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ASSESSMENT OF THE ANTIFERTILITY ACTIVITY OF THE ETHYLACETATE LEAF EXTRACT OF *LUPINUS ARBOREUS* IN MALE ALBINO RATS

Ohadoma S. C.* and Lawal B. A. S.

Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria.

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***Corresponding Author**
Dr. Ohadoma S. C.
Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria.

ABSTRACT

Objective: To investigate the anti-fertility tendency of the ethylacetate leaf extract of *Lupinus arboreus* in rodents. **Methods:** the ethylacetate extract of Lupinus arboreus leaf were assessed using biochemical estimation carried out in the testis and epididymis viz total cholesterol content, glucose – 6-phosphate dehydrogenase (G-6-PD) and Δ^5-3β-Hydroxysteroid dehydrogenase (HSD) activities. **Results:** weight of the testis and epididymis reduced significantly while no significant change was observed in the body weight. Fructose content in the seminal vesicles, sperm motility and sperm density diminished significantly in extract treated rats. The activities of the two key enzymes implicated in androgen biogenesis-G-6-PD and Δ^5 -3β-HSD, were significantly inhibited in the extract-treated rats. **Conclusion:** the results demonstrated that the leaves of *L.arboreus* arrests spermatogenesis as well as inhibits steroidogenesis consequently acting as antifertility agent in male rats.

KEYWORDS: Lupinus arboreus, chikadoma, antifertility, Δ^5-3β-hydroxysteroid, Glucose-6-phosphate dehydrogenase.

INTRODUCTION

Lupinus arboreus (Fabaceae) is commonly cultivated in Nigeria as ornamental plant where it is called Chikadoma.[1] It is a bushy shrub, with bright yellow sweet-smelling flowers blended with purple and white colours measuring up to 1.8 m (6 ft) tall. The English name is yellow bush.[2] The decoction of *L.arboreus* leaves in South-eastern Nigeria is being utilized in the ethnomedical management of various ailments, such as pain and inflammation.

Documented evidences exist showing that *L.arboreus* exert antimicrobial[3], antinociceptive and anti-inflammatory effects[1], as well as have plethora of phytochemicals.[4] Though the phytochemical constituents of L.arboreus vary according to the geographical origin yet quinolizidine alkaloids remain the chemotaxonomical markers of the plant genus.[5,6] Stigmast steroids (stigmastene 3,6-dione), triterpene hydroxyl acid (ursolic acid), a phenolic acid (ellagic acid) and a flavonol glycosides(tetrahydroxy flavones-3α -rhamnoside) have been reported to be present in *Lupinu arboreus.*[7]

Flavonol glycosides such as quercitin have been reported to possess antigonadotrophic activity[8] Hence, this study investigated the antifertility effect of *L.arboreus* in rodents.

MATERIALS AND METHODS

Plant materials and preparation of extract are in accordance to earlier documented report.[9]

Plant material: Leaves of *Lupinus arboreus* were collected from Owerri, Imo State, Nigeria. The botanical identification was officially done by Dr. Osuala, F.N. of Pharmacognosy Department, Madonna University, Elele, Nigeria. The leaves were air-dried at room temperature for 28 days. The leaves were grounded and make coarsely powder (2 kg). the powdered drugs were extracted by cold maceration method with methanol for 48 hr. after filtration, the crude methanol extract (CME) was concentrated using rotary evaporator.

Column Chromatographic Separation of CME

Two hundred grams of activated silica gel (70-230 mesh) was packed to two-third length of a glass column. One hundred grams (100 g) of the dry methanol extract was dissolved in methanol:water mixture (1:2 v/v) and introduced into the column. The column was eluted with 1.5 L hexane, 1.2 L ethylacetate and 1.0 L methanol in succession to yield hexane fraction (HEF), ethylacetate fraction (EAF) and methanol fraction (MEF).

The ethylacetate fraction (EAF) was chosen for furtherance of this work because of the confirmed presence of flavonol glycoside.[9]

Animals: Mature, healthy male rats (200-220 g) were employed in this study. The rats were maintained under standard laboratory conditions and had free access to water and standard pellets (Guinea Feeds Plc, Nigeria). The animals on transfer to work area were allowed two weeks to acclimatize.

