

## Evaluation of reproductive toxicity profile of ethanol stem-bark extract of *Newbouldia laevis* on hormonal profile and pregnancy outcome in Wistar rats

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### ABSTRACT

**Background:** The use of medicinal-plants for both curative and preventive-purposes has grown significantly throughout the world in recent years, although, contrary to conventional-therapy, herbal-medicine generally lacks detailed research on its safety and possible-risks.

**Objective:** The side-effects of crude-extract of *Newbouldia laevis* stem-bark in non-pregnant and pregnant-rats were investigated.

**Methods:** For the hormonal-profile study, thirty-two (32) male and (32) female rats were divided into four treatment-groups and treated with distilled-water and graded doses: 250, 500, and 1,000 mg/kg (selected based on LD<sub>50</sub> result) of crude-extract of *Newbouldia laevis* stem-bark daily for 28 days. Blood-samples were collected on day 29 to evaluate FSH, LH, progesterone, and estrogen serum levels in females and FSH, LH, and testosterone in male rats.

Forty (40) pregnant-rats were divided into 4 treatment-groups and treated orally for 14 days with distilled-water and crude-extract of *Newbouldia laevis* stem-bark (250, 500, and 1,000 mg/kg). On the 15th day, 5 rats were sacrificed from each group and opened up for evaluation of implantation-sites and the physical-appearances of the fetuses. The remaining five rats were left until parturition for pregnancy-outcome evaluations.

**Results:** Oral-administration of *Newbouldia laevis* stem-bark decreased progesterone, estrogen and LH levels in female rats with increased FSH levels. A decrease in the levels of testosterone and LH with an increase in FSH was observed in the male rats. No adverse fetal-development or pregnancy-outcome was seen following a 14-day administration of the crude-extract in pregnant-rats.

**Conclusion:** Even though the crude-extract did not exhibit any structural-abnormalities in the pregnant-rats, pregnant-women and men should exercise caution when consuming this plant because it can be toxic to the reproductive-system and may affect reproduction.

**Keywords:** Reproductive hormones, pregnancy outcome, *Newbouldia laevis*, pregnant rats, teratogenicity

## Évaluation du profil de toxicité reproductrice de l'extrait éthanolique d'écorce de tige de *Newbouldia laevis* sur le profil hormonal et l'issue de la grossesse chez les rats Wistar

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### RÉSUMÉ

**Contexte:** L'utilisation des plantes médicinales à des fins curatives et préventives a connu une croissance significative dans le monde ces dernières années, même si, contrairement à la thérapie conventionnelle, la phytothérapie manque généralement de recherches détaillées sur sa sécurité et ses risques éventuels.

**Objectif:** Les effets secondaires de l'extrait brut d'écorce de tige de *Newbouldia laevis* chez les rates non enceintes et enceintes ont été étudiés.

**Méthodes:** Pour l'étude du profil hormonal, trente-deux (32) rats des deux sexes ont été divisés en quatre groupes de traitement et ont reçu de l'eau distillée et des doses graduelles : 250, 500 et 1 000 mg/kg (sélectionnées en fonction du résultat DL50) d'extrait brut d'écorce de tige de *Newbouldia laevis* quotidiennement pendant 28 jours. Des échantillons de sang ont été prélevés le 29<sup>e</sup> jour pour évaluer les taux sériques de FSH, de LH, de progestérone et d'œstrogène chez les femelles et de FSH, de LH et de testostérone chez les rats mâles.

Quarante (40) rates gravides ont été réparties en 4 groupes de traitement et traitées par voie orale pendant 14 jours avec de l'eau distillée et un extrait brut de *Newbouldia laevis* écorce de tige (250, 500 et 1 000 mg/kg). Le 15<sup>e</sup> jour, 5 rats de chaque groupe ont été sacrifiés et ouverts pour l'évaluation des sites d'implantation et de l'apparence physique des fœtus. Les cinq rats restants ont été laissés en liberté jusqu'à la parturition pour des évaluations de l'issue de la grossesse.

**Résultats:** Administration orale de l'écorce de *Newbouldia laevis* a diminué les niveaux de progestérone, d'œstrogène et de LH chez les rats femelles avec une augmentation des niveaux de FSH. Une diminution des niveaux de testostérone et de LH avec une augmentation de FSH a été observée chez les rats mâles. Aucun effet indésirable sur le développement du fœtus ou sur la grossesse n'a été observé après une administration de 14 jours de l'extrait brut chez des rates gravides.

