Histological Studies Of The Teratogenic Effects Of Oral Administration Of Aspilia Africana (Asteraceae) Leaf Extract On The Developing Kidney Of Wistar Rats

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Citation

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Abstract

Histological studies of the teratogenic effects of oral administration of extract of Aspilia africana, used in ethno medical practice in Africa for the management of various ailments, on the developing kidneys of pregnant matured female Wistar rats were studied. The rats (n=24), average weight of 180g were randomly assigned into two treatment (n=16) and a control (n=8) group. The rats in the treatment groups received 0.5g/kg and 1g/kg of aqueous extract of Aspilia africana orally through orogastric tube in the first seven days of gestation, while the control rats received equal volume of distilled water without the extract of Aspilia added. The rats were fed with growers' mash and were given water liberally. After parturition, the kidney sections were obtained from the pups or neonates and processed for routine histological investigation.
Histological changes observed in the kidney sections revealed loss of renal corpuscle and varying degree of cyto-architectural distortion of the cortical structures, with degenerative and atrophic changes. This suggests the direct cytotoxic action of aqueous extract of Aspilia africana resulting from placental transfer during pregnancy to the neonates. This study highlights the possible abnormalities that could result in a newborn when a pregnant animal is exposed to an aqueous extract of Aspilia africana during the seven days of gestation. The results of this experiment suggest that the functions of the kidney could also have been affected as a result of the massive cyto-archictectural distortion. It is recommended that further studies aimed at corroborating these observations be conducted.

INTRODUCTION

Birth defects are known to occur in 3-5% of newborns [1]. They are the leading cause of infant mortality during the first year of life [2]. Approximately 7-10% of all newborns will require extensive medical care to diagnose or treat a birth defect [3]. Although significant progress has been made in identifying etiological causes of some birth defects, approximately 65% have no known or identifiable cause [4]. In ethno medical practice plant materials has been used as sources of medical compounds and had played a dominant role in the maintenance of human health in most rural communities in developing countries in time past. 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 [5].

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 [6]. This concern was frequently expressed at the International Workshop to Evaluate Research Needs on the Use and Safety of Medicinal Herbs (1998) could not be assumed safe because they are “natural” [7].

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 [8]. 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 [9].

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 [10] . It has also been established that Aspillia africana has an anticoagulant activities when applied topically to wounds [11] . Infusion of a liquid made from the leaves is taken by children and can also be mixed with clay as a medicine for stomach trouble [12] . It has been reported that the plant is effective against malaria (P. falciparum) infection [13] . It has been classified among substances with a low potential for toxicity, with an LD_{50} averaging 6.6g/Kg body weight [14] . The methanolic and aqueous extracts of the leaves of Aspilia africana have exhibited differential anti-infective activities on both Gram-positive and Gram-negative bacterial species [15,16] . Aspilia africana has many other additional uses such as palliative properties as documented by Okoli CO et al that the leaves of A. africana possess constituents capable of arresting wound bleeding, inhibiting the growth of microbial wound contaminants and accelerating wound healing which suggest good potentials for use in wound care [17,18] , 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 has been reported that Aspilia africana has some contraceptive or anti-fertility properties [19] .

The kidney is a paired organ located in the posterior abdominal wall whose functions include removing waste products from the blood and regulating the amount of fluid in the body. As in humans the majority of drugs administered to animals are eliminated by a combination of hepatic metabolism and renal excretion [20] . The kidney also plays a major role in drug metabolism. However its major importance to drug disposition remains its excretory function.

This work was performed in order to investigate potential teratogenic effects of Aspilia africana leaf extracts on the developing kidneys of pregnant Wistar rats.

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 liters of distilled water for 48hrs at 4 ^{o} C in a refrigerator. The extract was sieved and the juice was filtered using Whatman N ^{o} 1 filter paper. The filtrate was placed in a stainless-steel tray, and concentrated in an air-circulating oven at 42 ^{o} C until totally dry. The resultant extract (8g) was placed into small glass dishes and stored at 28 ^{o} C in an incubator for further studies.

