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Ethanolic Extract and Juice of *Chrysophyllum albidum* Fruit Improve Hormonal and Spermatogenic Indices of Male *Wistar* Rats

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Abstract: **The aim of this study was to investigate the effect of ethanolic extract and juice of *Chrysophyllum albidum* fruit on hormonal and spermatogenic indices of male *wistar* rats as proviron served as a reference standard. Twenty- four male *wistar* rats (150-190 g) were randomly distributed into four groups of six rats each. Group 1 served as control; group 2 received ethanolic extract of *Chrysophyllum albidum* fruit (ECAF) [100 mg/kg orally (p.o)]; groups 3 received juice of *Chrysophyllum albidum* fruit (JCAF) [100 mg/kg p.o] and groups 4 received proviron (PRV) [25mg/kg p.o]. Administration of ECAF and JCAF significantly ($p<0.05$) elevated the serum Testosterone, FSH, LH by 40%, 57% and 51% respectively and lower prolactin by 65% for ECAF and 32.3%, 40.3% and 29% respectively and Prolactin lowered by 31.3% for JCAF relative to the control. In addition, administration of ECAF and JCAF significantly ($p<0.05$) elevated Sperm Motility, Volume and Count by 40.3%, 60% and d35% respectively for ECAF and 30%, 25% and 38% respectively for JCAF. Conclusions: ECAF and JCA improved hormonal and spermatogenic indices of male *wistar* rats but ECAF is a more efficient intervention in ethnomedicine against infertility and male erectile dysfunction.**

Keywords: Chrysophyllum albidum, **hormonal indices, spermatogenic indices, proviron, ethnomedicine.**

I. INTRODUCTION

In order to rescue traditional medicinal plants and knowledge about them from imminent loss as well as the need for health for all, the need to study medicinal plants, according to [1] cannot be overemphasized for numerous reasons including *inter alia* widespread use of plants in folk medicine. Since the first earth summit in Rio de Janeiro, there has been a sustained global awareness of the importance of the plethora of biodiversity and natural resources from tropical forests for several purposes [2]. This seems not only from the ecotourism potentials, the forest products derivable there from, but also from the ethno botanical and ethno medicinal uses attached to the plant genetic resources obtained from these forests [3].

Chrysophyllum albidum, from the sapotaceae family, is commonly found in the Central, Eastern and Western Africa [4], [5]. They are distributed in Nigeria, Uganda, Niger, Cameroun and Cote d' Ivoire [6]. It is often called the white star apple and distributed throughout the southern part of Nigeria [7]. Across Nigeria, it is known by several local names and is generally regarded as a plant with diverse ethno-medicinal uses [4]. In South-western Nigeria, the fruit is called "agbalumo" and known as "udara" in South-eastern. The bark of the plant is used for the treatment of malaria and yellow fever, while the leaf is used as an emollient and for the treatment of skin eruption, stomach ache and diarrhoea [8]. The cotyledons from the seeds of *C. albidum* are used as ointments in the treatment of vaginal and dermatological infections in Western Nigeria. The fruit pulp is rich in vitamin C and iron and an excellent source of raw material for industries [6]. Furthermore, the fruit has been used in folklore medicine to treat infertility in those days although there has not been any scientific proof of that fact and that motivated this research work. Tannins, flavonoids, terpenoids, proteins, carbohydates and resins are the phytochemicals that have been reported in *C. albidum* according to [4]. Eleagnine, tetrahydro- 2 - methylharman and skatole have been isolated from this plant and eleagnine was the main compound responsible for its antimicrobial activity [7]. The seed cotyledon has been reported to possess anti-hyperglycemic and hypolipidemic effects [9].

Infertility can be defined as the inability of a couple to achieve pregnancy after one year of regular and unprotected sexual intercourse [10]. In a society like Nigeria with traditional and extended families, there are lot of pressure on the couple to have a baby. The major role of a woman is still seen to be one of perpetuating the family name. Hence, the diagnosis of infertility can be an assault on self-image, sexuality and relationship. The prevalence of infertility has been noted to be highly variable in Sub- Sahara Africa ranging from 20 – 46%. In contrast, an average rate of 10-15% have been consistently quoted in Nigeria [11]. This has been attributed to high rate of sexually transmitted diseases, complications of unsafe abortion and puerperal pelvic infections.

