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

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Citation

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Abstract

Histological studies of the teratogenic effects of oral administration of extract of <I>Aspilia africana</I>, used in ethno medical practice in Africa for the management of various ailments, on the developing liver of neonatal Wistar rats were carefully studied. The rats (n=24), average weight 0f 180g 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 <I>Aspilia africana</I> 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 <I>Aspilia</I> added. The rats were fed with growers' mash purchased from Edo feeds and Flour Mill Ltd, Ewu, Edo state and were given water liberally. After parturition, the liver sections were obtained from the pups or neonates 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 liver showed evidence of dilatations of the central veins, which contained lysed red blood cells and cyto-architectural distortions of the hepatocytes, centrilobular hemorrhagic necrosis, atrophic and degenerative changes with the group that received 1g/kg of the extract of <I>Aspilia africana</I> more severe. These findings suggest the direct cytotoxic effect of aqueous extract of <I>Aspilia africana</I> resulting from placenta 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 aqueous extract of <I>Aspilia africana</I> in the first few days of pregnancy. The results of this experiment suggest that the functions of the liver could also have been affected as a result of the massive cyto-architectural distortion. It is recommended that further studies aimed at corroborating these findings be carried out.

INTRODUCTION

Birth defects are known to occur in 3-5% of newborns[1]. They are the leading cause of infant mortality in the first year of life[2]. 7-10% of all 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]. 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[5].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[6].

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[7]. 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”[8].

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[9]. 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[10].

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[11]. It has also been established that Aspilia africana has an anticoagulant activities[12]. Infusion of the leaves is taken by children and can also be mixed with clay as a medicine for stomach trouble[13]. It has been reported that the plant is effective against malaria infection[14]. It has been classified among substances with low toxicity, with an LD_{50} averaging 6.6g/Kg body weight[15]. 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[16, 17]. 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 has been reported that Aspilia africana have some contraceptive or anti-fertility properties[18].

The liver is the largest glandular organ of the body, weighing between 1.4-1.6kg. It lies below the diaphragm in the thoracic region of the abdomen. It plays a major role in metabolism and has a number of functions in the body, including glycogen storage, plasma protein synthesis, production of bile; an alkaline compound which aids in digestion, and detoxification of most substances[19]. Since the liver is involved in the performance of these varied functions it may be susceptible to injury particularly in situation of toxicity. It would therefore be worthwhile to examine some probable teratogenic effects of Aspilia africana leave extract on the developing liver 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 litters of distilled water for 48hrs at 4oC in a refrigerator. The extract was sieved and the juice was filtered using Whatman No 1 filter paper. The filtrate was put in a stainless-steel tray, and concentrated in an air-circulating oven at 42oC until total dryness. The resultant extract was put into small glass dishes and stored at 28oC in an incubator for further studies.

ANIMALS: Twenty four, (24) adult Wistar rats; comprising of eighteen (n=18) pregnant matured female Wistar rats, and six (n=6) adult male rats with 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) serves as treatments groups while Group C (n=8) is the control. Each of the experimental and control groups had 2 (n=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 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 and 1g/kg extract of Aspilia africana orally through orogastric tube, respectively in the first seven days of gestation on a daily basis. The control group © received equal volume of distilled water without the extract of Aspilia africana for the same period. At parturition, the neonates from each group were sacrificed the following day and the liver dissected out and fixed in a freshly prepared 10% formal saline for routine histological study.

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, 17, 18, 20]

HISTOLOGICAL STUDY: The liver tissue was dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 6 microns thick were obtained using a rotatory microtome. The deparaffinized 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 control sections of the liver tissue of the neonates in the control group showed normal histological features with the hepatic lobules showing irregular hexagonal boundary defined by portal tract and sparse collagenous tissues. The hepatic portal veins, bile ductules and hepatic artery within the portal tract were all visible. (Figure1).

The treatment sections of the liver showed some histological changes that were at variance with those obtained in the control. There were evidence of dilatations of the central veins, which contained lysed red blood cells and cyto-architectural distortions of the hepatocytes and centrilobular hemorrhagic necrosis. There were atrophic and degenerative changes with the group that received 1g/kg of Aspilia africana more severe (Figure 2 & 3).

