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Effects of Ethanolic Extract of Moringa Oleifera Seeds and Leaves on Pregnancy

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Original Research Article

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Abstract: Infertility has remained a major health problem among couples and sexually active individuals who seek to procreate, and the search for therapeutic solutions have remained endless. This led to the consumption of moringa oleifera as fertility aid. Therefore, this study investigated the effects of ethanolic extract of moringa oleifera seeds and leaves on pregnancy. Eighty-four (84) albino rats comparing of 56 females and 28 males, which acclimatised for two weeks and mated in ratio of 2 females to 1 male, the pregnant female rats were then divided into 7 Groups of 7; All rats were weighed on a daily basis throughout the research period. Group 1- Control (10ml/kg body weight/day of vehicle [Tween 80] orally). Groups 2-4 (Seed extract at dose level of 100, 200 and 400mg/kg body weight/day respectively), Groups 5-7 (Seed extract at dose level of 100, 200 and 400mg/kg body weight/day respectively). Administration was carried out throughout the gestation period. The blood samples were collected for hormonal assay and under standard aesthetic conditions. From the results it was observed that the moringa oleifera leave and seed extract caused resorption of the foetus with decrease in weight in a dose dependent manner; however, there was no disruption of the normal gestation. The levels of FSH and LH for animals treated with 400mg/kg were significantly lower than those of 200mg/kg, 100mg/kg and the control group. In conclusion, the ethanol extract of both the leaf and seed of moringa oleifera has shown abortifacient effect and therefore not advise for consumption during pregnancy.
Keywords: Moringa oleifera, abortifacient effect, albino rats, reproductive system.

INTRODUCTION

For centuries and up until date, plants have remained an important and dependable source of medicine. World Health Organization (WHO) estimated that about 80% of the global population depends absolutely tradomedicine [1]. The therapeutic value of these plants are because of the variety of active phytochemicals and their essential composition. The role medicinal plants play in fighting and managing diseases have been attributed to presence of antioxidant in their constituents, often linked to numerous types of polyphenolic compounds [2]. Thus, the global interest in understanding the nature and dynamic of these natural antioxidants obtained from therapeutic plant materials for health care use has continued to grow.

Among the numerous medicinal plants which have shown great potentials is *Moringa oleifera* Lam (*Moringa oleifera*); commonly known as Moringa [3]. Moringa is a versatile tropical tree popularly known for its culinary uses; however, it has wide range of application in the industry, medicine and agriculture, including animal feeding. For this purposes it has become increasingly popular in Asian, European and African continents, where its economical valuable is unprecedented [4]. It has been dubbed the "Miracle tree" or "tree of life" by the media [4, 5]; as every part of *Moringa oleifera* have been reported for one or more theurapetic uses as well as pharmaceutical and industrial byproducts [2].

Aside the culinary [6] and other local uses [4, 5], various researchers [7, 2, 6] have reviewed the numerous biochemical properties of various parts of *Moringa oleifera* in the past. These parts contain both macro- and micro-nutrients, which are rich sources of natural antioxidants, which wide range of hormone modulation [8-10].

Across the globe, the importance of the reproductive system cannot be overlooked, as it is one of the most significant characteristics of humans and essential for the continuity of life, because of the continued exposure to life and attacks from environmental agents. The disease of the reproductive system is infertility and it has resulted in large cases of marital problems [11]. This high burden of infertility has lead couples and individuals, who yearn but are

unable to realize and sustain desired pregnancy to sort for assistance in tradomedicine especially in low resources countries.

The key hormones of the reproductive system are the Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) is a gonadotropin (glycoprotein based) released by the anterior pituitary as a result of stimulation by gonadotropin-releasing hormone (GnRH) and released by the hypothalamus. FSH and luteinizing hormone (LH) regulates the testis and ovary gonadal function by enhancing sex steroid production and gametogenesis. In women, follicle-stimulating hormone stimulates the growth of ovarian follicles in the ovary before the release of an egg at ovulation, and promotes oestra-diol production.

In the bid to manage infertility, various medicinal plants were often locally consumed and there have been reports of effective activities [12]; However, with respects to the reproductive system of females, there have been reported to biochemically and physiologically alter the reproductive cycle of female Wistar rats [13-15]. Hence the sole aim of this research work, which is to investigated the effects of ethanolic extract of moringa oleifera seeds and leaves on pregnancy. The objectives of this work are: to determine the level of influence the extract exerts on the pups' delivery and hormone of the pregnant albino rat following administration.

