TERATOGENIC EFFECTS OF AQUEOUS EXTRACT OF AZADIRACHTA INDICA LEAF ON HISTOLOGY OF THE LIVER OF NEONATAL WISTAR RATS

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ABSTRACT

Aqueous extracts of Azadiracta indica leaf is a common herbal medication used in West African, especially by rural dwellers in treatment of malarial, even in pregnant state. It has been documented to induce liver derangement in adult wistar rats. This therefore necessitate that a finding of its' effect on the neonatal wistar rat be investigated. Seventeen adult female wistar rats obtain from Zoology Department, Ambrose Alli University, Edo state, Nigeria, were used for this study. They were randomly assigned into four groups A, B and C of five rats respectively and these served as the treatment groups, while group D was assigned only two rats and served as the control group. Group D received feed marsh and saline water ad libitum, while group A received 350mg/kg/day of Aqueous extract of Azadirachta indica, group B received 700mg/kg/day of Aqueous extract of Azadirachta indica, and group C received 1100mg/kg/day of aqueous extract of Azadirachta indica. The extract was administered orally with the aid of a cannular inserted into the oral cavity. The animals were allowed feed and water liberally. Drug administration commenced from 10th day of gestation to 13th day after parturition. The neonatal rats were then weighed after which they were sacrificed for tissue processing. Histopathology results revealed that aqueous extract of Azadirachta indica at a dose of 1100mg/kg/day induced liver derangement which was severe, and evidenced by Enlarged nuclei containing lysed red blood cells, haemolysed blood cells in the vessels, and cirrhosis. But the derangement induced on the neonates of rats administered 700mg/kg/day of aqueous extract of Azadirachta indica was evidenced by enlarged nuclei of hepatocyetes and coarse showing prominent nucleoli. Some hepatocytes were binucleated and denuded, some parts of the liver are covered with fibrosis and there is evident of leucocytes infiltrate mainly lymphocytes and macrophages. The neonates of animals treated with 350mg/kg/day showed a mean body weight of (17.0 ± 0.2), and the mean weight of the liver was (6.81g) which when compared to the control was statistically not significant. The neonates of animals treated with 700mg/kg/day of aqueous extract of Azadirachta indica leaf showed a mean body weight of (16.0 ± 0.5g), and the mean weight of the liver was (5.88g) which when compared to the control was statistically not significant, while the neonates of animals treated with 1100mg/kg/day of aqueous extract of A.indica leaf showed a mean body weight of (14. ± 0.1g) and mean weight of liver was (2.64g), which when compared to the control was statistically significant ($P < 0.001$). The neonates of animals treated with 350mg/kg/day of aqueous extract of Azadirachta indica leaf revealed a normal histoarchitecture and body weight which when compared to the neonates in the control group was statistically not significant. We suggest that these findings reflect teratogenic impairment in the histoarchitecture of the liver of neonatal rats. Conclusively, the use of aqueous extract of Azadirachta indica in treatment of diseases including malarial during pregnancy may be the aftermath of liver dysfunction in neonates after birth. Therefore the use of aqueous extract of Azadirachta indica leaf during pregnancy should be avoided except at a considerable low dose (≤ 350mg/kg/day) and should be done under the supervision of a physician.

INTRODUCTION

In gynaecology several drugs are often used in pregnancy usually out of necessity despite their reported toxicities and negative side effects[1]. The case of Thalidomide was a big disaster in the 1960s[2]. However in recent times drug administration is done with utmost care[3]. The clinical conditions necessitating the use of drugs during pregnancy include hypertension, thromboembolism, hyperthyroidism, epilepsy, diabetes

mellitus, preterm labour, arthristis, pain, fever and malarial especially in Tropical African Countries where the environs provides breeding ground for the parasite to thrive well. The first written account of the use of herbs originated in China, while the first indication that Azadirachta indica was used as a medicinal plant was about 4500 years ago, and the tree can live for upto 200 years[4]. It has been documented that any substance taken into the body especially those that constitute chemical compounds such as Herbal plants should be regarded as drugs because they are capable of altering the normal biological system in the body.[5]