Spermatogenesis in mammals requires the actions of a complex assortment of peptide and steroid hormones, each of which plays an important role in the normal functioning of the seminiferous epithelium. These hormonal messengers are critical not only for regulation of male germ cell development, but also for the proliferation and function of the somatic cell types required for proper development of the testis [12]. In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair [13]. In addition, testosterone is essential for health and well-being [14] as well as the prevention of osteoporosis and normal sperm development. Prolactin (PRL) (luteotropic hormone or luteotropin) is secreted from the pituitary gland in response to eating, mating, estrogen treatment, ovulation, and nursing. Prolactin provides the body with sexual gratification after sexual acts [12]. It is a protein that in humans is best known for its role in enabling female mammals to produce milk, however, it is influential over a large number of functions with over 300 separate actions of PRL having been reported in various vertebrates [15]. However, highly elevated levels of prolactin decrease the levels of sex hormones, estrogen in women and testosterone in men (hyperprolactinamia) [16]. Therefore, the aim and objective of this study was to investigate the effect of ethanolic extract and juice of *Chrysophyllum albidum* fruit on hormonal and spermatogenic indices of male *wistar* rats as proviron served as a reference standard.

II. MATERIALS AND METHODS

Plant Material:

Fresh ripe fruits of *C. albidum* were purchased in rural parts of the south western Nigeria. Routine pharmacognostic investigations were carried out to confirm authenticity of this material. The fruits were divided into two, the first part was dried and cut into small pieces and crushed to a coarse powder using an electric blender. Coarse powder was subjected to extraction in cold maceration with ethanol according to the method of Ramados *et al.* [17]. This extract was concentrated using rotary evaporator to get ethanolic fruit extract of *C. albidum* [ECAF] and juice was collected from the second part (JCAF). They were stored in well-packed container at 4^0C for future use.

Animals:

Twenty - four (24) male *wistar* albino rats (four to five week-old), weighing between 150 g and 190 g, were purchased from the Animal House of the Physiology Department, University of Ibadan, Ibadan, Nigeria and were grouped into four (4) of six animals each. The animals were kept in well-ventilated cages in the departmental animal house at room temperature(28–30°C) and under controlled light cycles (12-hr light:dark). They were maintained on normal laboratory chow (Ladokun Feeds, Ibadan,Nigeria) and water. All experiments were conducted without anaesthesia and the protocol conforms to the guidelines of the National Institutes of Health (NIH publication no. 85–23, 1985) for laboratory animal care and use.

Experimental Protocols:

The animals were randomly divided into four (4) groups of six (6) animals each. Group I served as control; group II received ethanolic extract of *Chrysophyllum albidum* fruit (ECAF) [100 mg/kg orally (p.o)]; group III received juice of *Chrysophyllum albidum* fruit (JCAF) [100 mg/kg p.o] and group IV received proviron (PRV) [25mg/kg p.o]. The animals were treated for 30 days. At the end of treatment period, the rats were sacrificed by cervical dislocation and blood samples were collected *via* cardiac puncture using heparine [3.8%w/v] as anticoagulant and the plasma separated were used for the determination of diagnostic marker enzymes. The marker enzymes ALT and AST, and reproductive hormones (Testoterone [TSR], Follicle Stimulating Hormone [FSH], Leuterizing Hormone [LH] and Prolactin [PRL]) were assayed in serum using standard kits supplied from Randox. Also, the epididymis was excised immediately; semen was taken from it for sperm analysis.

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Statistical Analysis:

All values are presented as mean ± standard error. Statistical analysis was done using SPSS version 20, One-way ANOVA test and probability values <0.05 were considered to be statistically significant.

III. RESULTS

The administration of the ethanolic fruit extract (ECAF) and Juice of *C. albidum* (JCAF) significantly (P< 0.05) shoot up the sperm volume and count specifically by 50% and 30% for total volume and 49.34% and 28.4% for sperm count respectively relative to the control (Table 1). However, there were no significant difference in the serum activities of AST and ALT following the administration of ECAF and JCAF (Table 1). Furthermore, Significant differences (P< 0.05) were also noticed in the serum Follicle Stimulating Hormone (FSH) and Leuterizing Hormone (LH) following the administration of ECAF and JCAF. ECAF elevated the serum FSH and LH by 57.3% and 43.2% and JCAF also elevated them by 49.3% and 29% respectively with respect to the control (Table 2).

