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Histological Evaluation of the Embryotoxic and Neurotoxic Effects of *Mangifera indica* in Prenatally Exposed Wistar Rats.

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ABSTRACT

The different parts of *Mangifera indica* (Mango) tree are commonly used as food and for their medicinal benefits. The current study investigated the effects of its stem bark on very young Wistar rats following prenatal exposure. Twenty female Wistar rats were mated and divided into four groups. 1 ml of the crude aqueous extract of *Mangifera indica* stem bark was administered orally to each animal in the treatment groups for 3 consecutive days of each trimester, such that Group A received the extract on Days 3-5 of the first trimester, Group B received the extract on Days 10-12 of the second trimester, Group C received the extract on Days 17-19 of the third trimester, while Group D served as the Control, and received water throughout the gestational period. Upon delivery, some of the litters were sacrificed by cervical dislocation on postnatal day 1, and the rest on postnatal day 14. The frontal cortex of the litters was excised, fixed in 10% formalin, and processed for light microscopy, using the Haematoxylin and Eosin staining techniques. The study revealed that administration of the crude aqueous extract during the 1st trimester led to foetal loss in all pregnant rats, and various degrees of histological abnormalities in the frontal cortex of litters in the 2nd and 3rd Trimesters.

Keywords: frontal cortex, litters, *Mangifera indica*, prenatal

Mangifera indica (Mango), like other plants and herbs, has been used over the years by man for medicinal purposes. The different parts of the *Mangifera indica* tree such as the leaves, fruits, juice, seeds and stem bark, have proven beneficial in this regard. Various studies have shown that the decoction has analgesic, anti-inflammatory, antimalarial, anti-oxidant, antiseptic and hypoglycaemic properties (Aderibigbe *et al* 1999; Martinez *et al* 2000; Stoilova *et al* 2005; Pardo-Andreu *et al*, 2005; Bhowmik *et al* 2009; Márquez *et al* 2010).

Locally, the fruits and stem bark are sometimes used in pregnancy as they are believed to function as blood builders, in correcting anaemia associated with the pregnancy state, probably due to its high content of iron. Similarly, Traditional Medicine Practitioners claim that the stem bark extract of *Mangifera indica* is used for the treatment of anaemia but it appears there are no scientific works to verify this claim (Ogbe *et al* 2010).

The unripe, fully developed mangoes of pickling varieties contain citric, malic, oxalic, succinic and two unidentified acids. The ripe fruits constitute a rich source of vitamin A; some varieties contain fairly good amounts of vitamin C also. Carotene and xanthophyll are the principal pigments in ripe mango; Mangiferin is present in the leaves, while both tannins and Mangiferin are present in the stem bark of the mango tree (Chattopadhyay *et al* 1986; Ogbe *et al* 2010).

The aqueous extract of Mango stem bark has pharmacological properties, and is a mixture of polyphenols, triterpenoids, steroids, fatty acids, micro-elements and mangiferin. *Mangifera indica* polyphenols have protective effects on human T lymphocytes against activation-induced cell death. Furthermore, alcoholic extract of stem bark containing 2.6% of Mangiferin was found to have in vivo immunostimulatory effects (Chattopadhyay *et al* 1986; Makare *et al* 2001).

The aim of the current study was to determine the effect of prenatal administration of crude aqueous extract of mango (stem) bark on fetal survival, and the histology of the frontal cortex of prenatally exposed young Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Twenty adult female Wistar rats with an average weight of 173.5 ± 1.5 g were used; their weight difference was 6 g. They were grouped into four in different cages, each group containing five rats. The animals were allowed to acclimatise for 7 days under normal atmospheric conditions and in a good hygienic

environment, before commencement of the study. They were fed with pelletised guinea feed (growers mash) procured from Bendel Feeds and Flour Mill®, and clean water liberally.

Determination of Estrus Cycle Phase

Before introduction of the male rats, the female rats were daily examined to identify the Estrus cycle phase of each rat, using the vaginal smear method (Marcondes *et al*, 2002). This was used in knowing the exact day to expose the females to the male rats. The Estrus cycle length in rats is four to five days from the onset of sexual maturity up to the age of twelve months (Marcondes *et al*, 2002).

Mating and Confirmation of Pregnancy

As soon as the ovulation period had been confirmed, the male rats were introduced to the females in the evening, were left together overnight and separated the following morning. Five adult Wistar rats were used in mating the female rats. Pregnancy was thereafter confirmed through vaginal smear, to examine for the presence of spermatozoa in the smear.