Several authors have documented that Azadirachta indica constitute several active compounds. Although a large number of compounds have been isolated from various parts of neem, a few of them have been studied for biological activity. Antipyretic activity has also been reported and confirmed in nimbidin[6]. Oral administration of nimbidin demonstrated significant hypoglycaemic effect in fasting rabbits[7]. Significant antiulcer effect was observed with nimbidin in preventing acetylsalicylic acid, indomethacin, stress or serotonin-induced gastric lesions as well as histamine or cysteamine-induced duodenal ulcers.[8,9]. Nimbidin can also suppress basal as well as histamine and carbachol-stimulated gastric acid output and may act as an antihistamine by blocking H2 receptors, thereby helping as an antiulcer agent[10]. The spermicidal activity of nimbidin and nimbin was reported in rats and human as early as 1959.[11,12]. Nimbidin also demonstrated antifungal activity by inhibiting the growth of Tinea rubrum13. In vitro, it can completely inhibit the growth of Mycobacterium tuberculosis and was also found to be bactericidal[14]. Diuretic activity was also reported for sodium nimbidinate in dogs[15]. Nimbolide has been shown to exert antimalarial activity by inhibiting the growth of Plasmodium falciparum16, 17. Sulphur-containing compounds such as cyclic trisulphide and tetrasulphide isolated from the steam distillate of fresh, matured neem leaves have antifungal activity against Trichophyton mentagrophytes[18]. Several polysaccharides from Azadirachta indica exhibit various biological effects. Neem oil proved spermicidal against rhesus monkey and human spermatozoa in vitro[19]. In vivo studies showed that intravaginal application of neem oil prior to coitus can prevent pregnancy[19]. Antifertility effect of neem oil has also been studied and suggested to be a novel method of contraception[20,21]. Antifertility effect in mice was also reported[22]. Purified Neem seed extract (Praneem) has also been demonstrated to abrogate pregnancy in both baboons and bonnet monkeys, when administered orally[23]. From the hexane extract of Neem seed, an active fraction containing six components has been found to completely abrogate pregnancy in rodents when given orally up to a concentration of 10%, with no apparent side effect[24] and it was concluded that the effect is possibly due to activation of cell-mediated immune reaction. The mechanism of action of Neem oil appears to be non-hormonal, probably mediated through its spermicidal effect and may have fewer side effects than steroidal contraceptives[24]. Aqueous extract of Azadirachta indica leaves was also reported to significantly decrease blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia[25]. The aqueous leaf extract when orally fed, also produces hypoglycaemia in normal rats and decreased blood glucose levels in experimentally-induced diabetes in rats[26]. Aqueous leaf extract also reduces hyperglycaemia in streptozotocin diabetes and the effect is possibly due to presence of a flavonoid, quercetin[27]. A significant hypoglycaemic effect was also observed by feeding neem oil to fasting rabbits[28]. Recently, hypoglycaemic effect was observed with leaf extract and seed oil, in normal as well as alloxan-induced diabetic rabbits[28]. The possible mechanisms underlying the hypoglycaemic activity of the aqueous leaf extract have also been discussed[29]. Though the precise mechanism of the action of Azadirachta indica leaf has not been reported.

This present study is aimed at investigating the teratogenic effect of aqueous extract of Azadirachta indica on the histology of the neonatal liver and the effect on the liver weight of adult wistar rats.

MATERIALS AND METHODS

Animals

Seventeen female adult wistar rats weighing between 140g – 170g were randomly divided into four groups (A, B, C & D) of five rats each. Animals in group D received distilled water orally and served as control. The animals treated with aqueous extract of Azadirachta indica (group A) received doses of 350mg/kg/day, (group B) received doses of 700mg/kg/day, and (group D) received doses of 1100mg/kg/day respectively by gavage. The animals were allowed feed and water liberally. The treatment commenced from 10th day of gestation to 13th day after parturition.