Table 1: Effect of Ethanolic fruit extract and Juice of *C. albidum* on the sperm volume, sperm cell count and serum activities of aspertate transaminase (AST) and Alanine transaminase (ALT).

GROUPS	VOLUME(ml)	COUNT(Million/ml)	AST(U/L	ALT(U/L)
CONTROL	3.54 ± 0.23	88.71 ± 5.23	141.67 ± 20.3	321.34± 50.32
ECAF	5.61 ± 0.48*	139.6 ± 8.5*	139.61 ± 31.4	310.75 ± 43.54
JCAF	4.42 ± 0.67*	131.4 ± 6.52*	120.29 ± 25.37	290.98 ± 49.32
PRV	5.21 ± 0.31*	131.4 ± 6.52*	135.56 ± 30.4	309.51 ± 35.12

***** *Significantly different (P< 0.05) from the control*

ECAF= ethanolic extract of Chrysophyllum albidum fruit , JCAF= Juice of Chrysophyllum albidum fruit , PRV= Proviron

Table 2: Effect of Ethanolic fruit extract and Juice of *C. albidum* on the serum Follicle Stimulating Hormone (FSH), Leuterizing Hormone (LH), weight of testes and epididymis

GROUPS	FSH(MIU/M)	LH(MIU/M)	WGTTT (g)	WGTEPDM(g)
CONTROL	23.34 ± 2.43	41.34 ± 2.7	0.83±0.01	0.068±0.003
ECAF	36.13 ± 0.97*	55.36 ± 3.5*	1.03±0.02	0.095±0.002
JCAF	29.34 ± 3.1*	52.23 ± 1.6*	0.99±0.01	0.089±0.001
PRV	33.17 ± 1.2*	58.81 ± 4.7*	1.16±0.04	0.091±0.002

***** *Significantly different (P< 0.05) from the control*

ECAF= ethanolic extract of Chrysophyllum albidum fruit , JCAF= Juice of Chrysophyllum albidum fruit , PRV= Proviron, FSH = Follicle Stimulating Hormone, LH = Leuterizing Hormone, WGTTT = weight of testes, WGTEPDM = weight of epididymis.

Significant differences (P< 0.05) were also noticed in the serum TSR and PRL following the administration of ECAF and JCAF. ECAF elevated the serum TSR by 43.2% and lowered PRL by 37.4% relative to the control and JCAF also elevated TSR by 44.5% and lowered the serum PRL by 33% respectively when compared with the control (Figure 1). Also, administration of ECAF and JCAF significantly (P< 0.05) shoot up the sperm motility and live/dead ratio specifically by 61.8% and 33.4% for motility and 59.56% and 32.87% for live/dead ratio respectively relative to the control (Figure 2).

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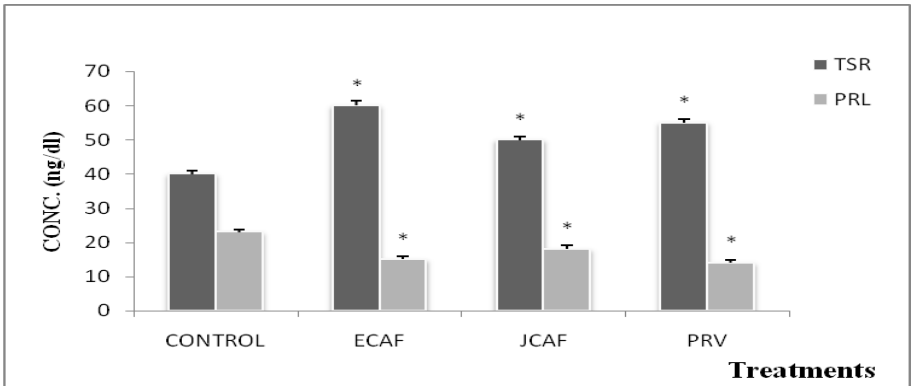

Figure 1: Effect of Ethanolic fruit extract and Juice of *C. albidum* on the serum Testosterone (TSR) and Prolactin (PRL).
** Significantly different (P< 0.05) from the control*
ECAF= ethanolic extract of *Chrysophyllum albidum* fruit , JCAF= Juice of *Chrysophyllum albidum* fruit , PRV= Proviron