**Conclusion:** Même si l'extrait brut n'a présenté aucune anomalie structurelle chez les rates enceintes, les femmes enceintes et les hommes devraient faire preuve de prudence lorsqu'ils consomment cette plante, car elle peut être toxique pour le système reproducteur et peut affecter la reproduction.

**Mots clés:** Hormones de reproduction, issue de la grossesse, *Newbouldia laevis*, rates gravides, tératogénicité.

## INTRODUCTION

Medicinal plants have long been used to cure various diseases and illnesses, given that they contain various bioactive constituents such as terpenoids, phenolics, saponins, flavonoids and alkaloids. In addition, they have significantly aided in the development of pharmaceutical drugs.<sup>1-2</sup> Despite the well-known hazards and benefits, roughly 80 per cent of people on earth, specifically those of reproductive age, rely on medicinal plants for healthcare, given that they are easily accessible and cost-effective.<sup>3</sup> Although several studies have documented the noteworthy benefits of phenolic compounds found in medicinal plants in the fight against many chronic illnesses and ailments, such as cancer, diabetes, asthma, ageing, and tissue inflammation, to name a few,<sup>4-5</sup> reports have shown that medicinal plants are not entirely safe, as some of their phytochemical constituents can be toxic to humans, interfere with normal reproductive cycle functions, alter the morphology of reproductive organs and change the body's hormone composition and concentration, which can lead to abnormal ovulation and infertility.<sup>6-8</sup>

Several physiological changes that lead to a variety of illnesses and medical disorders, including nausea and vomiting, pain, edema, gastrointestinal problems, pre-eclampsia, infections and respiratory illnesses, may occur during pregnancy. Most of the time, these conditions call for immediate medical attention.<sup>9-10</sup> The majority of pregnant women preferred using herbal drugs or supplements to treat pregnancy-related problems and/or to improve the mother's well-being, regardless of any potential adverse effects associated with the use of the plant, because they believe medicinal herbs are safer than synthetic drugs.<sup>11</sup> In addition to treating these illnesses, specific secondary metabolites found in herbal medicines have been shown to have a variety of biological potentials, including both beneficial and detrimental effects. They are also known to cross the placenta readily, which can have an adverse impact on the developing fetus and the pregnancy, leading to uterine contractions, an unfavorable pregnancy outcome and abortion.<sup>12</sup>

Several tropical plants have shown beneficial therapeutic potentials, including fertility enhancement, antimicrobial and anti-oxidant activities.<sup>13</sup>

*Newbouldia laevis* (P. Beauv) seem of the family *Bignoniaceae* and order *Scrophulariales* is a fast-growing tropical and hedge shrubby tree with large glossy and

deep green leaves. It has various ethnic names in Nigeria; Akoko in Yoruba, Aduruku in Hausa and Ogirisi in Igbo. In Ghana and the Ivory Coast, it is called Sesemasa and Sokunde.<sup>14</sup> Despite its unknown harmful effects, this plant, commonly referred to as a fertility plant, is being utilized for maintenance of pregnancy. Various ethno-medicinal applications and folkloric uses of different parts of *Newbouldia laevis* have been scientifically validated and reported, this include antiulcer activity,<sup>15</sup> anti-plasmodia activity,<sup>15,20</sup> anti-diarrhoeal activity,<sup>16</sup> anxiolytic and antidepressant-like activities,<sup>17</sup> antimicrobial activity,<sup>18</sup> toxicological and antiplasmodial properties among others.<sup>19</sup> In addition to numerous vitamins and minerals (Vitamins A, B1, B2, B6, B9, C, D, K and E; calcium, zinc, magnesium, potassium and sodium) present in *Newbouldia laevis*, several secondary metabolites such as saponins, terpenoids, flavonoids, tannins, alkaloids, cardiac glycosides, steroids which are of pharmacological importance have also been isolated and reported.<sup>18,20</sup>

WHO has now strongly recommended the safety evaluation of medicinal plants owing to the increase in their global exploit.<sup>21-22</sup> It is therefore essential to justify the traditional use of the *Newbouldia laevis* plant, which some women in African countries use to treat infertility issues, given that studies have indicated the involvement of plants' bioactive elements in reproductive abnormality. This study investigated the reproductive toxicity profile of ethanol stem-bark extract of *Newbouldia laevis* in both pregnant and non-pregnant rats.