MATERIALS AND METHODS

Purchase, Identification and Extraction of Plant (*Moringa oleifera*)

Moringa oleifera seed and leaf were purchased at Moringa House, 14, McAkini Road off Ada-George Road, Port Harcourt, Nigeria. The plant parts were harvested, identified and authenticated by a botanist. The seeds and leaves of *Moringa oleifera* were properly processed, grinded to powdered and subjected ethanol soxhlet extraction. The seed and leaf extracts were evaporated to near dryness on rotary evaporator (40°C), weighed and preserved at -4°C in a refrigerator until needed.

Experimental design

Fifty-six (56) non-pregnant female albino rats (weighing between 180-200g) and twenty-eight (28) male albino rats (weighing between 200-220g) were obtained from the animal farm, University of Port Harcourt. The rats were housed four per cage and maintained under natural conditions. They were fed with laboratory feeds and clean tap water. They were allowed to acclimatize to laboratory environment for 14 days before commencement of research. All experimental protocols were in line with the approved guidelines of the University's Research Ethics Committee. After the 2weeks of acclimatization, the female animals were mated with males (2:1). Animals were checked for the presence of vaginal plug to confirm pregnancy. The pregnant rats were separated out for the main research. The pregnant female rats were divided into 7 groups of 7 animals each. All rats were weighed on a daily basis throughout the research period. The animals were treated with different dose of *Moringa oleifera* seed and leaf extract. Group 1 represented the control group, which was administered 10ml/kg body weight/day of vehicle (Tween 80) orally. Groups 2-4 were administered suspension of ethanolic extract of *Moringa oleifera* seed in tween 80 orally at dose level of 100, 200 and 400mg/kg body weight/day respectively. Groups 5-7 were administered suspension of ethanol extract of *Moringa oleifera* leaf in tween 80 at dose level of 100, 200 and 400mg/kg body weight/day respectively. Extracts and vehicle were administered throughout the gestation period. The mothers were sacrificed after delivery under deep diethyl ether anaesthesia and blood collected for hormonal profile.

Determination of lethal dose

Doses used were based on the LD_{50} of the plant and previous studies done. The oral LD_{50} of the leaves of the plant in rats has been recorded as 6616.67mg/kg [16] while it is 5000mg/kg for the seeds [17]. The doses chosen are approximately 2, 4 and 8% of the LD_{50} respectively.

Hormonal assay

The blood samples collected from the animals were subjected to hormonal analysis.

Data analysis

All data generated were computed and analysed using Microsoft Office Excel 2013 and IBM SPSS version 23.0. Data was presented in tables of descriptive statistics as mean±SEM (Standard Error of Mean). Analysis of Variance (ANOVA) was done to determine if significant difference exist between the groups, while Dunnett's multiple comparison test was done to determine the pair that differs (each group will be compared against the control; typical of Dunnettts). Comparison was carried out at three significant levels (95%, 99% and 99.9%). Hence $P< 0.05$, $P< 0.01$ and $P< 0.001$ respectively will be considered significant.

RESULTS

Table-1: Weight of pregnant animals treated with leaf extract of *Moringa oleifera*

Group	Mean±SEM (g)			
	Week 0	Week 1	Week 2	Week 3
Control	209.57±0.43	219.14±0.80	235.29±1.30	270.71±0.84
100mg/kg	209.57±0.53	214.71±0.36*,**	220.71±0.57*,**,***	239.43±1.23*,**,***
200mg/kg	206.14±1.55*	212.57±1.15*,**,***	220.29±0.61*,**,***	232.57±0.95*,**,***
400mg/kg	211.14±0.77	213.71±0.61*,**,***	219.00±0.79*,**,***	227.14±0.86*,**,***

SEM =Standard Error of the Mean, *= P < 0.05, ** = P < 0.01, *** = P < 0.001

Table-2: Weight of pregnant animals treated with seed extract of *Moringa oleifera*

Group	Mean±SEM (g)			
	Week 0	Week 1	Week 2	Week 3
Control	209.57±0.43	219.14±0.80	235.29±1.30	270.71±0.84
100mg/kg	209.86±0.59	215.29±0.57*,**	221.43±0.65*,**,***	243.14±2.18*,**,***
200mg/kg	207.29±0.97	213.43±0.75*,**,***	220.29±0.61*,**,***	232.43±0.87*,**,***
400mg/kg	209.71±0.71	214.14±0.67*,**,***	219.43±0.65*,**,***	226.14±0.67*,**,***

