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Effect of *Grewia mollis* Stem Bark Extract on Gonadal Function of Wistar Rats

[1]Obidah, W., [1]Wakawa, H. Y., [2]Ja'afar, J. N. and [3]Ezeanyika, L. U. S.
[1]Department of Biochemistry, Faculty of Life Sciences, Modibbo Adama University Yola, Nigeria
[2]Department of Biotechnology, Faculty of Life Sciences, Modibbo Adama University Yola, Nigeria
[3]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka
Ziammeandrew@gmail.com; jnjaarfar@mau.edu.ng; Lawrence.ezeanyika@unn.edu.ng

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Corresponding author
Obidah, W.
wilson.obidah@mau.edu.ng

Abstract:
Grewia mollis (*G. mollis*) stem bark is used traditionally as a natural food additive in baked foods and condiments. However, there is no sufficient information on the effect of the stem bark on the gonadal organs in rodents. The aim of this study is to determine the effect of dietary administration of G. mollis stem bark in rats. The effect of subchronic dietary administration of *G. mollis* stem extract on gonadal organs was evaluated in male and female Wistar rats. The animals were assessed on behaviour, body weight and food intake, and serum gonadal hormone concentration, and the gonadal organs were assessed for histopathological changes. The results showed no behavioural changes in rats administered *G. mollis* stem bark extract. Administration of *G. mollis* stem bark extract caused no alteration in the parameters determined, except in the male groups administered 1.5% *G. mollis* stem bark extract, where serum luteinizing hormone was elevated. Thus, dietary administrations of *G. mollis* stem bark extract caused subtle or no effect on the gonadal organs of Wistar rats. This suggests that *G. mollis* stem bark is safe for consumption as a thickener in food and related products. The findings support the traditional use of G. mollis stem bark extract in locally baked foods and condiments.

Keywords: *Grewia mollis*, Stem bark, Toxicity, Gonads, Hormones, Rat

1. Introduction

Grewia mollis (Malvaceae) is a plant growing wild in Nigeria and many other African countries. *G. mollis* stem bark is used as a natural food additive in many locally made foods. The stem bark is used as a thickener and binder in baked foods and condiments. The mucilage obtained from the stem bark is mixed with beans or corn flour to enhance the texture of locally baked foods. *G.mollis* stem bark is also used as a binder in "Dawadawa", a locally made condiment in Nigeria. The stem bark is used in traditional medicine for the treatment of constipation, snake bites, and wound healing [1, 2]. In Chad, *G. mollis* stem is used in traditional brewing to enhance the sedimentation of suspending particles and production of clear beer. *G. mollis* stem bark has been reported to have antimicrobial activity against many microbes [3].

The available toxicological information shows that *G. mollis* stem bark has low toxicity in rodents [2]. The median lethal dose (LD50) of the ethanol extract of *G. mollis* stem bark administered intraperitoneally to rats was 1500 mg/kg body weight [4] while the LD50 of the aqueous extract in mice is greater than 9600 kg/kg [5]. Subacute administration of the stem bark had minimal toxicity in rats and mice [5, 6]. However, there is no sufficient report on the potential toxic effect of *G. mollis* stem bark on the gonadal organs of rats. The aim of the study is to determine the effect of *G. mollis* stem bark extract on the gonadal function of male and female rats.

2. Materials and Methods

2.1 Materials and equipment

2.1.1 *Equipment*

The instruments used were an analytical balance (Ohaus-Adventurer SL, India), a table centrifuge (Heraeus Christ), a spectrophotometer (Heraeus Christ), a hot oven (Gallenkemp), an Acurex plate washer (India), and an Acurex ELISA plate analyser (India).

2.1.2 Chemicals and Reagents

The chemicals used in this study were of analytical grade, obtained from Sigma-Aldrich Chemical Corporation USA and BDH Poole, England. The test kits for the determination of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), progesterone, and oestrogen were purchased from Monobind Inc., U.S.A.

2.1.3 Experimental animals

Male and female Wistar rats aged 5-7 weeks old were procured from the animal house of the Faculty of the Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria. The animals were housed in improvised

cages at room temperature and were fed standard laboratory feed (Grand Cereals limited). The animals had free access to food and water. They were allowed to acclimatise for one week prior to commencement of the study. Animal care was according to standard methods [7].