## MATERIALS AND METHODS

### Drugs and chemical reagents

All the drugs and chemical reagents used for this study were of analytical grade.

### Collection and identification of the plant material

The stem-bark of *Newbouldia laevis* was collected from Ajebo, Abeokuta, Abeokuta South Local Government Area, Ogun State, Nigeria. The plant was taken to Zaria where it was identified and authenticated by Malam Namadi Sunusi of the Department of Botany, Ahmadu Bello University, Zaria and a voucher specimen was deposited in the herbarium section of the Department and voucher number ABU02881 given.

### Extraction of the plant material

The fresh stem-bark of the plant was air dried under shade until a constant weight was obtained and then reduced to coarse powder using a grinding machine. Five

hundred grams (500 g) of powdered plant material was cold macerated in 4 litres of 90 % ethanol for 72 hours with intermittent shaking and then filtered with Whatman (No. 3) filter paper. The resultant filtrate was concentrated using a rotatory evaporator, dried over a water bath at 55°C and stored in air tight container (universal bottle) prior to use.

#### Extract reconstitution

The extract was reconstituted by suspending 3 g of crude extract in 10 ml of distilled water to obtain a stock solution of 300 mg/ml.

#### Experimental animals

Adult Wistar rats (120-160 g) of both sexes obtained from the Animal House of Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for the study. The animals were kept in clean cages for two weeks' acclimatization and all through the study. The animals were fed with standard rodent pellet diet and water ad libitum for the duration of the study and used with strict adherence to the Ethics Guidelines and Research Policy of Ahmadu Bello University, Zaria. Ethical Approval (ABUCAUC/2021/084) was obtained from the ABU Committee on Animal Use and Care. All animal experimentation was carried out according to the guidelines of Institutional Animal Ethics Committees (IAEC).

#### Experimental design for Hormonal assay

After acclimatization 32 male and 32 female rats were weighed and divided into four groups of eight (8) rats per group. The control group received distilled water (1 ml/kg) and the other groups were given graded doses of the extract orally for 4 weeks.

#### Treatments were as follows;

Group 1: Normal control (1 ml/kg distilled water).

Group 2: Crude extract of *Newbouldia laevis* stem-bark at dose of 250 mg/kg per Bwt.

Group 3: Crude extract of *Newbouldia laevis* stem-bark at dose of 500 mg/kg per Bwt.

Group 4: Crude extract of *Newbouldia laevis* stem-bark at dose of 1000 mg/kg per Bwt.

Five rats from each group were sacrificed at the end of the experiment, and blood samples were collected. Using the enzyme linked immune-sorbent assay (ELISA) technique, the obtained blood samples were centrifuged at 2500 rpm for 5 minutes at 10-25°C to obtain the serum for the analysis of estrogen, progesterone, testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) levels as described by.<sup>23</sup>

#### Effect of ethanol crude-extract of *Newbouldia laevis* stem-bark on foetal development and pregnancy outcome in rats

One hundred (100) female and 50 male rats of reproductive age were mated in cages of 6 females to 3 viable males. Pregnancy was checked the morning following mating and the confirmation of which was the presence of spermatozoa in the vaginal smear of rats found with vaginal plug. Forty (40) successful pregnant rats were selected and assigned day zero (0) of conception, the day following day zero of conception was counted as day one of pregnancy, the rats were then divided into 4 treatment groups of 10 rats each and treated orally for 14 days with distilled water 1 ml/kg (group 1) and crude extract of *Newbouldia laevis* stem-bark at doses of 250, 500 and 1,000 mg/kg for groups 2, 3 and 4 respectively; on the 15th day of pregnancy, 5 rats were sacrificed from each group and opened up for evaluation of implantation sites (undeveloped, resorption sites). Available fetuses were weighed together with the placenta (total weight) and separately (placenta weights and weights of fetuses); physical appearances of the fetuses were also examined for abnormalities; the tail and crown-rump lengths were measured. The remaining 5 rats were left until parturition and used for pregnancy outcome evaluations including duration of gestation, litter size, weight of litters at birth, number of still and live births, and presence of physical defects (fetal viability) as well as growth profile in 6 weeks of live litters. The litters were weighed and mean litter weights calculated for each rat and for each group.