Preparation of Crude Aqueous Extract of *Mangifera indica*

Mango stem bark was obtained, weighed (483 g) and pulverised using a wooden mortar and pestle. The pulverised bark weighed 448.3 g. The extraction process was carried out by boiling the weighed pulverised in 2 L of water for 3 hours. It was thereafter allowed to cool down for a day, and then sieved and filtered to remove both large and fine particles respectively. The filtered extract measured 750 ml, and was stored in a brown-coloured bottle, to prevent any possible reaction to sunlight.

Administration of Crude Aqueous Extract of *Mangifera indica*

The animals were divided into 4 groups as shown below, and administration of the extract was based on the average weight of 173.5 ± 1.5 g (weight difference at the commencement of administration was 6 g).

Group A: received 1 ml of mango bark extract on Gestational Days 3, 4 and 5;
Group B: received 1 ml of mango bark extract on Gestational Days 10, 11 and 12;
Group C: received 1 ml of mango bark extract on Gestational Days 17, 18 and 19; and,
Group D: Control, given water throughout gestation.

Animal Sacrifice

The animals were weighed every three days through the gestational period till the day of delivery, using a weighing scale. After the female rats littered, the pups from each group were sacrificed by cervical dislocation on days 1 and 14, and the frontal cortex excised and fixed in 10% formalin for histological tissue processing, using the Haematoxylin and Eosin techniques.

RESULTS

Physical Observation

The weight changes during the gestational period were reported in Table 1 and Figure 2. Weight increase in the adult female rats during pregnancy was progressive in all the groups, consistent with their gestational status; however, Group A had a significantly low weight difference (Table 1; Figure 1). Litters in Group C whose mothers received the crude extract during the 3rd Trimester had a higher birth weight, compared with the Control, while the birth weight of litters in Group B was lower than those of the Control Group. All pregnant rats in Group A lost their foetuses, hence, there were no litters to examine (Table 2).

Table 1: Weight Difference of Adult Female Rats and Total Litters delivered

Groups	Initial Weight (g)	Final Weight (g)	Difference in Weight (g)	Total Litters (g)
A	175±4	186±8	11	Nil
B	175±26	228±36	53	18
C	169±23	227±24	58	14
D	175±28	229±24	54	14