Experimental design and protocol

Five groups comprising five rats each were employed.

Group I received normal saline (5 ml/kg body weight) and served as control.

Group II received propylene glycol (5 ml/kg body weight).

Group III received ethylacetate extract 100 mg/kg body weight.

Group IV received ethylacetate extract 200 mg/kg body weight.

Group V received ethylacetate extract 400 mg/kg body weight.

All treatment was done intraperitoneally on alternate days for 18 days.

The rats were weighed before and after the commencement of the experiment. Starting from the 19^{th} day, the rats were subjected to fasting for 18 hours after which they were sacrificed by cervical dislocation. The reproductive organ were immediately dissected, the adherent fat trimmed off, weighed and kept in 0.2 M cold sucrose buffer at 4^{o}c for further engagement.

Body weight, sperm motility and density

The sperm motility and density were assessed in cauda epididymis according to the standard method [10] and the body weight recorded throughout the experiment.

Biochemical estimations

The biochemical estimations were carried out in the testis and epididymis. Testis (10 mg) was weighed and homogenized (Potter Elvahjem) using chloroform: ethylacetate (2:1) mixture. The non-polar part was extracted and total cholesterol content was estimated according to standard method.[11] Testis (15 mg) was weighed and homogenized (Potter Elvahjem), then the ascorbic acid content was measured.[12] Testis (20 mg) was weighed and homogenized (Potter Elvahjem) using 1 ml of normal saline and of 0.1 M phosphate buffer (pH=7.4) and centrifuged. The activity of Δ^5-3β-hydroxysteroid dehydrogenase (HSD) was estimated according to standard method.[13] Tissue (20 mg) was weighed and homogenized (Potter Elvahjem). The activity of glucose-6-phosphate dehydrogenase (G-6-PD) was determined by standard method.[14]

STATISTICAL ANALYSIS

The results obtained were analyzed using student's t-test and one way analysis of variance (ANOVA) and difference between means were regarded significant at $p< 0.05$.

RESULTS

The body weight gain of extract-treated rats was similar to that of control animals. The weight of epididymis and testis reduced in the extract-treated rats compared to that of vehicle-treated rats (Table I). In the extract-treated animals, sperm motility and density were decreased (Table II). In the biochemical findings, fructose content in the seminal vesicles reduced significantly though in dose-dependent manner after the treatment of ethylacetate extract of *L. arboreus* (100, 200 and 400 mg/kg) in comparison with that of the vehicle control (Table II). Cholesterol and ascorbic acid levels were significantly elevated in testes. The two key steroidogenic enzymes-G-6-PD and Δ^5-3β-HSD, were significantly inhibited all the used doses in comparison to the vehicle-treated animals (Table III)

Table. 1: Effect of ethyl acetate extract of *L. arboreus* on body weight, weight of testis and epididymis in mature male rats.

Treatment	Body weight before treatment(g)	Body weight after Treatment(g)	Weight of testis treatment (g)	Weight of epididymis(mg)
Saline 5 ml/kg b.w.(i.p.)	165± 4.2	170± 5.5	144± 3.2	170± 2.5
Vehicle (PG) 5 ml/kg b.w.(i.p.)	170± 3.3	176± 4.8	145± 1.8	173± 1.4
EAF 100 mg/kg b.w.(i.p.)	169± 4.2	174± 45	131± 1.4*	150± 4.2*
EAF 200 mg/kg b.w.(i.p.)	162± 2.4	168± 3.3	124± 3.5*	138± 3.1**
EAF 400 mg/kg b.w.(i.p.)	165± 4.28	171± 4.2	98± 2.18***	108± 1.86***

EAF= ethylacetate fraction of *L. arboreus* leaves, PG= Propylene glycol, i.p- intraperitoneal. Treatment period = 18days. P<0.05, **P<0.001 significantly different from vehicle control. Values are mean ±S.E. of 5 rats.