SEM =Standard Error of the Mean, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

Table-3: Duration of gestation after treatment with extract of *Moringa oleifera*

Group	Mean±SEM	
	Leaf Extract	Seed Extract
Control	22.29±0.36	22.29±0.36
100mg/kg	21.86±0.26	22.00±0.22
200mg/kg	22.57±0.20	22.71±0.18
400mg/kg	-	-

SEM =Standard Error of the Mean, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

Table-4: Number of Pups delivered after treatment with extract of *Moringa oleifera*

Group	Mean±SEM	
	Leaf Extract	Seed Extract
Control	7.14±0.34	7.14±0.34
100mg/kg	3.29±0.29*,**,***	4.14±0.51*,**,***
200mg/kg	2.14±0.26*,**,***	2.14±0.26*,**,***
400mg/kg	-	-

SEM =Standard Error of the Mean, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

Table-4.6: Birth weight of pups delivered after treatment with extract of *Moringa oleifera*

Group	Mean±SEM (g)	
	Leaf Extract	Seed Extract
Control	5.75±0.03	5.75±0.03
100mg/kg	4.91±0.27*,**	4.92±0.05*,**,***
200mg/kg	3.44±0.13*,**,***	3.91±0.17*,**,***
400mg/kg	-	-

SEM =Standard Error of the Mean, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

Table-2: Level of FSH in albino rats treated with extract of *Moringa oleifera*

Group	Mean±SEM	
	Leaf Extract (µg/ml)	Seed Extract (µg/ml)
Control	9.89±0.03	9.89±0.03
100mg/kg	6.11±0.17*,**,***	7.47±0.14*,**,***
200mg/kg	5.44±0.15*,**,***	6.83±0.13*,**,***
400mg/kg	3.13±0.04*,**,***	4.26±0.05*,**,***

SEM =Standard Error of the Mean, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

Table-3: Level of LH in albino rats treated with extract of *Moringa oleifera*

Group	Mean±SEM	
	Leaf Extract(µg/ml)	Seed Extract(µg/ml)
Control	11.51±0.14	11.51±0.14
100mg/kg	9.76±0.07*,**,***	10.16±0.03*,**,***
200mg/kg	8.53±0.27*,**,***	8.62±0.09*,**,***
400mg/kg	5.34±0.10*,**,***	6.82±0.02*,**,***

SEM =Standard Error of the Mean, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

DISCUSSIONS

Ethanol extract of Moringa oleifera was involved in the study, with their effects examined on the following: maternal weight gain, duration of gestation, number of pups delivered, pups birth weight as well as FSH and LH.

From the current study, a time dependent decrease in weight was observed in the animals rather than an expected increase in the weight of the animals treated with the extract. This could have resulted due to the observed resorption of the foetus. This is an abortificient effect, and Varsha *et al.*, [18] has also reported similar effect on the ethanol extract of the stem bark of Moringa oleifera. On the contrary, the control group animals had an increase in weight which is usual in pregnancy (Tables 2 and 3).

The duration of gestation was not affected for the animals that did not experience resorption; they all have similar duration of gestation (Table-4).

However, the ethanolic extract of the seed and leaf of *Moringa oleifera* was found to decrease the number of pups delivered in a dose dependent manner (Table-1). A similar observation was made with the weights of the litters (Table-6). These may be related to the reported abortificient effect of the plant. On this regard several works have reported similar effect. Some of them include the report of Awe *et al*., [19] and Zade & Dabhadkar [20]. These works have also reported a dose dependent effect of the stem bark of the plant of the number of resorption of the pregnant animals, i.e. the higher the dose the greater the number of animals that experience resorption.

Table 2 and 3 revealed that there was also a dose dependent decrease in the levels of FSH and LH in the tested animals; higher doses produce low hormonal level while the lower doses produce higher hormonal levels. The levels of FSH and LH for animals treated with 400mg/kg were significantly lower than those of 100mg/kg, 200mg/kg and the control group. This is indicating that the ethanolic extract of both leaf and seed of moringa oleifera have the capacity of reducing the level of both hormones (FSH and LH) in biological systems.

CONCLUSIONS

The ethanol extract of both the leaf and seed of moringa oleifera has shown abortificient effect therefore, pregnant women are advised not to use the plant no matter the anticipated beneficial effect. More research should be conducted to know the effects on other organs and hormones not covered in this work.

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