2.2 Methods

2.2.1 Collection and preparation of *G. mollis*

Matured fresh plant material (*G. mollis*) was collected from Bati village, Yadim Development Area, Fufore Local Government Area of Adamawa State, Nigeria. The plant was identified and authenticated by a Botanist in the Department of Plant Sciences, Modibbo Adama University, Yola, Adamawa State. The fresh inner stem bark was removed from the outer part, shredded, and dried under shade at room temperature and later in the oven at 50°C until a fairly constant weight was obtained. The dried shredded sample was kept in glass bottle lidded containers at room temperature. The dried sample material was used for the aqueous extraction.

2.2.2 Aqueous extraction of *G. mollis*

The dried shredded stem bark of *G. mollis* was extracted in distilled water. About 4 litres of distilled water were transferred into a 12 litre graduated plastic container. Then, 500 g of dried stem bark was added continually with constant stirring using a glass rod. The infusion was made up to 8 litres by adding more distilled water and covered and kept at room temperature for 12 hours for substantial recovery of the mucilage. The infusion was filtered through a muslin cloth to remove the extraneous materials. The filtrate was lyophilised to powder and stored in lidded glass containers.

2.2.3 Diet preparation

The lyophilised *G. mollis* aqueous extract was made to powder using an Endrumer mill. The powdered sample was mixed with standard laboratory diet (*Grand cereals Limited, Jos*)at 0, 0.5, 1.5 and 5%. Fresh dietary mixtures were prepared every week. The control diet was not mixed with the *G. mollis* stem bark extract.

2.2.4 Experimental design

Twenty-four (24) male and twenty-four (24) female Wistar rats were allocated to four groups (6 rats/group/sex) and fed a diet containing *G. mollis* aqueous extract powder at 0, 0.5, 1.5 or 5% dietary levels for 90 days (Table 1). The animals were observed at least once daily for overt signs of toxicity.

Table 1: Animal treatment protocol

Animal group/sex	Dietary treatment and administration
Group 1 (control)	Diet only.
Group 2	0.5% *G. mollis* aqueous extract in diet.
Group 3	1.5% *G. mollis* aqueous extract in diet.
Group 4	5% *G. mollis* aqueous extract in diet.

(n= 6/sex)

2.2.5 *Sample collection and preparation*

The blood samples were collected in dry glass centrifuge tubes by cardiac puncture under diethyl ether anaesthesia. The blood samples were allowed to coagulate (clot) at room temperature and centrifuged at 3000 r.p.m. for 15 minutes for separation of serum. The serum samples were used for hormonal assay.

2.2.6 Determination of rat body weights

Animal body weights were determined by gravimetric technique using a tabletop weighing balance. The rats were weighed before commencement of the study, at weekly intervals, and at the end of the study before sacrifice.

2.2.7 Determination of food intake

Food intake was measured by the gravimetric method using a weighing balance. Food intake was measured daily. The amount of food consumed was recorded in grams (g/rat/day).

2.2.8 Determination of serum gonadal hormones

Serum samples were used to assay hormones indicative of gonadal function using Enzyme-linked Immunosorbent Assay method. The hormones were follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), oestrogen, and progesterone levels. The hormones were assayed using test kits purchased from Monobind Inc., U.S.A.

2.2.9 Histopathological analysis of organs

The testes and ovaries were excised from the control and treated groups and examined microscopically for histopathological changes using standard procedures **[8].**

2.2.10 Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Statistical data were analysed using one-way analysis of variance (ANOVA) and Dunnett's multiple range test (SPSS 26.0). Treated groups were compared with same-sex controls. The level of significance was set as $p < 0.05$.

3. Results and Discussion

3.1 Signs of toxicity

There was no mortality or adverse clinical signs in animals administered *G. mollis* stem bark extract or the control group.

3.2 Body weight and food intake

Table 2 shows the changes in body weight of animals administered *G. mollis* stem bark extract in rats. There were no significant changes in body weight between animals administered *G. mollis* and the control. A change in body weight gain is related to the general health condition of experimental animals [9]. The absence of significant alterations in weight gain is an indication that the health status of the animals administered *G. mollis* stem bark extract is comparable to the control group. Dietary administrations of *G. mollis* stem bark extract had no effect on the food intake of rats.