Table. 2: Effect of ethylacetate extract of *L. arboreus* on sperm density, sperm motility and seminal vesicle fructose content in mature male rats.

Treatment	Sperm density (millions/ml)	Sperm motility (%)	Seminal vesicle fructose (mg/g)
Saline 5 ml/kg b.w.(i.p.)	53.6± 2.1	67.3± 1.6	4.2± 0.3
Vehicle (PG) 5 ml/kg b.w.(i.p.)	54.5± 2.6	66.4± 1.7	4.3± 0.2
EAF 100 mg/kg b.w.(i.p.)	38.5± 2.4	54.6± 2.2*	4.0± 0.1*
EAF 200 mg/kg b.w.(i.p.)	28.2± 2.6**	39.4± 2.7**	3.2± 0.2**
EAF 400 mg/kg b.w.(i.p.)	18.3± 3.8***	24.4± 1.8***	2.4± 0.1***

EAF= ethylacetate fraction of *L. arboreus* leaves, PG= Propylene glycol, i.p- intraperitoneal. Treatment period = 18days. P<0.05, **P<0/01, ***P<0.001 significantly different from vehicle control. Values are mean ±S.E. of 5 rats.

Table 3: Effect of ethylacetate extract of *L. arboreus* on content of cholesterol, ascorbic acid and G-6-PD and Δ^5-3β-HSD activities in testis of mature male rats.

treatment	Cholesterol (μg/mg of tissue)	Ascorbic acid (μg/mg of tissue)	G-6-PD (U/mg of protein	Δ^5-3β-HSD (U/mg of protein)
Saline 5 ml/kg b.w.(i.p.)	73.3± 0.8	114.3± 0.8	4.2± 0.8	170± 0.06
Vehicle (PG) 5 ml/kg b.w.(i.p.)	70.6± 1.1	110.2± 1.6	4.0± 0.12	1.1± 0.06
EAF 100 mg/kg b.w.(i.p.)	109.2± 2.62**	138.4± 2.1*	3.7± 0.05	0.9± 0.04*
EAF 200 mg/kg b.w.(i.p.)	119.3± 2.5***	153.3± 2.6**	3.1± 0.04***	0.7± 0.02**
EAF 400 mg/kg b.w.(i.p.)	167± 4.6***	194± 6.6***	22± 0.02***	0.5± 0.008***

EAF= ethylacetate fraction of *L. arboreus* leaves, PG= Propylene glycol, i.p- intraperitoneal. Treatment period = 18days. P<0.05, **P<0/01, ***P<0.001 significantly different from vehicle control. Values are mean ±S.E. of 5 rats.

DISCUSSIONS

The result showed that sperm motility and density were reduced. This may not be unconnected with the reduction in the fructose content in the seminal vesicles as fructose is the energy source for sperm motility and agrees with the report that sperm motility and

density were reduced in aluminum chloride treated mice due to lack of fructose in the seminal vesicles of mice.[15] Cholesterol is an important precursor in the biosynthesis of steroid hormones and it is involved in steroidogenesis in testes hence its level is related to the fertility of individuals.[16] The elevated level of cholesterol may be due to decreased androgen synthesis, which resulted in accumulation of cholesterol in testes hence impaired spermatogenesis.[17] This may suggest non-utilization of lipid towards biosynthesis of testosterone.[18] These results indicate that the ethylacetate extract of *L. arboreus* inhibited spermatogenesis by reducing sperm motility, density and fructose content as well as elevation of substrate level and reduction of the two named enzymes. Previously, tetrahydroxy flavones-3α-rhamnoside has been isolated from this plant.[9] The presence of this constituent which is a flavonol glycoside, may be responsible for its antifertility effect since Quercitin another major flavonol glycoside has been found and reported to possess antigonadotrophic activity.[19,8]

CONCLUSION

The ethylacetate extract of *Lupinus arboreus* leaves exhibited inhibition of spermatogenesis and testicular steroidogenesis in male rats thereby acting as an antifertility agent.

CONFLICT OF INTEREST

The Authors have not declared any conflict of interest.

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