ORIGINAL ARTICLE

Retarded hippocampal development following prenatal exposure to ethanolic leaves extract of *Datura metel* in wistar rats

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ABSTRACT

Background: *Datura metel* contains atropine alkaloids and has been used to treat complication like asthma and, bronchitis, because of its anticholinergic properties. **Aim:** This study aimed to determine the prenatal effects of ethanolic extract of *D. metel* leaves exposure on the development of hippocampus. **Materials and Methods:** Twenty rats (12 females and 8 males) were purchased. The females were grouped into four groups (A_D). Group A were given 500 mg/kg body weight of the extract on the first day of fertilization to the end of gestation period, Group B were given 500 mg/kg body weight on the 8th day of fertilization to the end of gestation period, Group C were given 500 mg/kg body weight on 15th day of fertilization to the end of gestation period and Group D were given normal saline throughout the gestation period. **Results:** Rats in Group A showed no implantation, rats in Group B had abortion on the 7th day after administration, and rats in Group C gave birth with their litters showing retarded hippocampus development and neural degeneration and rats in Group D (control) showed normal development. **Conclusion:** Ethanolic extract of *D. metel* leaf is teratogenic in the late stage of pregnancy, is abortificient and can serve as a contraceptive.

Key words: *Datura metel,* fertilization, hippocampus, parturition, prenatal

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INTRODUCTION

A good number of drugs and chemicals consumed by pregnant women today have been found to be teratogenic (i.e. having the ability to induce congenital anomalies).[1] Drugs such as thalidomide and phenytoin (to mention few) have been shown to have this ability, among other chemical agents. The mechanisms surrounding these congenital anomalies are enclosed in a study referred to as teratology. This study has received widespread attention from experimental embryologists in recent years. The type and degree of defects produced by these agents depends on the dosage of the agents ingested and the stage of embryonic development of body structures at the time of exposure. Such teratogens mainly induce defects during the most critical embryonic period of development (period of organogenesis) that is between the 3rd and 8th weeks of development in man and 6th to 14th days in Wistar rats.[2] These teratogens can induce defects by crossing the placenta to disrupt the developmental patterns of the embryo directly or by altering the physiology of the pregnant animal such that this alteration consequently leads to developmental defects in the conceptus.[2]

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Plants have been the source of medicines for thousands of years,[3] *Datura metel* is a medicinal plant whose use dates back as far as 3000 years.[4] Today, it is mainly used in traditional Chinese medicine as a treatment for asthma, chronic bronchitis, chronic pain, seizures, and coma.[4] *D. metel* has also been used for its anaesthetic, or pain-killing, properties.[5] It is known as an anticholinergic, that is, it reduces spasms by blocking the transmission of nerve impulses. In addition, *D. metel* is a well-known plant with delirium properties;is capable of causing hallucinations. In Vietnam *D. metel* is added to asthmatic cigarettes.[4] Great care must be taken when using this herb, as the toxic dose is very close to the medicinal dose. The wrong dosage can induce hallucinations, severe intoxication, and even death.

MATERIALS AND METHODS

Twenty adult Wistar rats comprising of 12 females and 8 males were used for this study.

All experimental investigations were done in compliance with humane animal care standard outlined in the 'Guide to the care and use of animals in research and teaching', as approved by the Institute of Laboratory Animal Resource. National Resource Council. DHHS, Pub. No. NIH 86-23.[6]

Fresh *D. metel* leaves were collected in Ilorin metropolis. It was identified by the local people and was authenticated at the Plant Biology Department, University of Ilorin. The leaf was plucked from the stem, and weighed (weight was 590 g). The leaf was pounded in a mortar and pestle to form mesh. The mesh was dissolved in 5900 ml of 75% alcohol. The solution was left for 24 h and then sieve; the filtrate was then evaporated to dryness in an oven at 60°C. The paste was stored in closed bottle and kept inside a refrigerator at –4°C.

The pregnant rats were administered ethanolic extract of *D. metel* with the aid of orogastric tube

The female rats were divided into four groups (A_D) with each group comprising of 3 females. Vaginal smear was done every day to determine the proestrous stage of the rats using the method of Marcondes *et al.,* 2002. Once in proestrous, the female rats are introduced to male for mating for 24 hours. The next day, vaginal smear was done to confirm mating; this was evidenced by the presence of sperm cells in the vaginal smear in the form of a tiny threadlike structure under microscope using 10x magnification.

Group A: Treated group with 500 mg/kg body weight of *D. metel* extract from the first day of fertilization to parturition.

Group B: Treated group with 500 mg/kg body weight of *D. metel* extract from the 8th day of fertilization to parturition.

Group C: Treated group with 500 mg/kg body weight of *D. metel* extract from the 15th day of fertilization to parturition.