Table 2: Effect of *G. mollis* on weight gain and food intake of rats

Group	Initial body weight (g)	Final body weight (g)	Mean body Weight gain (%)	Food consumption (g/rat/day)
Male				
Control	112.67± 3.09	265.83 ± 6.47	135.94	24.83 ± 2.44
0.5% GSE	110.83± 5.15	268.27 ± 7.12	142.06	23.58 ± 4.51
1.5% GSE	111.41± 4.03	263.22 ± 4.51	136.26	25.22 ± 4.43
5% GSE	113.82± 4.45	258.40 ± 5.54	127.03	24.17 ± 3.70
Female				
Control	101.67± 3.50	222.33 ± 4.23	118.68	20.67 ± 3.78
0.5% GSE	102.17± 3.45	220.83 ± 6.70	116.14	20.35 ± 1.25
1.5% GSE	101.33± 3.20	225.41 ± 4.33	122.45	20.42 ± 1.57
5% GSE	100.56± 2.46	216.17 ± 6.58	114.97	18.74 ± 1.56

Values are mean ± standard error; n= 6

Change in food intake is considered to indicate the overall health condition of experimental animals [10]. Additionally, alterations in food intake may occur due to low palatability of the admixed diet. The reduction in food intake may result in low nutrient intake, which may have implications on the nutrition status of the experimental animals.

3.3 Serum hormonal levels

Figure 1 shows the effect of *G. mollis* stem extract on serum gonadal hormone levels of male rats. Serum LH concentrations increase significantly in the group administered 1.5% *G. mollis* stem bark extract. However, the concentration of LH in the male rats administered 5% was comparable to the same-sex control. LH stimulates Leydig cells to secrete testosterone, which plays a more critical role in spermatogenesis in males by direct activation of the androgen receptor in the testes [11, 12]. High blood concentration of LH with a corresponding decrease in serum testosterone indicates Leydig cell dysfunction [13, 14]. However, the serum testosterone levels in the male rats fed 1.5% *G. mollis* aqueous extract were comparable to the control group. Moreover, the effect was not observed in the male rats fed with a high dose (5%) of *G. mollis* aqueous stem bark extract.

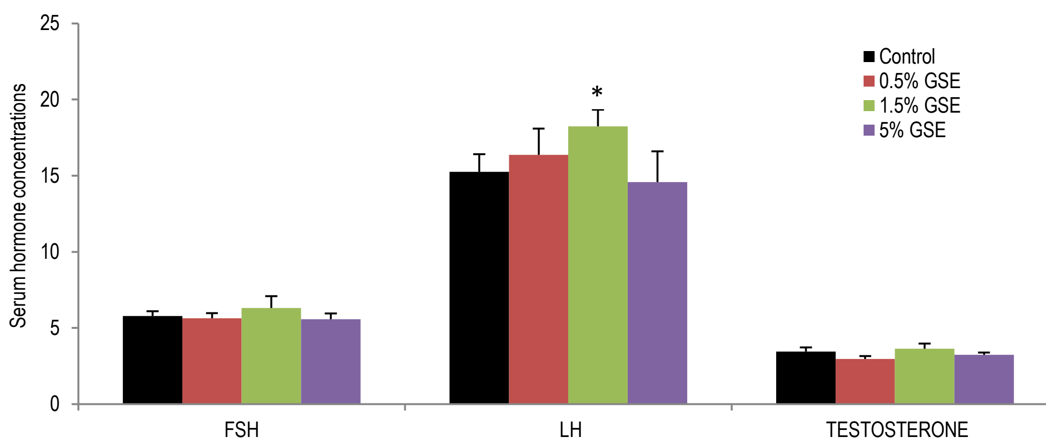

Figure 1: Effect of *G. mollis* stem bark extract on serum gonadal hormones concentrations of male rats GSE, *Grewia mollis* stem bark extract.