Figure 1

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

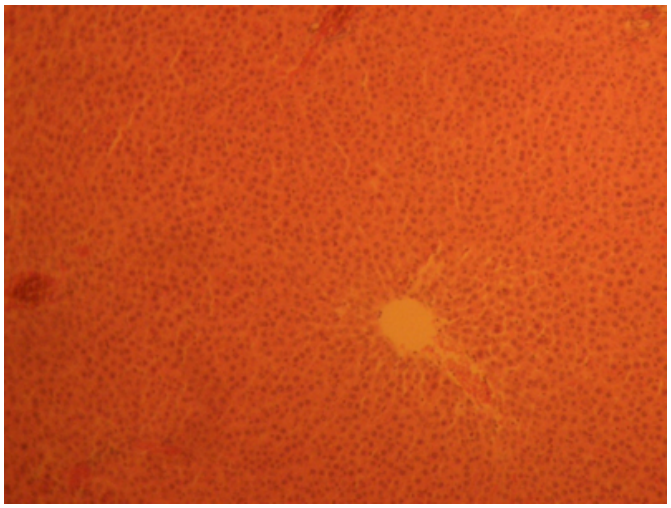


Figure 2

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

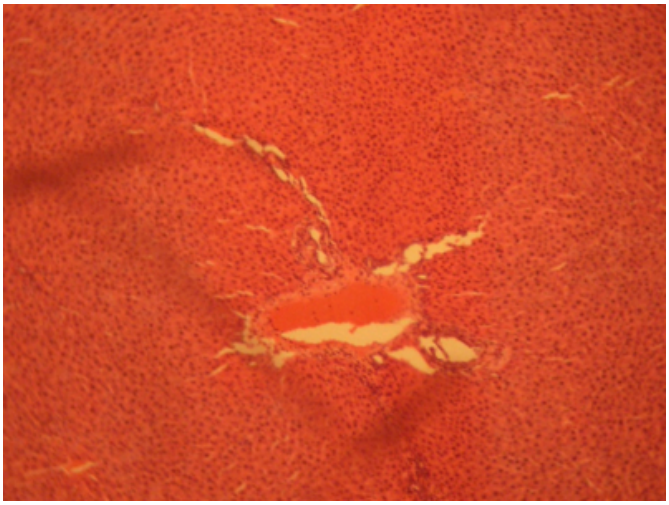
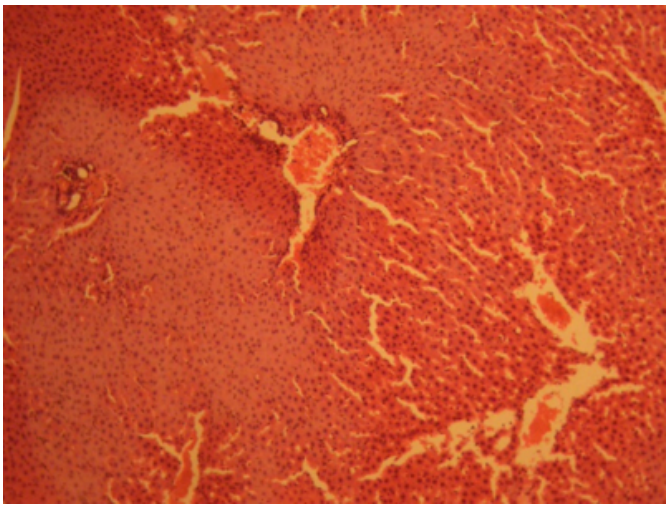


Figure 3

Figure 3: Photomicrograph of the neonatal liver 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 degree of cyto-architectural distortion of the hepatocytes; with evidence of dilatations of the central veins, which contained lysed red blood cells and centrilobular hemorrhagic necrosis in the neonates of the treated groups compared to the control group. There were degenerative and atrophic changes observed in the neonatal liver of rats that received the high dose (1g/kg) of Aspilia africana extract.

It may be inferred from the present results that higher dose

of Aspilia africana extract given during the period of gestation resulted in degenerative and atrophic changes observed in the liver cell. The actual mechanism by which Aspilia africana induced cellular degeneration observed in this experiment needs further investigation. The necrosis observed is probably due to the high concentration and teratogenic effects of Aspilia africana on the developing liver.

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[21]. 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[22]. In this experiment Aspilia africana extract could have acted as toxins to the developing liver cells. 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[23].The principle holds true for toxicological insults to the brain and other organs[24]. 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 liver 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 of the hepatocytes, dilatations of the central veins, which contained lysed red blood cells, centrilobular hemorrhagic necrosis, atrophic and degenerative changes in the developing liver of Wistar rats. With these results, it is probable that the functions of the liver 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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