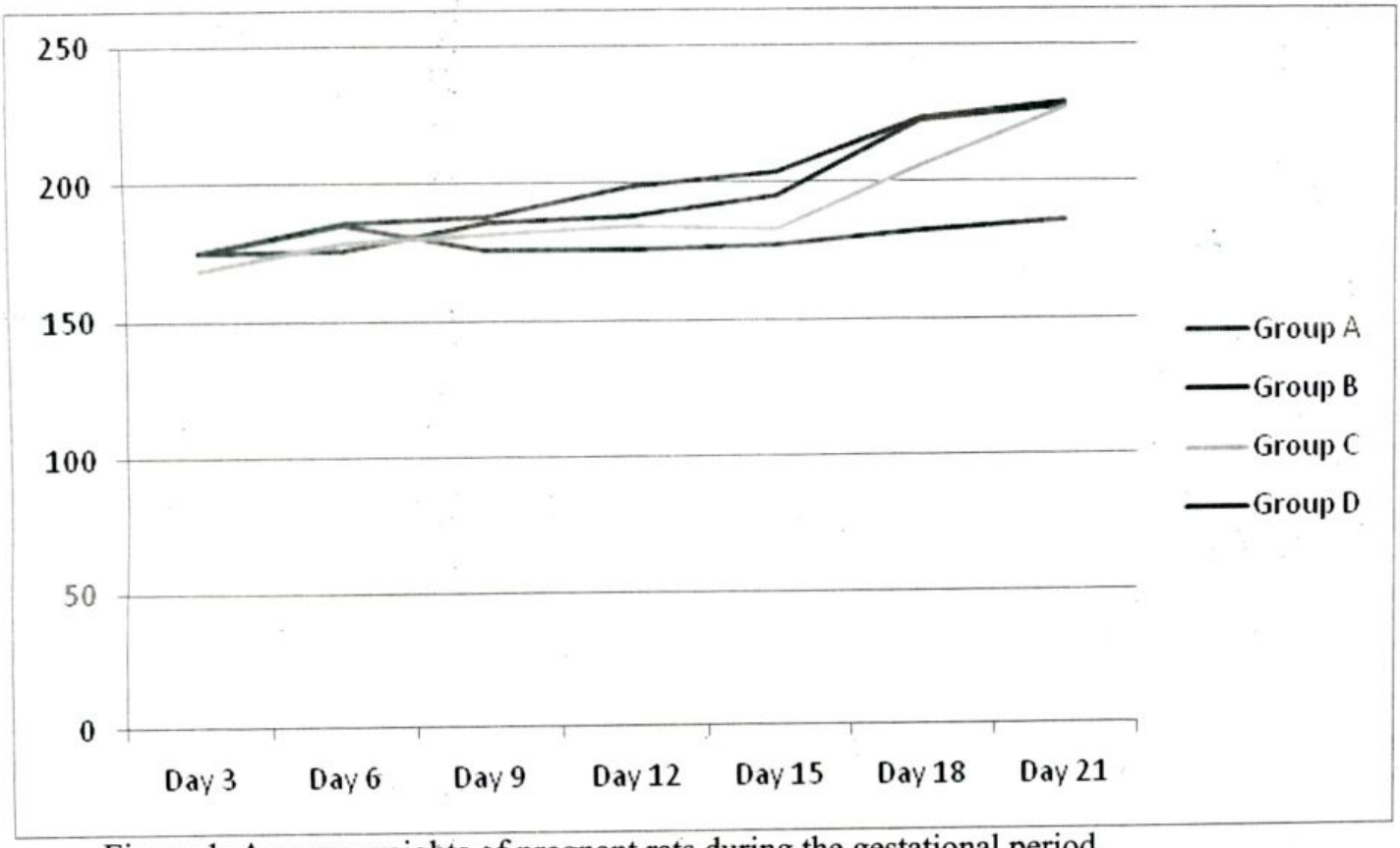

Figure 1: Average weights of pregnant rats during the gestational period.

Table 2: Average Weights of Litters and Cerebral Cortex (weight in grams)*

Groups	Day 1 Litters	Day 1 Cerebral Cortex	Day 14 Litters	Day 14 Cerebral Cortex
A	-----	----	----	----
B*	5.45	0.19	19.40	1.05
C*	7.05	0.23	26.75	1.28
D*	6.14	0.22	20.95	1.16

Histological Observation

The structure of the frontal cortex on the 1st day of life showed poorly stained cells with large neurons and vacuolations in litters of both Group B and C exposed to the Mango decoction during the 2nd and 3rd Trimester respectively, while the control group showed normal microarchitecture with the presence of small pyramidal and stellate cells, cell processes, and no vacuolar spaces (Figures 2-4).

The microarchitecture of the frontal cortex of litters sacrificed on Day 14 showed some neuronal cells appearing smaller in size in Group B compared to the Control Group D; also present were vacuolations, large perinuclear spaces, scattered neurons, distorted cell architecture, and lightly stained cell processes, when compared with the Control (Figures 5, 7). Moreover, in comparison with the Group B litters whose mother rats received the crude extract during the 2nd Trimester, litters in Group C had reduced presence of vacuolations, and the neurons were lightly stained (Figures 6,

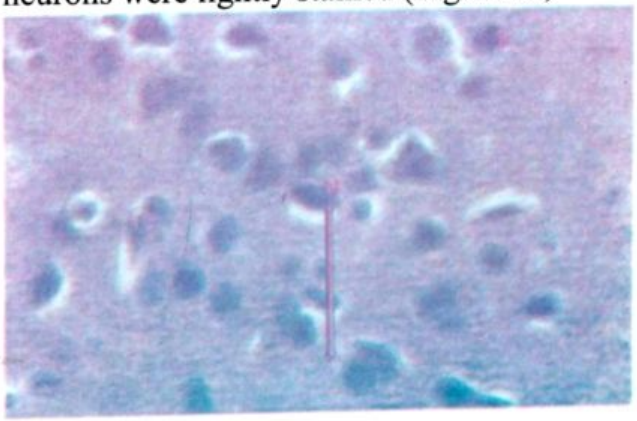

Figure 2: Photomicrograph of the frontal cortex of a one day old litter (Group B) following prenatal exposure to 1 ml of crude aqueous extract of *Mangifera indica* in the 2nd Trimester, showing large-sized neurons (N) with poorly defined architecture, and many vacuolar spaces. H&E x480.