Experimental Procedure

The reproductive status and oestrous period of the animals were determined by obtaining their virginal smears. After two complete regular cycles, timed mating of the female animals was done on the night of the pro-oestrous (N) phase of the cycle. In the morning

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following mating, vaginal smears were taken again. The presence of spermatozoa and squamous cells in the smear confirmed mating and fertilization of ovulated spermatozoa. The sperm positive morning was thus designated day 0 of pregnancy. Each rat was weighed at an interval of three days, before the experiment, up to thirteen days after the parturition. On the 13th day after parturition, the body weights of the neonatal rats were measured. The neonatal rats in the three experimental and control groups were sacrificed by cervical dislocation in order to obtain their liver for weight measurement and histological processing. The liver was then taken for tissue processing and after which the Photomicrographs were then produced. The weight of each treated group and the control was then tested for a statistical significant difference by using the students T test to compare two variants (Group A vs. group D; Group B vs. group D; Group C vs. group D)

RESULTS

The neonates of the control group of animals (Group D) that was given Saline water showed normal liver architecture that was evidenced by normal Hepatic cells (Fig. 4), and the litre size for this group of animals (group D) was Six. The neonates of the maternal rats that were treated with 350mg/kg/day of aqueous extract of Azadirachta indica (group A) revealed normal liver architecture that was evidenced by normal Hepatic cells (Plate 1), and the litre size for this group of animals (group A) was six. The neonates of the maternal rats that were treated with 700mg/kg/day of Azadirachta. indica (group B) revealed a deranged liver architecturethat was evidenced by haemorrahage, pyknotic nuclei of cell, centolobular infiltration, paranchymal degeneration and focal necrosis (Fig. 2), and the litre size for this group of animals (group B) was six. The neonates of the maternal rats that were treated with 1100mg/kg/day of Azadirachta. indica (group C) revealed a deranged liver architecture that was evidenced by Parenchyma degeneration, haemorrhage, pyknotic nuclei of cells and chronic venous congestion (Plate 3). The liver of the neonates of maternal rats in the control group (D) given saline water and feed mash had a mean weight of 6.63g and the litre size for this group of animals (group D) was five. The liver of the neonates of maternal rats treated with 350mg/kg/day (group A) had a mean weight of 6.81gm (Table 1) when compared to the weight of the neonates in the control group (Table 1) the difference in weight was not statistically significant. The liver of the neonates of maternal rats treated with 700mg/kg/day (group B) had a mean liver weight of 5.88gm (Table 1) when compared to the weight of the neonates in the control group (Table 1), the difference in weight was not statistically significant. The liver of the neonates of maternal rats treated with 1100mg/kg/day (group C) had a mean weight of 2.64g (table 1) when compared to the mean liver weight of the neonates in the control group, the difference in weight was statistically significant ($P < 0.001$). The mean Body weight of the neonates treated with 350mg/kg/day (group A), and 750mg/kg/day (group B) was 17.0 ± 0.2g and 16 ± 0.5g respectively. When these weight was respectively compared to the body weight of the neonatal control rats (17.1 ± 1.1g), it was statistically not significant. The mean body weight of the neonatal rats treated with 1100mg/kg/day (group C) was 14 ± 0.05g, when compared to the weight of the neonatal control animals it was statistically significant ($P < 0.001$). This is shown in Table 1.

Table 1: Effect of aqueous extract of Azadirachta indica leaf on the mean weight of the neonatal liver and mean body weight of the neonatal rats

Animal Group	Dose Administered	Mean Weight of Neonatal liver (gram)	Mean Body Weight of The Neonates (gram)
A	350mg/day of Neem Extract	6.81	17.0 ± 0.2
B	700mg/day of Neem Extract	5.88	16 ± 0.5
C	1100mg/day of Neem Extract	**2.64	**14.10 ± 0.1
D	Feed Mash + H_2O (Control)	6.63	17.1 ± 1.1

*Significantly different from value of control (**P < 0.001; all weights in gram)

Mean ± S.D

LEGEND TO FIGURES

1. Cross-section of neonates of maternal rats treated with 350mg/kg/day shows a normal liver architecture evidenced by normal Hepatic cells (Fig. A.). Stains: Haematoxylin & Eosin. Mag. X400.

2. Cross-section of neonates of maternal rats treated with 700mg/kg/day shows a deranged liver architecture; enlarged Nuclei of hepatocyetes and coarse showing prominent nucleoli. Some hepatocytes are binucleated and denuded (naked nuclei). There is complete lose of liver architecture due to disorientation and loss of hepatocytes. Some parts of the liver are covered with fibrosis and there is evident of leucocytes infiltrate mainly lymphocytes and macrophages. The liver therefore is cirrhotic (liver cirrhosis) Fig.2. Stains: Haematoxylin and Eosin. Mag. X400

3. Cross-section of neonates of maternal rats treated with 1100mg/kg/day shows a deranged liver architecture; enlarged nuclei containing lysed red blood cells, haemolysed blood cells in the vessels, and cirrhosis. Stains: Haematoxylin & Eosin. X400.

