Histological Studies Of The Effects Of Oral Administration Of Aspilia Africana (Asteraceae) Leaf Extract On The Ovaries Of Female Wistar Rats

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Abstract

Histological studies of the effects of oral administration of extract of Aspilia africana, used in ethno medical practice in Africa for the management of various ailments, on the ovarian tissues of matured female Wistar rats were carefully studied. The rats (n=24), average weight 0f 182g were randomly assigned into two treatments (n=16) and control (n=8) groups. The rats in the treatment groups received 0.5g/kg and 1g/kg of aqueous extract of Aspilia africana orally through orogastric tube for fourteen days, while the control rats received equal volume of distilled water without the extract of Aspilia added. The rats were fed with grower's mash purchased from Edo feeds and Flour Mill Ltd, Ewu, Edo state and were given water liberally. The rats were sacrificed on day fifteen of the experiment. The ovary was carefully dissected out and quickly fixed in 10% formal saline for routine histological study after H&E method.

The histological findings after H&E methods indicated that the treated sections of the ovary showed some cellular hypertrophy of the Theca folliculi, complete distortion/destruction of the basement membrane. Degenerative and atrophic changes were observed in the oocyte and zona granulosa; these were more pronounced in those that received 1g/kg of aspilia africana extract. There were marked vacuolations appearing in the stroma cells when compared to the control sections. These findings indicate that Aspilia africana consumption may probably have adverse effects on the ovaries by its deleterious effects on the oocytes and stroma cells of ovary of adult Wistar rats. It is recommended that further studies aimed at corroborating these observations be carried out.

INTRODUCTION

Plant materials as sources of medical compounds continue to play a dominant role in the maintenance of human health since antiquity. Over 50% of all modern chemical drugs are of natural plant product origin, and is essential in drug development programs of the pharmaceutical industry[1]. Like any therapeutic agent, when overdosed or incorrectly used they also have the potential to induce adverse effects. The historic role of medicinal herbs in the treatment and prevention of disease, and their role as catalysts in the development of pharmacology do not, however, assure their safety for uncontrolled use by an uninformed public[2].

There has been minimal research to address possible adverse reproductive, immunologic, or neurological effects or even systemic toxicity and/or carcinogenicity that might be associated with high doses or prolonged use of these products[3]. This concern was frequently expressed at the International Workshop to Evaluate Research Needs on the Use and Safety of Medicinal herbs could not be assumed safe because they are “natural”[4].

In Benin City, Nigeria, many plants are used in herbal medicine to cure diseases and heal injuries. Such medicinal plants include Aspilia Africana (Asteraceae), a perennial herb varying in height from 60cm to about 1.5m depending on rainfall. It is a common weed of field crops in West Africa and sometimes found in fallow land, especially the forest zones[5]. It is ligneous at the base, its fruit quadrangular akenes and leaves opposite and hairy. The plant is a weed grazed by cattle and sheep and is mostly used in the western state of Nigeria as food for rabbits and hares[6].

Aspilia Africana is widely used in ethno medical practice in Africa for its ability to stop bleeding, even from a severed artery, as well as promote rapid healing of wounds and sores and for the management of problems related to cardiovascular diseases[7]. It has also been established that Aspillia Africana has an anticoagulant activities[8]. Infusion of the leaves is taken by children and can also be mixed with

clay as a medicine for stomach trouble[9]. It has been reported that the plant is effective against malaria infection[10]`. It has been classified among substances with low toxicity, with an LD_{50} averaging 6.6g/Kg body weight[11]. The methanolic and aqueous extracts of the leaves of Aspilia Africana has exhibited differential anti-bacterial activities on both Gram-positive and Gram-negative bacterial species[12,13]. Aspilia Africana has so many other uses, like palliative for alleviating menstrual cramps and dysmenorrheal, which are not documented, probably because empirical studies had not been carried out on them to prove or disprove their efficacy. In some communities in Nigeria women boil and filter the leave of Aspilia Africana, which they drink to prevent conception. It is therefore suggestive that Aspilia Africana may have some contraceptive or anti-fertility properties,

The ovary is a paired, egg-producing reproductive organ found in female organisms. The ovaries also functions in the production of various steroid and peptide hormones like estrogen and progesterone which sub serve many functions in the reproductive system[14].

This work is carried out to investigate some probable histological effects of Aspilia Africana leave extract on the ovary and its likely involvement in female infertility in Nigeria, by its varied use in the treatment of other medical conditions, on adult female Wistar rats. About 15% of cases of female infertility investigation will show no abnormality. In these cases abnormalities are likely to be present but not detected by current methods[15].