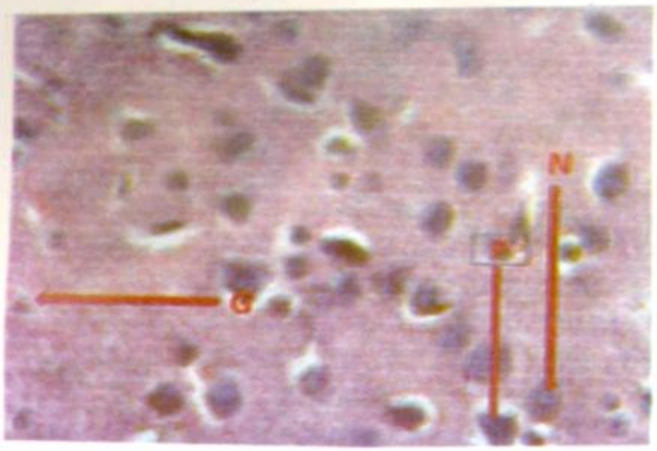

Figure 3: Photomicrograph of the frontal cortex of a one day old litter (Group C) following prenatal exposure to 1 ml of crude aqueous extract of *Mangifera indica* in the 3rd Trimester, showing neurons (N), pyramidal cells (P), glial cells (G) and vacuolations. Neuronal cells appear smaller in size compared with those of Group B. H&E x480.

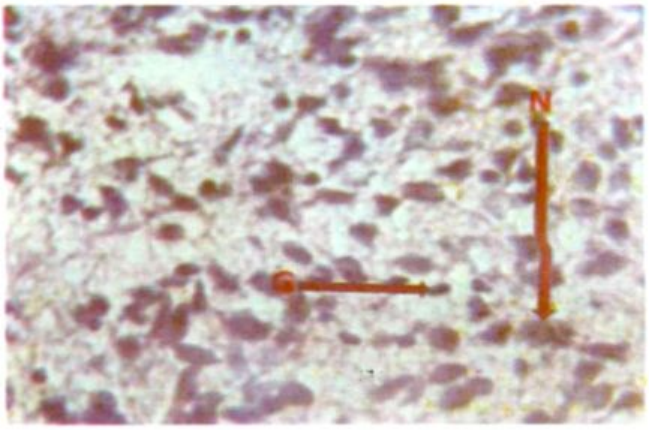

Figure 4: Photomicrograph of the frontal cortex of a one day old litter in the Control Group (Group D) showing apparently regular and ordered cellular arrangement, glial cells (G) and neurons (N). The processes of neurons could be seen arising from the neurons. Vacuolations were absent, compared to Groups B and C. H&E x480.

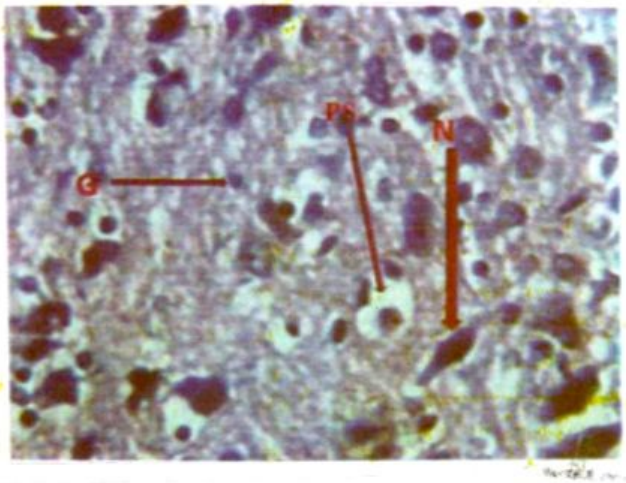

Figure 5: Photomicrograph of the frontal cortex of a 14 day old litter (Group B) following prenatal exposure to 1 ml of crude aqueous extract of *Mangifera indica* in the 2nd Trimester, showing numerous perinuclear spaces (PS) and vacuolations. H&E x480.

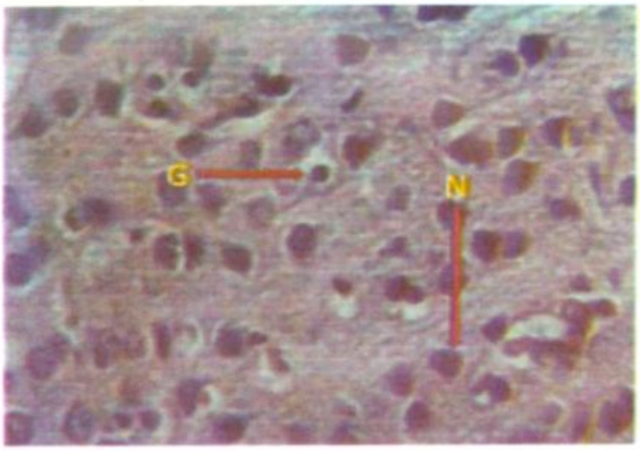

Figure 6: Photomicrograph of the frontal cortex of a 14 day old litter (Group C) following prenatal exposure to 1 ml of crude aqueous extract of *Mangifera indica* in the 3rd Trimester, showing large-sized neurons (N) with many vacuolations. H&E x480.