Animals: Twenty four, (24) adult Wistar rats; comprised of eighteen (18) pregnant mature female Wistar rats, and six (6) adult male rats with an average weight of 180g were randomly assigned into three groups A, B and C of (n=8) in each group. Groups A and B of (n=16) served as treatment groups while Group C (n=8) was the control. Each of the experimental and control groups contained 2 matured male rats. 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 growers' mash obtained from Edo feed and flour mill limited, Ewu, Edo state and given water liberally. The rats gained maximum acclimatization (2 weeks) before actual commencement of the experiment.

Aspilia africana Administration: Oral administration of the extract of Aspilia africana commenced with the establishment of pregnancy. This was carried out by taking the vaginal smear of the rats following the introduction of the male rats into their cages and mating confirmed. The rats in the treatment groups (A and B) were given 0.5g/kg/day and 1g/kg/day extract of Aspilia africana orally through an orogastric tube, respectively during the first seven days of gestation. The control group (C) received an equal volume of distilled water without the extract of Aspilia africana for the same dosing period. At parturition, the neonates from each group were sacrificed the following day and the kidneys were dissected out and fixed in a freshly prepared 10% formal saline solution for routine histological assessment.

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 [14,15,16,17,18,21]

HISTOLOGICAL STUDY

Renal tissues were dehydrated in an ascending grade of alcohol (ethanol 70%), cleared in xylene and embedded in paraffin wax after the method of Drury and Wallington 1980 [22] . 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 micrograph of the kidney tissue of the neonates in the control group showed a well-detailed cortical parenchyma. The renal corpuscles appear as dense rounded structures with the glomeruli surrounded by narrow Bowman's spaces (Figure 1)

The kidney tissue of the neonate of the animals in group A treated with 0.5g/kg of aspilia africana extract revealed some shrunken and even loss of renal corpuscles within the renal parenchyma with the glomerulus showing densely packed blood cells. (Figure 2)

The kidney sections of the neonate of the animals in group B treated with 1g/kg of aspilia africana extract revealed marked distortion of cyto-architecture of the renal cortical structures, tubular atrophy and tubular necrosis. The glomerulus showed densely packed red blood cells and degenerative and atrophic changes. (Figure 3)

About the eight day of the experiment the animals in the treatment groups were observed to be restless and lethargic, with those in groups B more aggressive. Some animals in groups B were observed to have diarrhea at about the fifth day of experiment but this effect was transient. There were no significant weight changes between the control and the treated animals and between the dosed males and the pregnant females in the study groups.