4. Control sections of the Liver given only saline water orally and feed mash. Shows a normal liver architecture with well defined Hepatic cells. Stains: Haematoxylin & Eosin. Mag. X400.

FIGURES

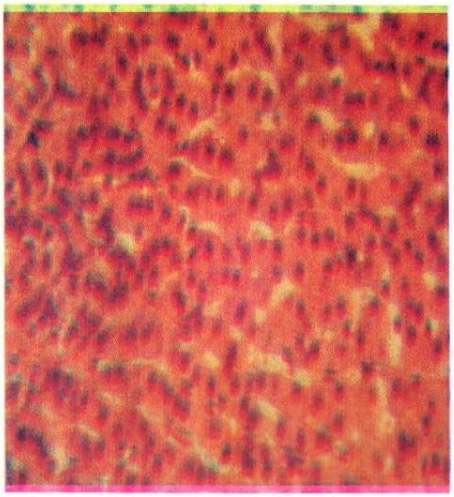

Figure 1: Cross-section of liver treated with 350mg/kg/day of aqueous extract of Azadirachta .indica. Stains. Heamatoxylin & Eosin. Mag. X400

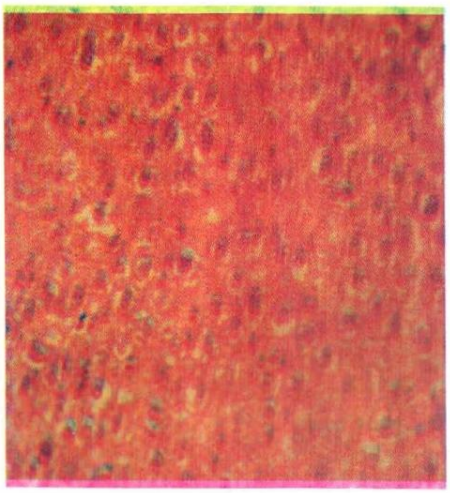

Figure 2: Cross-section of the liver treated with 700mg/kg/day with aqueous extract of Azadirachta indica. Stains: Haematoxylin & Eosin. Mag. X400

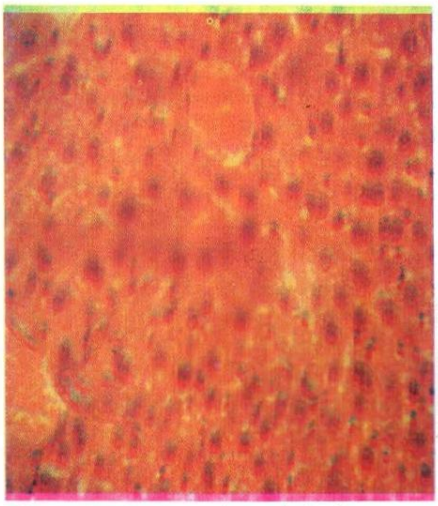

Figure 3: Cross-section of the liver treated with 1100mg/kg/day of aqueous extract of Azadirachta indica. Stains: Haematoxylin & Eosin. Mag. X400

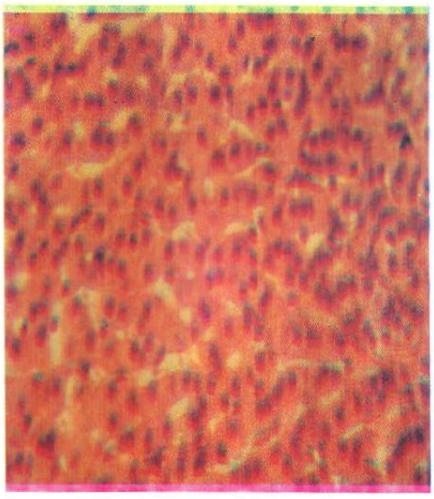

Figure 4: Control sections of the Liver given only saline water and feed marsh. Stains: Haematoxylin and Eosin. Mag. X400.