Group D: Control group were given 1ml of normal saline throughout the gestation period

The weights of the pregnant rats were taken on the first day of fertilization, 8th day after fertilization, 15th day after fertilization and the day of parturition.

The gestation period in female rats is normally about 21-23 days, although these can vary.[7] The rats were allowed to give birth; the mothers and litters were transferred to the weaning cage. After 21 days of gestation period, the pregnant rats in the control group (Group D) littered 6 pups averagely; those treated 14 days after fertilization (Group C) littered 6 pups averagely; those treated 7 days after fertilization (Group B) had abortion (evidenced by vaginal bleeding on gestational day 14 and 15). Those treated from the first day of fertilization (Group A) showed no signs of pregnancy. Administration of extract was stopped for Group C after delivery, for Group B after noticing the abortion and for Group A after 25 days. Two litters in each group were sacrificed on the day of parturition (day 0) and 14 days after parturition. The litters were sacrificed by cervical dislocation. After sacrifice the brain was harvested and fixed in 10% formocalcium as promptly as possible; this procedure was subsequently carried out on other days of sacrifice.

The brains are fixed in 10% formocalcium, hippocampus was excised and processed for haematoxylin and eosin (H & E) staining.[8,9] The tissues were embedded in paraffin and processed for routine histological studies. Slices of 5 µm size were sectioned with the Letiz rotary microtome. The sections were mounted and examined with the light microscope and the photomicrography of each slide was recorded.

Results

Body weight [Figure 1]

From the first day of fertilization all rats in both the treated and the control group showed the same weight.

On day 7 after fertilization, Groups B and C showed the highest increase in weight, followed by Groups D and A being the lowest.

On day 14 after fertilization, Group C showed the highest increase in the level of weight changes followed by Groups D, B and A being the lowest.

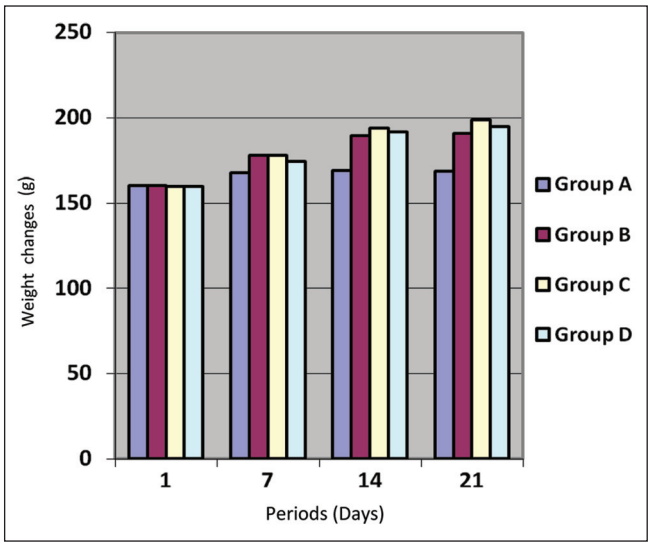

Figure 1: Graphical representation of the weight changes in the Pregnant Rats during gestation period

On day 21 after fertilization, Group C showed the highest level of weight changes, followed by Groups D, B and A being the lowest.

Litters number [Table 1]

Group A did not give birth after 25 days

Group B had abortion after 7 days after administration of ethanolic extract of *D. metel* leaf.

Rats in Group C gave birth to 6 litters averagely.

Rats in Group D gave birth to 6 litters averagely.

Litters weight

Table 2 shows that litters of rats in Group C weighed more than that in Group D

Hippocampal Neurohistology

From the photomicrographs, the following can be observed:

At day 0 [Figure 2], the hippocampus is not fully developed, but using the third ventricle as a landmark, the lumen of the third ventricle varies with litters of Group C showing large lumen as compared to that of litters of the control group.

At day 14 [Figure 3], the pyramidal cell layer of litters in Group C appears dense as compared to that in the control group which shows normal cell density.

The granular cell layer of the litters in Group C shows more vacuoles as compared to litters of Group D, more astrocytes is also seen in the litters of Group C than that of Group D in the granular cell layer.

DISCUSSION

The hippocampus, like the rest of nervous tissue, is made up of neurones and neuroglia.[10] The neurons are the excitable functional units of the nervous tissue.

In this study, rats in Group A treated with 500 mg/kg body weight from the day of fertilization to parturition did not show any signs of pregnancy; this may be because of the failure of the fertilized eggs to implant, which may be as a result of the toxic effect of the extract administered.