#### Statistical analysis

The experimental data were presented as Mean  $\pm$  standard error of the mean (SEM). Data were analyzed using One-way analysis of variance (ANOVA) and followed by Dunnett's/Tukey's post-hoc tests using Graph Prism 6 Software (Graph Pad Soft Inc., CA, USA). The results were considered significant at  $P < 0.05$ .

## RESULTS

#### Hormonal assay

#### Effect of 28 days oral administration of ethanol crude extract of *Newbouldia laevis* stem-bark on male and female reproductive hormones in rats

The progesterone level in the female rats was dose dependently reduced and significant ( $p < 0.05$ ) at 500 and 1,000 mg/kg doses of the extract. The estrogen level was significantly ( $p < 0.05$ ) and dose dependently reduced at all dose levels. The testosterone in male rats decreased

dose dependently, which was significant ( $p < 0.05$ ) at 1,000 mg/kg. The LH level dose dependently reduced in both male and female rats, but significantly ( $p < 0.05$ ) at higher doses. The FSH level on the other hand was significantly

( $p < 0.05$ ) increased in female rats at 1000 mg/kg and also significantly ( $p \leq 0.05$ ) increased at 250 mg/kg in male rats (Table 1).

**Table 1: Effect of 28 days oral administration of ethanol crude extract of *Newbouldia laevis* stem-bark on serum reproductive hormone levels in male and female rats**

Parameters investigated	Treatment groups (mg/kg)							
	Female rats				Male rats			
	Distilled	ethanol crude extract			Distilled	ethanol crude extract		
	H <sub>2</sub> O (1 ml/kg)	250	500	1000	H <sub>2</sub> O (1ml/kg)	250	500	1000
<b>Progesterone (nmol/L)</b>	20.03± 9.98	14.43± 4.93	8.06± 1.84*	7.49± 1.13*	-	-	-	-
<b>Testosterone (ng/mL)</b>	-	-	-	-	1.76 ± 0.88	1.50± 0.46	1.34± 0.27	1.20± 0.04*
<b>LH (IU/mL)</b>	21.90 ± 0.40	16.15± 0.35	12.65± 0.25*	9.45± 0.25*	8.67± 0.47	8.16± 1.02	5.92± 0.35	4.61± 0.05*
<b>(FSH) (IU/mL)</b>	1.29 ± 0.26	1.19± 0.53	1.92± 0.79	3.34± 1.85*	0.41± 0.04	2.56± 0.12*	1.43± 0.34	1.32± 0.09
<b>Estrogen (pg/mL)</b>	15.10 ± 3.85	8.41± 0.77*	6.66± 1.11*	4.47± 1.58*	-	-	-	-

n = 8; Values are Mean ± SEM, Statistics: One Way ANOVA followed by Dunnet’s Post hoc test at \*  $p = 0.05$  compared to control. FSH = Follicle Stimulating Hormone; LH = Luteinizing Hormone

**Pregnancy outcome**

**Effect of ethanol crude extract of *Newbouldia laevis* stem-bark on fetal development following 14 days' oral administration in rats**

There was no still birth from any of the 5 pregnant rats used in all the groups as all the litters were observed to be alive. The implantation sites were all intact with no

resorption; and the slightly reduced mean fetal and placental weights of the treated groups with respect to control were not statistically significant ( $p \leq 0.05$ ). The mean crown rump length and tail length of the fetuses were also insignificantly ( $p \leq 0.05$ ) reduced at all doses administered compared to control group (Table 2).

Table 2: Effect of ethanol crude extract of *Newbouldia laevis* stem-bark on fetal development

Parameters investigated	Treatment groups (mg/kg)			
	Distilled H <sub>2</sub> O (1 ml/kg)	Ethanol crude extract		
		250	500	1000
Percent (%) Live Foetus	100	100	100	100
Average No. of Live Foetus	8.67 ± 0.33	8.33 ± 0.88	8.00 ± 0.58	7.87 ± 0.35
No. of Resorption Sites	0	0	0	0
Mean Weight (g) of Foetuses with Placenta	3.94 ± 0.01	3.82 ± 0.01	3.79 ± 0.03	3.71 ± 0.02
Mean Foetal Weight (g)	3.01 ± 0.01	2.99 ± 0.01	2.94 ± 0.02	2.89 ± 0.01
Mean Placental Weight (g)	0.91 ± 0.01	0.86 ± 0.01	0.84 ± 0.02	0.82 ± 0.01
Mean Crown -rump Length (cm)	3.81 ± 0.03	3.78 ± 0.01	3.63 ± 0.01	3.32 ± 0.02
Mean Tail Length (cm) of Foetuses	2.42 ± 0.05	2.27 ± 0.03	2.23 ± 0.01	2.19 ± 0.02