Discussion

The current investigation and literature review reveals that aqueous extract of Azadirachta indica leaf are commonly use in pregnancy as a curative treatment for malarial most especially by the rural dwellers in tropical Africa countries, despite the fact that the side effect and toxicity is unknown[5].

Photomicrophs of liver sections from the neonates of the control rats showed a normal hepatic cell architecture (Fig.4). The neonates of maternal rats administered 350mg/kg/day of aqueous extract of Azadirachta indica also revealed a normal liver histoachitecture (Fig. 2) when it was compared to the histoachitecture of the control rats (Fig. 4). This is in support with the report that Herbal medications when taken in their natural form and dose have no harmful effect in the body[30]. Secondly; it is also in consistent with the report that aqueous extract of Azadirachta indica is safe at a dose ≤ 350mg/kg/day[31]. The neonates of maternal rats administered 700mg/kg/day of aqueous extract of Azadirachta indica revealed that the Nuclei of hepatocytes are enlarged and coarse showing prominent nucleoli, Some hepatocytes are binucleated and denuded (naked nuclei), there is complete lose of liver architecture due to disorientation and loss of hepatocytes, Some parts of the liver are covered with fibrosis and there is evident of leucocytes infiltrate, mainly lymphocytes and macrophages. The liver therefore is cirrhotic "liver cirrhosis"((Fig. 2)), and the neonates of maternal rats administered 1100mg/kg/day of aqueous extract of Azadirachta indica revealed enlarged nuclei containing lysed red blood cells, haemolysed blood cells in the vessels, and cirrhosis of the liver (Fig.3). Therefore the deranged Liver histoarchitecture observed in figures 2 and 3 is in conformity with the report that aqueous extract of Azadirachta indica at a dose >350mg/kg/day might induce liver derangement in Mice31, and also supports the finding that Herbal medications are drugs because they contain chemical compounds which when ingested can alter the normal biological system in the body[30].

Our literature review revealed that Azadirachta indica leaf contain Biologically active compounds: Sulphur-containing compounds such as cyclic trisulphide and tetrasulphide isolated from the steam distillate of fresh, matured neem leaves and was reported to have antifungal activity against Trichophyton mentagrophytes18. The toxicity effect induced on the neonatal liver is further in conformity with the report that aqueous extract of Azadirachta indica at a dose of 1100mg/kg/day induced severe derangement of the liver of adult female mice, which resulted to death of 75% of the rats in that particular experimental group of adult female mice[31]. This result further confirms the report on the toxic effect of Azadirachta indica seed extract in pregnancy that purified Azadirachta indica seed extract (Praneam) has also been demonstrated to abrogate Pregnancy in both Baboons' and Bonnet monkeys, when administered orally[23]. From the hexane extract of Azadirachta indica seed, an active fraction containing six components has been found to completely abrogate pregnancy[23]. The mean weight of the neonatal liver and the mean Body weight of the neonate administered with 350mg/kg/day and 700mg/kg/day was statistically not significant when compared to that of the neonates of the control animals (Table 1). But the neonates of the maternal rats treated with 1100mg/kg/day of aqueous extract of Azadirachta indica had a body weight of (14.1 ± 0.1g) and liver weight of (2.64g) which was statistically significant when compared to that of the neonates of the control rats ($P < 0.001$). This also conforms to the report that there is a relation between total organ volume and organ weight expressed in a straight line on a logarithmic scale[33]. This also agrees with the report that Body weight and organ weight is an indicator of the Health status of an individual[30]. This finding also reveals that aqueous extract of Azadirachta indica when administered at a dose of 700mg/kg/day in pregnancy induces liver derangement in neonates but does not induce a significant weight loss in the neonate and when administered to pregnant rats at a dose of 1100mg/kg/day induces both liver derangement and significant lose in body weight in the neonates ($P < 0.001$) Table 1. We suggest that Azadirachta indica is more toxic when administered at a dose of 1100mg/kg/day than at a dose of 700mg/kg/day in pregnancy.

Conclusively, aqueous extract of Azadirachta indica leaf at a dose greater than 350mg/kg/day is capable of inducing teratogenic effects on the liver histology, liver weight and body weight of neonatal rats. Therefore the use of aqueous extract of Azadirachta indica leaf during pregnancy in treatment of malarial and other diseases at a dose >350mg/kg/day should be avoided. It is recommended that further studies be carried out to corroborate these findings.

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