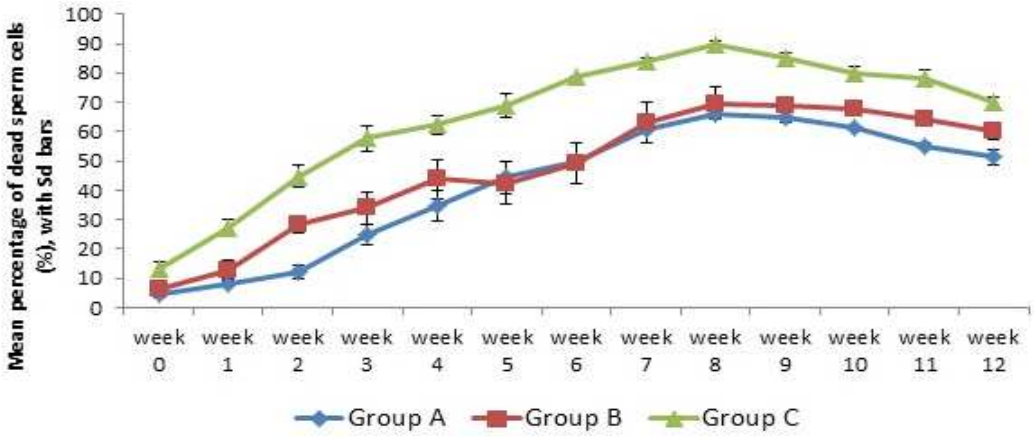

Figure 4. Percentage of dead sperm cells in semen ejaculates of goat buck groups treated with varied doses of ethanolic extract of *Carica papaya* seed for eight weeks.

Discussion

Treatment with the different doses of the extract as used in the study did not affect semen volume. It is thought that the lack of significant effect on semen volume may be as a result of the fact that semen volume can be made up from tissue fluids and does not necessarily translate to sperm concentration or viability, just as blood volume can be made up from tissue fluids in cases of loss of blood. The change in semen colour from milky to watery is probably be due to the reduction in the cell concentration. The low sperm cell value as recorded in the present study agrees with the reports of Harsha *et al (1996)*. Harsha *et al*. (1995) also showed that frequent ejaculation also could have contributed to low sperm cell concentration. It is possible that treatment with the ethanolic extract of *Carica papaya* seeds affected the androgenic hormone that is responsible for spermatogenesis and thus the level of sperm cell production or that the extract negatively affected the germinal layer of the seminiferous tubule thus, disrupting sperm cell production.

The dose dependent effect of the varying doses of the extract on sperm cell concentration, sperm motility and percentage of dead sperm cells as recorded in this study, with the highest dose exhibiting the most severe effect, agrees with what was reported by Chinoy and Padman (1997) in rats. The significantly higher number of dead sperm cells recorded for the Group C bucks in this study, is thought to be due to the higher dose used in treating them. This finding concurs with the reports in available literature, which have shown that oral administration of aqueous, methanol, ethanol, ethyl acetate and choloroform extracts of *C. papaya* seeds has reversible contraceptive effects, adversely affects sperm motility and leads to sperm deaths. (Lohiya *et al*., 2002; 2006; 2008).

While Papaya is known to be a good source of fibre and vitamins, and it is renowned to be good for digestive health; eating too much of it has been reported to have a laxative effect (Muss *et al*., 2013; Soputri and Panjaitan, 2022). The diarrhoea recorded in the Group C rats concurs with these reports.

It is thought that the findings in this study shall be significant in control breeding practices especially in the small ruminant livestock industry for certain reasons. Firstly, *Carica papaya* fruit and seeds are commonly available in homes and household of goat farmers. The information on its effect on male reproduction is therefore very vital to farmers especially at small holder level, since the adverse effects on ejaculate parameters may likely manifest at this level. Secondly, the extract promises to be a good male contraceptive for goats, and this is an advantage when we consider its possible use in controlled breeding programme.

Based on the results of the study, it was concluded that treatment with extract of *Carica papaya* seeds as used in the study led to significant adverse effects on sperm cell concentration, sperm motility and the percentage of dead sperm cells, especially at the higher dose of 0.5 mg/kg. Further studies on the effect of the ethanol seed extract of *Carica papaya* on male hormones and testicular morphology, body weight, serum enzyme markers of organ damage in these animals need to be done in order to find explanations for the results obtained and possibly relate the present findings to hormonal and testicular alterations.

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