n = 5; Values are Mean ± SEM, Statistics: One Way ANOVA

#### Effect of ethanol crude extract of *Newbouldia laevis* stem-bark on pregnancy outcome in rats

There was no statistically significant ( $p < 0.05$ ) difference in litter size in all the treatment groups, and the mean birth weight was slightly reduced but none was significant ( $p < 0.05$ ) compared to the control group. The pups of extract treated groups showed slight dose related reduction in mean weight at the first 3 weeks. From week 4, a consistent increase in weight was recorded when compared with the control and was significant ( $p < 0.05$ ) in weeks 5 and 6 (Table 3).

Table 3: Pregnancy outcome in 6 weeks old pups of dams of rats treated with ethanol crude extract of *Newbouldia laevis* stem-bark for 14 days

Treatment groups	Mean ± SEM				Weight (g) changes of litters at weeks					
	Litter Size	Birth Weight	Still Birth	Physical Defects	1	2	3	4	5	6
Distilled H <sub>2</sub> O (1 ml/ kg)	8.67 ± 0.33	3.91 ± 0.02	-	-	8.74 ± 0.02	21.67 ± 0.01	40.02 ± 0.01	56.88 ± 0.1	62.80 ± 0.05	71.32 ± 0.02
250 mg/kg ECE	8.33 ± 0.88	3.77 ± 0.02	-	-	8.63 ± 0.01	21.45 ± 0.01	39.96 ± 0.01	57.79 ± 0.01	64.66 ± 0.01*	73.54 ± 0.01*
500 mg/kg ECE	8.00 ± 0.58	3.62 ± 0.02	-	-	8.40 ± 0.01	20.98 ± 0.01	39.28 ± 0.01	57.45 ± 0.01	64.31 ± 0.02*	73.21 ± 0.01*
1000 mg/kg ECE	7.85 ± 0.35	3.45 ± 0.01	-	-	8.31 ± 0.02	20.72 ± 0.02	38.87 ± 0.01	57.28 ± 0.02	64.18 ± 0.01*	73.07 ± 0.02*

n = 5; Values are Mean ± SEM, Statistics: Repeated measure ANOVA followed by Bonferroni's post hoc test at \* $p < 0.05$  compared to control

ECE= ethanol crude extract

**Effect of ethanol crude extract of *Newbouldia laevis* stem-bark on the uterus and placenta of 15 days pregnant rats treated for 14 days**

Histological examination of the uterus from pregnant rats administered with the ethanol crude extract of *Newbouldia laevis* stem-bark (250, 500 and 1000mg/kg) for 14 days revealed changes as seen in figure 1.

Normal uterus at 250 mg/kg, but slight to moderate hypertrophy of the uterine endometrium at 500 and 1000 mg/kg of the extract; normal placental gland at 250 mg/kg, but slight lymphocyte hyperplasia and adipocyte infiltration at 500 mg/kg and 1000 mg/kg doses of the extract were observed.