Figure 1

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

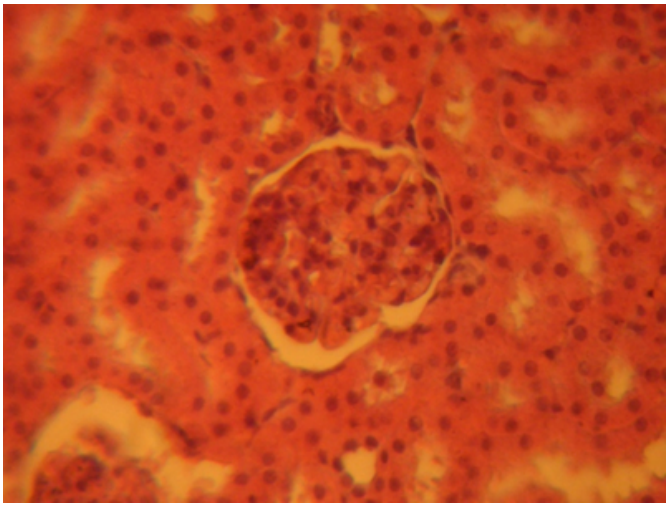


Figure 2

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

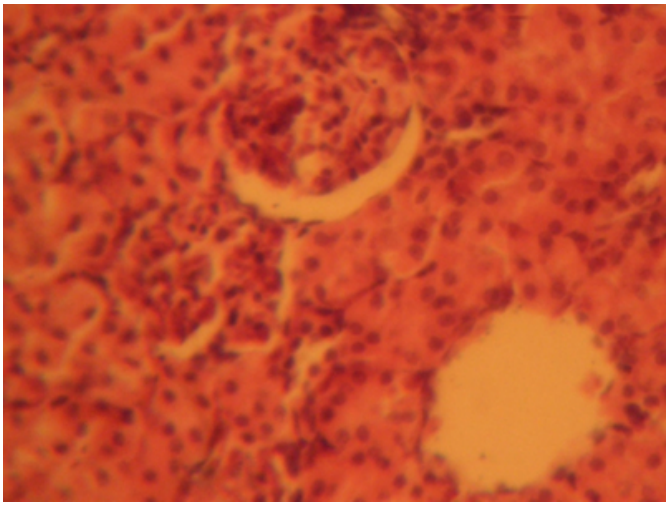
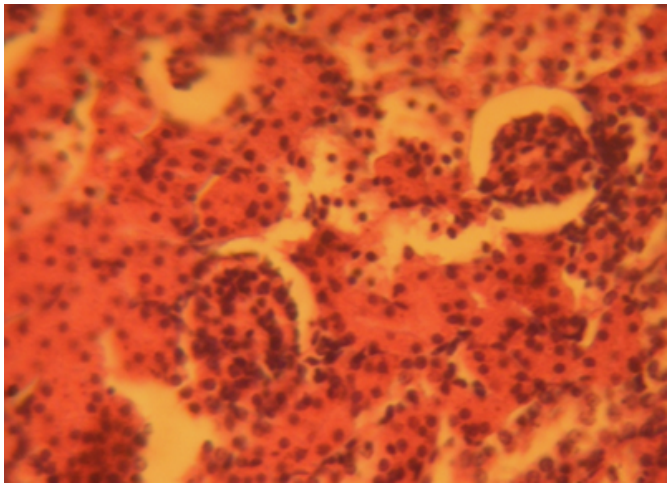


Figure 3

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



DISCUSSION

The results (H & E) reactions showed that administration of Aspilia africana extract to pregnant Wistar rats during the first seven days of gestation caused varying degrees of cyto-architectural distortion in the neonates of the treated groups compared to the control group. There were dose-dependent degenerative and atrophic changes observed in the neonatal kidneys of rats that were marked in animals that received the high dose (1g/kg) of Aspilia africana extract.

It may be inferred from the present results that higher doses of Aspilia africana extract given during the period of gestation could result in even greater degenerative and atrophic changes that could occur in the renal corpuscle. The actual mechanism by which Aspilia africana induced cellular degeneration observed in this experiment required further investigation. The necrosis observed is probably due to the high concentration and teratogenic effects of Aspilia africana on the developing kidney. Aspilia Africana extract is known to affect intracellular Calcium levels [10] . This could be the mechanism responsible for the observed necrosis in this study.

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 [23] . 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 [24] . In this experiment Aspilia africana extract may have acted as toxins to the developing kidney. The process of cellular necrosis involves disruption of membrane's structural and functional integrity. 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 [25] .The principle holds true for toxicological insults to the brain and other organs [26] . It may be inferred from the present results that intake of Aspilia africana extract during the first seven days of gestation resulted in teratogenic effects on the developing kidneys of pregnant Wistar rats, with that of higher dose more marked.

CONCLUSION AND RECOMMENDATION

In conclusion, our study revealed that Aspilia africana extract causes varying degree of cyto-architectural distortion, degenerative and atrophic changes in the developing kidneys of Wistar rats. With these results, it is probable that the functions of the kidneys of the neonate may be adversely affected, and the use of Aspilia africana in the management of other medical condition by alternative medical practitioners and rural dwellers should be with caution especially during pregnancy. It is recommended that further studies be carried out to examine these findings.

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