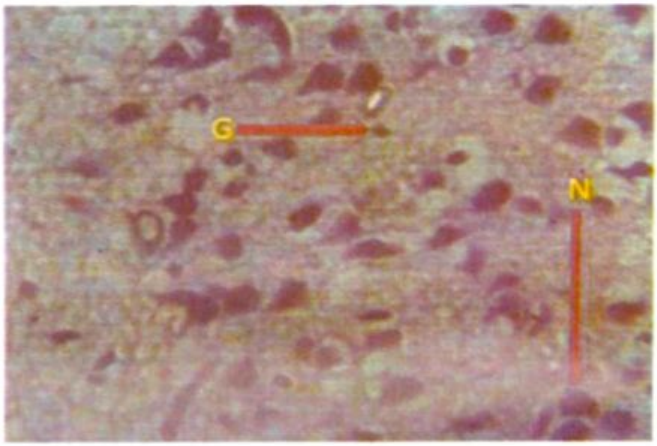

Figure 7: Photomicrograph of the frontal cortex of a 14 day old litter in the Control Group (Group D) showing apparently normal cytoarchitecture, glial cells (G) and neurons (N). H&E x480.

DISCUSSION

A good number of herbs and drugs consumed by pregnant women have chemicals known as teratogens, which are capable of inducing various forms of congenital anomalies. Although some herbal preparations may have some therapeutic benefits, due to unavailability of appropriate dosage regimen, they are often taken locally in excess quantities, leading to apparent adverse effects which could be sometimes fatal.

The type and degree of defects caused by teratogens depend on the stage of embryonic development of body structures at the time of exposure. Teratogens are more likely to induce defects during the most critical period of development, which in Wistar rats is between the 3rd to 14th day of gestation. This is the period of organogenesis.

Teratogens cross the placental barrier, thereby disrupting the developmental patterns of the embryo directly by altering the physiology of the pregnant animal, such that this alteration consequently leads to developmental defects in the conceptus (Moore and Persaud, 2006).

The findings from this study showed that the dose of the crude extract given during the gestational period was detrimental to the developing foetuses, ranging from mild, to moderate, to severe adverse effects. Animals given the crude aqueous extract during the 3rd Trimester appeared to be only mildly affected. The growth rate of these animals was greater than that of the Control animals as shown in the weight difference, and the litters of this same group had higher birth weights than the Control, which was maintained up till the second week of life. Furthermore, at the microscopic level, the degree of distortion to the normal frontal cortical histology was mild, compared with that of the litters whose mothers received the crude aqueous extract during the 2nd Trimester. The other extreme of the adverse effect of the crude aqueous extract was seen in animals that received the decoction during the 1st Trimester, beginning from the 3rd day of gestation; all the foetuses were lost. Although the exact mechanism is not known, it is evident the crude aqueous extract of *Mangifera indica* (Mango Stem bark) is embryotoxic and abortifacent, when given in the 1st Trimester to Wistar rats. This period is very critical for organogenesis in rats, and since this process lasts till the end of the 2nd Trimester, that could be the reason why the effects seen in the litters of mothers that received the crude extract in the 3rd Trimester were not as marked as in those that received it during the 2nd Trimester (which was still within the organogenetic period).

Pregnancy was confirmed in the adult female rats prior to the commencement of administration of the extract, and their weights monitored. Observation of animals in this category showed very low growth rate compared with other groups where pregnancy was positive. Weight increase over the period of gestation was 11 g only, whereas the weight increase in the other Groups B, C and D, was more than 50 g. In rats, organogenesis begins on the 3rd day of development, and this marked the day of commencement of administration of the crude extract. The daily increase in weights of adult rats in Group A was comparable to those of the other Groups, up till about the 6th day of gestation which marked the peak for Group A followed by a drastic drop in weight to near the pre-administration values; and this level was maintained almost throughout the 2nd trimester. This signified that, having previously confirmed a positive pregnancy status in each animals in this Group, it is most likely the crude aqueous extract of *Mangifera indica* was responsible for the foetal loss recorded in this group of animals during the 1st Trimester.

In addition, this study also observed an increase in the growth rate of brain tissue in relation to body weight. This, however, did not translate to increased rate of brain or neural development.

CONCLUSION

Mangifera indica affects the process of organogenesis in rats, causing fetal loss in the 1st Trimester and various degrees of neurotoxicity in other Trimesters.

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