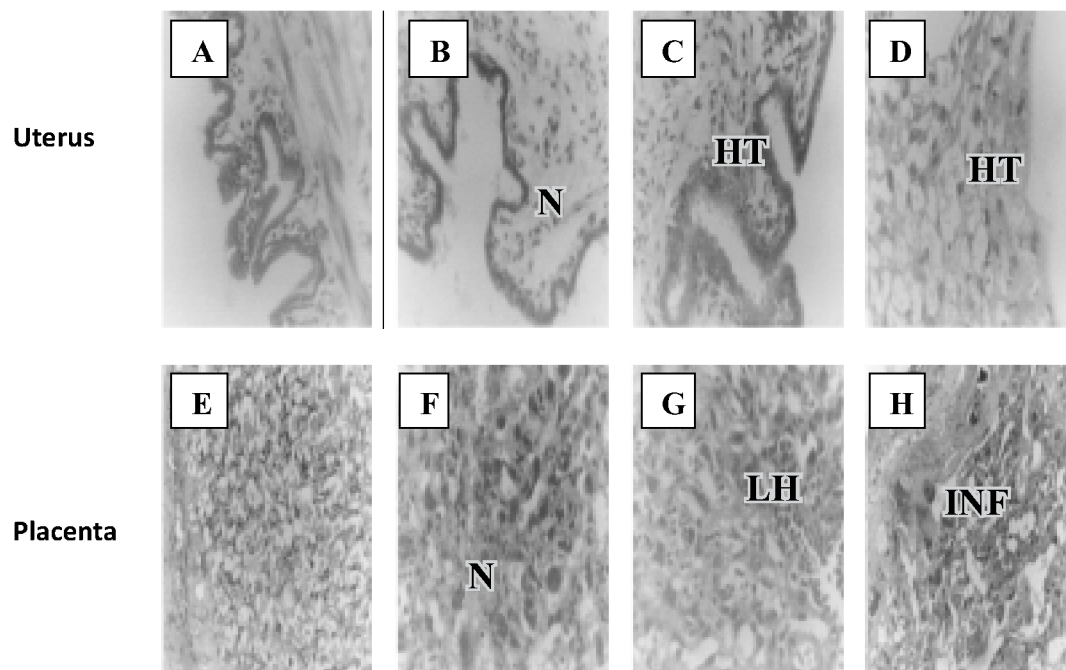


Figure 1: (A) Photomicrograph of pregnant rat uterus showing normal uterine tissue; (B) pregnant rat uterus showing normal uterine tissue; (C) pregnant rat uterus showing slight hypertrophy of the uterine endometrium; (D) pregnant rat uterus showing moderate hypertrophy of the uterine endometrium; (E) pregnant rat placenta showing normal placental gland; (F) pregnant rat placenta showing normal placental gland; (G) pregnant rat placenta showing slight lymphocyte hyperplasia; (H) pregnant rat placenta showing slight adipocyte infiltration (x400).

Legend: NL- *Newbouldia laevis* stem-bark extract; A & E - D/water, B & F - 250 mg/kg NL, C & G - 500 mg/kg NL, D & H - 1000 mg/kg NL

## DISCUSSION

The hormonal profile of the treated rats changed in a dose-dependent manner. In female rats, progesterone levels significantly reduced at 500 and 1000 mg/kg dosages, while estrogen levels significantly reduced at all doses. However, FSH levels increased significantly in female rats at 1000 mg/kg and in male rats at 250 mg/kg, while testosterone levels in male rats significantly reduced at 1000 mg/kg. In contrast, at higher doses, LH levels significantly decreased in both male and female rats.

The study revealed that progesterone, estrogen and testosterone levels in the treated rats decreased after 28 days of crude extract administration. Fertility and the maturity of reproductive organs are often influenced by the reproductive hormones progesterone, estrogen, and testosterone produced in female ovaries and male testicles.<sup>24-25</sup> An imbalance in these hormones' levels may interfere with their normal physiological or homeostatic regulatory processes. Testosterone is the primary sex hormone essential for sexual functions in males; a decrease in this hormone is a significant indicator of infertility and can also cause erectile dysfunction and loss of libido;<sup>26</sup> estrogen is a female hormone that plays a significant role in the development and regulation of the female reproductive system, secondary sex characteristics and menstrual cycle. It also has protective effects on mood and mental health, bone strength, and heart health and controls the growth of the uterine lining, low levels of estrogen have been linked to irregular menstruation, low sex drive, infertility, reduced bone density and insomnia;<sup>27</sup> progesterone is a naturally occurring hormone in females and is necessary for breast development, endometrial receptivity, embryo implantation and the successful establishment and maintenance of pregnancy, infertility and pregnancy loss has been reported as some of the consequences of low progesterone levels or an inadequate response to progesterone.<sup>28</sup>

Reduction in the levels of these hormones after 28 days of oral administration of the crude extract may be a result of the adverse effect of the extract on the gonads (ovaries and testicles) responsible for the production of gonadal hormones, thus resulting in reduced gonadal functions (hypogonadism), it may also be due to 9-tetrahydrocannabinol's suppression of the gonadotrophin-releasing hormone in the hypothalamus or as a result of inhibition of the enzyme aromatase responsible for the production of estrogen from

androgens.<sup>29</sup> Alkaloids in plants, present in the crude extract used in this study, have been reported to reduce the plasma concentrations of reproductive hormone levels<sup>30</sup> and are also known to inhibit aromatase enzyme activity.<sup>31</sup> Thus, there may have been an endocrine disruptor type of alkaloids in the extract, which may be responsible for the reduction observed.