MATERIALS AND METHODS

PLANT MATERIALS: Fresh leaves of Aspilia africana were collected in November, 2006 at Oluku Town in Ovia North-East local government area of Edo State. The plant was identified and authenticated at the Botany department of the University of Benin, Benin City. The harvested fresh leaves were sun dried and ground into a fine powder. The dried material (300g) was macerated in 6 litters of distilled water for 48hrs at 4°C in a refrigerator. The extract was sieved and the juice was filtered using Whatman N°1 filter paper. The filtrate was put in a stainless-steel tray, and concentrated in an air-circulating oven at 42°C until total dryness. The resultant extract was put into small glass dishes and stored at 28°C in an incubator for further studies.

ANIMALS: Twenty four, (24) adult female Wistar rats with average weight of 182g were randomly assigned into three groups A, B and C of (n=8) in each group. Groups A and B of (n=16) serves as treatments groups while Group C (n=8) is the control. The rats were obtained and maintained in the Animal Holdings of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Nigeria. They were fed with grower's marsh obtained from Edo feed and flour mill limited, Ewu, Edo state and given water liberally. The rats gained maximum acclimatization before actual commencement of the experiment.

ASPILIA AFRICANA ADMINISTRATION: The rats in the treatment groups (A and B) were given 0.5g/kg and 1g/kg extract of Aspilia africana orally through orogastric tube, respectively on a daily basis. The control group © received equal volume of distilled water without the extract of Aspilia africana added for fourteen days. The rats were sacrificed on the fifteenth day of the experiment. The ovaries were quickly dissected and fixed in 10% formal saline for routine histological techniques. The 0.5g/kg and 1g/kg extract of Aspilia africana doses were chosen and extrapolated in this experiment based on the indiscriminate use of the plant here in Nigeria and on previous work done with this plant[11,12,13,14,15,16].

HISTOLOGICAL STUDY: The tissue were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. The deparaffinised sections were stained routinely with haematoxylin and eosin. Photomicrographs of the desired results were obtained using digital research photographic microscope in the University of Benin research laboratory.

RESULTS

The ovaries of the control group showed normal histological features, illustrating a well defined zonal granulosa surrounding the oocyte and compact theca folliculi and the presence of some primordial follicles (Figure 1).

The ovaries of the treated groups showed some cellular hypertrophy of the Theca folliculi, complete distortion/destruction of the basement membrane separating the Theca folliculi from the zona granulosa. Degenerative and atrophic changes were observed in the oocyte and zona granulosa; these were more pronounced in those that received 1g/kg of aspilia africana extract. There were marked vacuolations appearing in the stroma cells (figures 2 and 3).

Figure 1

Figure 1: Photomicrograph of the ovary of control animals (Group C) (Mag. x400)

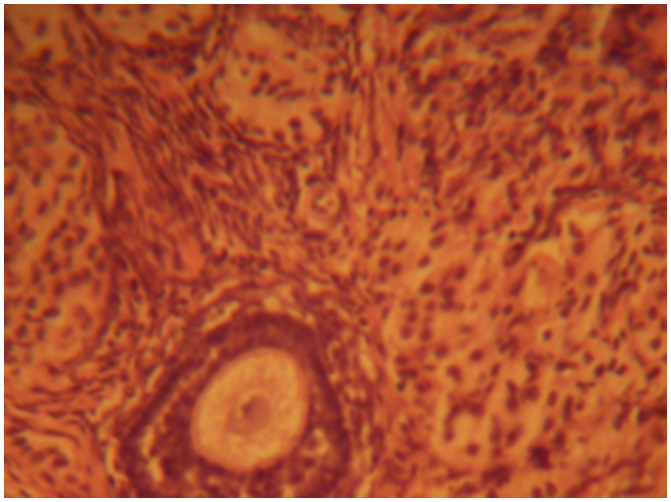


Figure 2

Figure 2: Photomicrograph of the ovary of rat treated with 0.5g/kg of extract. (Group A) (Mag. x400)