Rats in Group B treated with 500 mg/kg body weight for the last 2 weeks of gestation period (8-21st days of gestation), had abortion after 7 days of administration (day 15 of gestation). This may be due to the effect of the extract. This proves that the extract is harmful at the critical period of development in rats, which is between the 6th and 13th day of gestation.[2]

Rats in group C treated with 500 mg/kg body weight for the last week of gestation period (15-21st day of gestation) gave birth to an average of 6 litters, with no gross and morphological changes when compared to those of the control group.

Rats in group D treated with normal saline throughout the gestation period (control group) gave birth to an average of 6 litters with normal morphological appearance.

Average weight of litters inGroup C is slightly higher than that of the control group at birth and at day 14 which is approximately 0.5 g [Table 2]. This can be due to the effect of the extract administered.

Table 1: Showing the number of litters given birth per group

	Rat 1	Rat 2	Rat 3
Group A	—	—	—
Group B	—	—	—
Group C	9	6	4
Group D	8	3	8

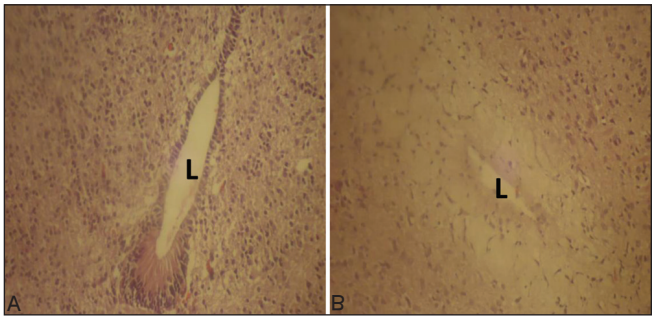

Figure 2: Photomicrograph of rat hippocampus at Day 0 H&E staining ×640. (L – Lumen). A – Litters of rats given 500 mg/kg body weight of *D. metel* during the third week of pregnancy till parturition, B – litters of rats given normal saline throughout gestation period

Table 2: Weight changes (g) in litters (Expressed as mean±SEM)

Days	Group C	Group D
0	5.33±0.03	4.83±0.05
14	14.78±0.54	15.20±0.61

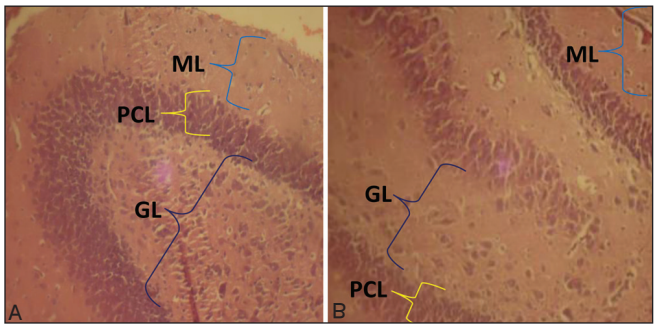

Figure 3: Photomicrograph of rat hippocampus at Day 14 H&E staining ×640. (PCL – Pyramidal Cell Layer, ML – Molecular Layer, GL – Granular Layer). A – Litters of rats given 500 mg/kg body weight of *D. metel* during the third week of pregnancy till parturition, B – litters of rats that were given normal saline throughout gestation period.

Histologically at day 0, the hippocampus of both the litters in Group C and Group D were not yet fully differentiated from the rest of the limbic system, hippocampus forming the peripheral border of the third ventricle.[11] The lumen of the third ventricle was larger in the histological observation of group C as compared to that in group D [Figure 2], which suggests retarded hippocampal development in the foetus of the animal treated with 500 mg/kg body weight of the extract in the last week of gestation. This may be due to the adverse effect of the extract on the neuronal development of the hippocampus. This can be as a result of retarded cell proliferation and differentiation in this part of the brain.

It was observed histologically that, at day 14, the pyramidal cell layer of litters of Group C appear dense as compared to the control group that showed normal density [Figure 3]. This can be due to late development of the pyramidal cells which may be due to the effects of the extract.

The granular cell layer of group C shows more vacuoles compared to the control group and there is presence of more glia in the granular cell layer of group C compared to group D [Figure 3]. This vacuolations and more glia may be due to neural cell death and gliosis in the area, which may be caused by the extract.[8]

CONCLUSION

In conclusion, it can be deduced that the ethanolic extract of *D. metel* leaves can be used as a contraceptive because there is no signs of pregnancy in rats that were given 500 mg/kg body weight from the day of fertilization to parturition. It can also be used as an abortive drug when used in early period of gestation as it caused abortion in rats that were given 500 mg/kg body weight for the last 2 weeks of gestation period (8-21st day of gestation).

It should be avoided in the late period of gestation, as, seen in the histological observation of tissues of litters in Group C, there was retarded hippocampal development, neuronal damage and neural cell death that will affect the normal functioning of the hippocampus.

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