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Research Article

Effects of Prenatal Exposure to Hyoscyamine Fraction of *Daturastramonium* Seeds on Physical Growth Indices and Bodyweight in Wistar Rat Pups (*Rattusnorvegicus*)

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ABSTRACT

The study aimed to evaluate the effects of prenatal exposure to hyoscyamine fraction of *Daturastramonium* (*D. stramonium*) seeds on physical growth indices and body weight in Wistar rat pups. Fresh seeds of *D. stramonium* were procured, identified, macerated and fractionated. Eight (8) Wistar rats weighed 150-250 grams comprised of equal gender were used for the study. The rats were mated and divided into control and treated groups. Equivalent body weight of normal saline and 200 mg/kgbwt of hyoscyamine fraction were orally administered to them respectively on gestational days (GD) 15-20. The litters obtained were observed for gross physical malformation and growth indices (i.e. ear-detachment, hair-growth, eye-opening and teeth-eruption). The animals each group were weighed weekly from neonate to adulthood (PND 8-84). The data obtained were expressed as mean ± SEM. Pearson's chi-square test of association and General Linear Model (GLM) repeated-measures ANOVA followed by Fisher's multiple comparisons post-hoc tests were used to show the mean differences using Minitab 17 (LLC., U.K.) statistical package software. $P<0.05$ was considered statistically significant. There was no association between prenatal exposure to hyoscyamine fraction of *Daturastramonium* seeds and physical growth indices ($p>0.05$). However, a statistically significant decrease in the postnatal weight gain was observed ($p<0.05$) between the groups. In conclusion, prenatal exposure to hyoscyamine fraction does not affect physical growth indices it, however, decreased postnatal body weight gain in Wistar rat pups.

Keywords: Bodyweight; *Daturastramonium*; Fraction; Hyoscyamine; Physical growth

INTRODUCTION

Over the last decade, epidemiological studies have shown that the intrauterine environment does not always sufficiently protect the fetus from environmental factors. Maternal stress, dietary factors, and exposure to environmental chemicals have a significant influence on fetal growth and fetal development with consequences on birth outcome, child development and adult health [1-3]. Evidence is accumulating that early life exposures induce changes in fetal growth patterns. Altered fetal programming leads to non-adaptive postnatal responses that become manifest as excessive weight gain and insulin resistance [4]. A herb is defined as a plant grown for culinary, medicinal, or in some cases spiritual value [5]. The use of herbal medicines (HMs), has increased significantly in the last two decades [6-9]. Abuse of certain plants can produce a wide range of clinical effects such as psychotic, stimulant, sedative, euphoric, and anticholinergic symptoms [5]. Women are the primary consumers of HMs and usually continue to use during pregnancy [10]. Pregnant women usually perceive herbal products as a safe, natural alternative to conventional drugs [11] and often use them to improve their wellbeing or for the treatment of non-life-threatening conditions (e.g., nausea, constipation). Previous research has shown that 5% of pregnant women use 1 or more addictive substances [12,13]. Approximately 1 in 20 infants are exposed to illicit drugs [14]. Marijuana, cocaine, heroin, hallucinogens, and inhalants are examples of illicit drugs [15]. Medicinal plants used in herbal medicines contain xenobiotic agents, i.e., substances foreign to the human body, and as such,

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their biotransformation products can be potentially toxic. In addition to the immediate effects, which are readily correlated with their intake, effects that are established in the long-term may be asymptomatic but can lead to a severe clinical picture, sometimes fatal [16].

Daturastramonium is one of the well-known ethno-traditional herbs with both poisonous and medicinal properties [17]. It has been used for relieving many which include coughing, asthma and controlling pain for a long time [18]. In Nigeria, the juice of the leaves in warm milk is used to expel intestinal worms including cestodes, while the seeds in palm oils are used externally for insect bites and stings [19-20]. In Hausa tribe, *D. stramonium* seeds are commonly abused by the youths in local the beverage (*Zobo*) and in porridge serves to unsuspecting attendees especially during festivities to induce hypnosis. Although caution has been drawn on the use of *D. stramonium* during pregnancy [21], however, pieces of literature have been scanty especially on its effect on physical growth indices and body weight in Wistar rats.