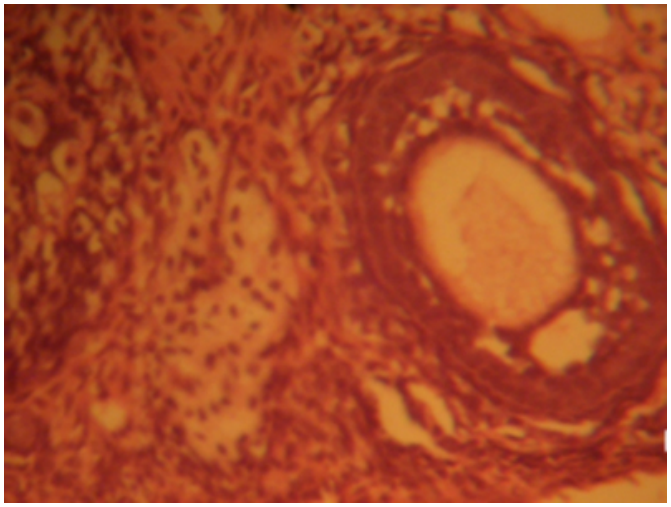
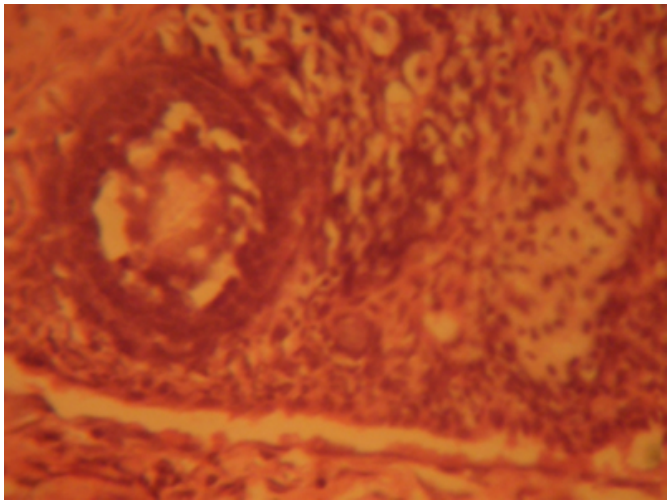


Figure 3

Figure 3: Photomicrograph of the ovary of rat treated with 1g/kg of extract. (Group B) (Mag. x400)



DISCUSSION

The results of the haematoxylin and eosin staining (H & E) reactions showed some cellular hypertrophy of the Theca folliculi, complete distortion/destruction of the basement membrane separating the Theca folliculi from the zona granulosa. Degenerative and atrophic changes were observed in the oocyte and zona granulosa; these were more pronounced in those that received 1g/kg of Aspilia africana. There were marked vacuolations appearing in the stroma cell.

The increase in cellular hypertrophy of the Theca folliculi in the treatment groups as reported in this study may have been as a result of cellular proliferation; the mechanism which is not yet clear. The vacuolation probably indicates the presence of mucous. Degenerative and atrophic changes which were observed in the oocyte and zona granulosa were more pronounced in the group treated with higher dose (1g/kg) of Aspilia africana extract.

It may be inferred from the present results that higher dose and prolonged administration of Aspilia africana extract resulted in degenerative and atrophic changes observed in the ovaries. The actual mechanism by which Aspilia africana extract induced cellular degeneration observed in this experiment needs further investigation.

Degenerative changes have been reported to result in cell death, which is of two types, namely apoptotic and necrotic cell death. These two types differ morphologically and biochemically[17]. Pathological or accidental cell death is

regarded as necrotic and could result from extrinsic insults to the cell such as osmotic, thermal, toxic and traumatic effects[18]. In this experiment Aspilia africana extract could have acted as toxins to the oocyte and follicular cells of the ovaries. The process of cellular necrosis involves disruption of membrane's structural and functional integrity which was also a landmark of this experiment. In cellular necrosis, the rate of progression depends on the severity of the environmental insults.

The greater the severity of insults, the more rapid the progression of cellular injury[19].The principle holds true for toxicological insults to the brain and other organs[20]. It may be inferred from the present results that prolonged intake of Aspilia africana extract resulted in increase toxic effects on the ovaries with that of higher dose more marked.

CONCLUSION AND RECOMMENDATION

In conclusion, our study revealed that Aspilia africana extract causes some cellular hypertrophy, complete distortion/destruction of the basement membrane, degenerative and atrophic changes, and vacuolations in the cells of the ovaries. With these results, it is probable that the functions of the ovary may be adversely affected, and Aspilia africana could be used for contraception or could be one of the factors causing female infertility by its varied use in the management of other medical condition by alternative medical practitioners and rural dwellers. It is recommended that further studies be carried out to examine these findings.

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