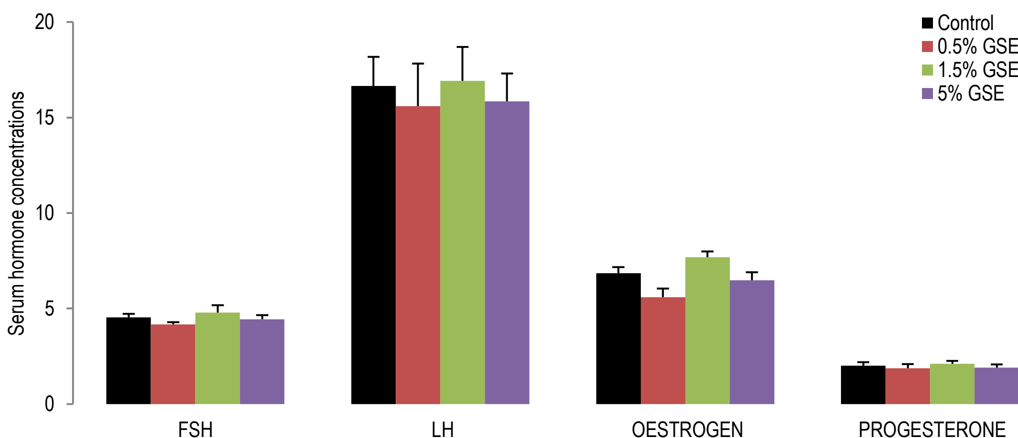

Figure 2: Effect of *G. mollis* stem bark extract on serum gonadal hormones concentrations of female rats GSE, *Grewia mollis* stem bark extract.

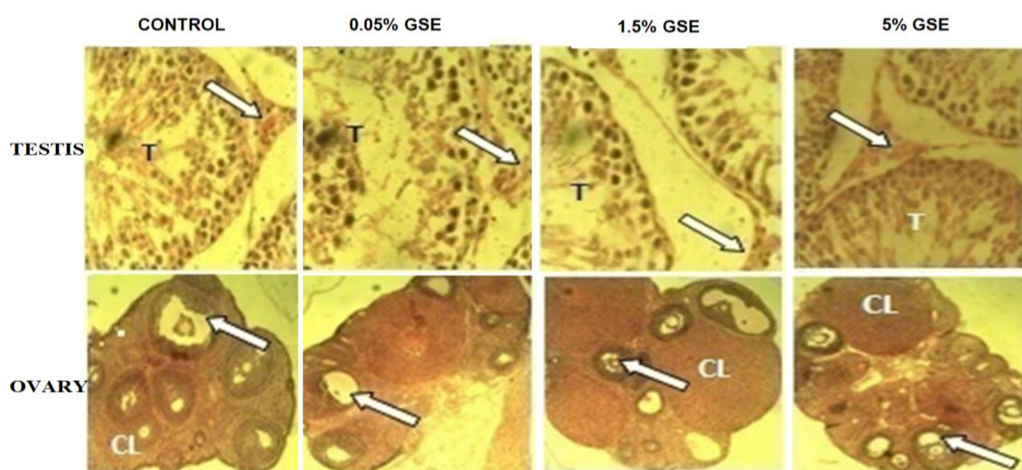

Figure 3: Photomicrographs of the testis and ovaries from the experimental male and female rats

Therefore, the elevated level of LH with no concomitant alteration in other gonadal hormones was considered an incidental or subtle effect with no serious alteration on the gonadal function.

Figure 3: show the photomicrographs of the testis and ovaries from the experimental male and female rats administered graded doses of *G. mollis* aqueous extract (GME) in diet showing normal organ architecture. In males, the photomicrograph shows the interstitium (arrows) containing the leydig cells, and seminiferous tubules (T) at various stages of development with no visible histologic abnormality. In females, photomicrographs show the presence of corpus luteum (CL) and various stages of follicular development in all the groups (arrows). H and Ex 400. GSE, *Grewia mollis* stem bark extract.

Microscopic examination of the testis and ovaries revealed no alteration in the gonads. The testes showed interstitium (arrows) containing leydigcells, and seminiferous tubules (T) with no visible histologic abnormality. The ovaries showed the presence of corpus luteum (CL) at various stages of follicular development. This finding further demonstrates that *G. mollis* aqueous stem bark extract had no adverse effect on rat gonadal organs.

4. Conclusion

Dietary administration of *G. mollis* stem bark extract caused no significant alteration in serum gonadal hormone levels and histopathology of gonadal organs. Thus, *G. mollis* stem bark extract had no effect on the gonadal organs of male and female rats. The findings support the existing application of *G. mollis* stem bark as a local food thickener.

Recommendation

Further studies are required on the effect of *G. mollis* stem bark extract on sperm parameters and fertility of male and female rats.

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