FSH helps regulate sexual development, growth, pubertal maturation, and reproductive processes in the human body. It directly stimulates estrogen synthesis and follicular development in females and indirectly stimulates the maturation of germ cells in both male and female subjects.<sup>32</sup> LH, on the other hand, triggers ovulation and development of the corpus luteum in females; in males, it stimulates testosterone production from Leydig cells in the testis, thus helping promote male reproductive functions.<sup>33-34</sup> The production of luteinizing hormone (LH), as with Follicle-stimulating hormone (FSH), is regulated by gonadotropin-releasing hormone (GnRH) of the hypothalamus, and both are produced by gonadotropic cells in the anterior pituitary gland.<sup>35</sup> The reduction in LH, therefore, may suggest an instability effect of the neuro-endocrine system mechanism (hormone control centres in the brain) due to the crude extract, probably related to either hypothalamic or gonadal suppression and /or blockade or antagonism of the GnRH pituitary receptors. Reports have shown the involvement of alkaloids and flavonoids in reducing LH. Thus, the reduced level of LH observed in this study may be related to the presence of these phytochemicals in the crude extract.<sup>30</sup>

However, in this study, the FSH level increased significantly in the female rats at higher doses and in male rats at a lower dose; both hormones (FSH and LH) are synthesized and secreted by the gonadotropic cells of the anterior pituitary gland regulated by the same mechanism. Thus, only the crude extract could block or affect the LH receptors. The extract at its lowest level used in this study increased the FSH level in the male rats; this suggests its ability to improve male reproductive function (sperm production) at lower doses only, as higher doses may result in deleterious effects. Higher doses of the extract may block the FSH functions, enhancing spermatogenesis induction and maintaining normal sperm production.<sup>36</sup> On the other hand, high doses of the crude extract may be more favourable in females, as seen in this study. The result of this study contrasts with the findings of,<sup>37</sup> who reported no difference in the LH level and an increase in the estradiol



level in the female rats treated with *Newbouldia laevis* leaf extract. On the other hand, the result agrees with the findings of,<sup>38</sup> in which there was an increase in FSH and a decrease in testosterone levels in the male rats treated with *Newbouldia laevis* leaf extract. The reduction observed in the levels of the reproductive hormones in this study may adversely affect conception and pregnancy in female rats and cause infertility in male rats. On the effect of 14 days administration of ethanol crude extract of *Newbouldia laevis* stem-bark on fetal development, pregnancy outcome and growth profile of pups in pregnant rats, there were no significant changes in mean crown-rump length and tail length of the fetuses or on placental and fetal weights compared to control, suggesting lack of fetal development related toxic effects. There was no resorption of implantation sites or dead fetuses, no abortion or preterm delivery, given that all the rats delivered within the reported standard gestation period of 21-23 days without stillbirths. The mean litter size and birth weights in all the extract-treated groups were not different from the pregnant non-treated control; instead, the pups of the treated groups gained more weight than the pregnant non-treated control group. No obvious teratogenic abnormality was found either on the fetuses or the litters. The obtained result suggests that the extract did not disrupt the placental functions of nutrient and oxygen transfer, which may have affected fetal development or pregnancy outcome.<sup>39</sup> These results indicate that the treatment had no significant adverse effects on fetal development or pregnancy outcomes. Histopathology data helps to investigate the hazards as well as the toxic mechanisms involved in toxicological studies; in this study, slight to moderate pathological changes were observed in the rats' uterus and placenta; the histological changes observed may have been the usual immune signs related to pregnancy as there were no histological changes found in the uterus of the non-pregnant rats treated with the crude extract for 28 and 90 days.<sup>20</sup>

## CONCLUSION

Even though the crude extract did not exhibit any structural abnormalities in the pregnant rats, pregnant women and men should exercise caution when consuming this plant because it can be toxic to the reproductive system and may affect reproduction, as evidenced by the study's findings of a decrease in the levels of reproductive hormones.

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