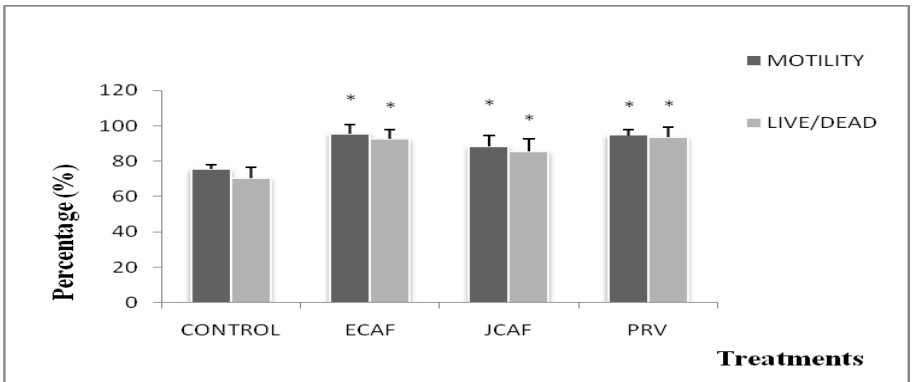

Figure 2: Effect of Ethanolic fruit extract and Juice of *C. albidum* on the motility and live/dead ratio of the sperm cells.
** Significantly different (P< 0.05) from the control*
ECAF= ethanolic extract of *Chrysophyllum albidum* fruit , JCAF= Juice of *Chrysophyllum albidum* fruit , PRV= Proviron

Significant differences (P< 0.05) were also noticed in the serum TSR and PRL following the administration of ECAF and JCAF. ECAF elevated the serum TSR by 43.2% and lowered PRL by 37.4% relative to the control and JCAF also elevated TSR by 44.5% and lowered the serum PRL by 33% respectively when compared with the control (Figure 1). Also, administration of ECAF and JCAF significantly (P< 0.05) shoot up the sperm motility and live/dead ratio specifically by 61.8% and 33.4% for motility and 59.56% and 32.87% for live/dead ratio respectively relative to the control (Figure 2).

IV. DISCUSSION

The finding of this study indicates that ethanolic fruit extract (ECAF) and juice of *C.albidum* (JCAF) causes increase in the weights of sexual organs such as testis and epididymis. The weight, size and secretory function of testes, epididymides and seminal vesicle are closely regulated by androgens [16]. The number of sperm cells in epididymis was also markedly increased. The increased sperm counts as well as increased weight of sexual organs are indicative of improved fertility because of extract treatment. In order to understand these observation measurements of testosterone, FSH, LH and prolactin levels after treatment was undertaken. Elevated levels of testosterone were noticed in treated animals. TSR is the main male gonadal hormone produced by the interstitial cells of the Leydig in the testis. It is also the major index of androgenicity. A certain concentration of androgens is also required for the initiation and maintenance of spermatogenesis and for the stimulation of growth and function of the prostate and seminal vesicles. TSR also helps in maintaining body shape, and increasing muscle mass and strength [18].

The levels of FSH and LH were also increased after treatment with the extracts. TSR is produced by leydig cells of the testes in response to LH, under the control of the hypothalamic–pituitary–testis axis [19]. It seems appropriate that the elevation in serum steroid hormone levels by administration of the extracts may be accountable for the observed effect on spermatogenesis. Both testosterone and FSH have an important function in spermatogenesis [16]. FSH has key roles in the development of a normal complement of functional Sertoli cells (and thus adult spermatogenic potential), in the maturation of sertoli cells at puberty and the maintenance of their cytoskeleton and cell junctions, and in the maintenance of spermatogonial development. Together, FSH and TSR support meiosis, exhibit an anti-apoptotic action on spermatocytes and round spermatids, and act co-operatively to promote spermatid maturation and sperm release [20].

V. CONCLUSION

Ethanolic fruit extract and juice of *C. albidum* increased steroid hormones as well as sperm count, the mechanism of which appears to be through stimulation of gonadotropin probably by hypothalamus activation. ECAF and JCAF improved hormonal and spermatogenic indices of male *wistar* rats but ECAF is a more efficient intervention in ethnomedicine against infertility and male erectile dysfunction.

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