This study aimed to evaluate the effects of prenatal exposure to hyoscyamine fraction of *D. stramonium* seeds on physical growth indices and body weight in Wistar rat pups (*Rattusnorvegicus*). The study may create awareness to the National Food and Drugs Agency and Control as well as National Drug Laws Enforcement Agency to look beyond the prohibition of conventional drugs of abuse to herbs of abuse such as *D. stramonium*.

MATERIALS AND METHODS

Collection of plant materials

Ethical approval was obtained from the Ahmadu Bello University Committee on Animal Use and Care(ABUCAUC/2018/042). Fresh *D. stramonium* seeds were procured from Sharada residential area of Nassarawa Local Government, Kano State, Nigeria. The seeds were identified and a voucher number (VN108) was issued at the herbarium of the Botany Department, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna state, Nigeria. The seeds were separated from the pods, washed thoroughly with clean tap water and air-dried under shade. Two thousand grams of the dried seeds were weighed using a digital Weighing machine, grounded to a pulp using an electronic blender. The powdered sample was collected into a sterile cellophane bag and kept in a cool dry place for extraction.

Ethanol extraction of crude *D. stramonium* seeds

The pulverized seeds powder was extracted with 70% ethanol using a modified procedure of cold maceration method as explained previously [22]. The 200 g of air-dried powdered of the seeds was placed in 1,500 ml of a 4.1 (v/v) mixture of ether and 95% alcohol shook vigorously and allowed to macerate for 72 hours. A 5g of magnesium oxide with 30 ml of water was mixed in an evaporating dish. The extract was added to the mixture and stirred vigorously. The mixture was then placed on water-bath and allowed to evaporate to dryness. A 250 ml portions of distilled water was poured into the dried residue three times and boiled, filtering each portion while still hot. A 5 ml of the portion of 10% sulphuric acid (H_2SO_4) was added to the combined filtrates and allowed to concentrate to the one – third of its original volume. The residue was later fractionated using chloroform.

Fractionation of hyoscyamine

The dried residue was dissolved in three 750 ml portions of boiled water and diluted with a 5 ml portion of 70% ethanol in separating funnel while still hot. A 5 ml portion of 10% sulphuric acid (H_2SO_4) was added to the combined filtrates and allowed to concentrate to the one – third of its original volume. The flocculant of the extract was precipitated while the filtrate was removed by filtration. The filtrate was then basified with 2 ml portion of ammonia hydroxide and partitioned with 5 ml portions of chloroform. The fraction was evaporated in a water-bath. The residue was purified by dissolution in chloroform and diluted hydrochloric acid. The acid was basified with a layer of ammonium hydroxide and hyoscyamine was re-purified with a layer of chloroform before being transferred into Eppendorf's tube. The tube was opened in dry space to allow the chloroform to evaporate, thus leaving crystals of the hyoscyamine in the tube.

The High-Performance Liquid Chromatography (HPLC)

HPLC condition: A reversed-phase Techsphere 50DS C_{18} HPLC column (25 cm × 4.6 mm i.d.) particle size 5 µm, Supelco, Bellefonte, PA, USA) with oven temperature, 40°C in conjunction with UV adsorption detector operating at 270 nm was employed. The mobile phase was a mixture of 20% acetonitrile, and 45% methanol, 35% water (H_2O) and 0.1 mol/L phosphoric acids which adjusted the pH to 7.0 and were used at a flow rate of 1 ml/min. A calibration curve for l-hyoscyamine was generated to determine the amount of the hyoscyamine in the sample fraction. All analyses were carried out at the Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria (Figure 1).

Experimental animals

Eight (8) healthy Wistar rats comprised of equal numbers of adult males and virgin females were procured from the Animal House of the Anatomy Department, Faculty of Basic Medical Sciences, Bayero University Kano. The animals were transported to the Animal House of the Pharmacology Department, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU) Zaria. The males were separated from the female, housed and allowed to acclimatize for two weeks at ambient temperature, with alternate day and night cycles natural condition. Rat chow (Vital feeds®) and tap water were made available to the animal's *ad libitum*. All the procedures carried out were in concert with the approval of Ahmadu Bello University (ABU) Zaria Animal's Right and Ethics

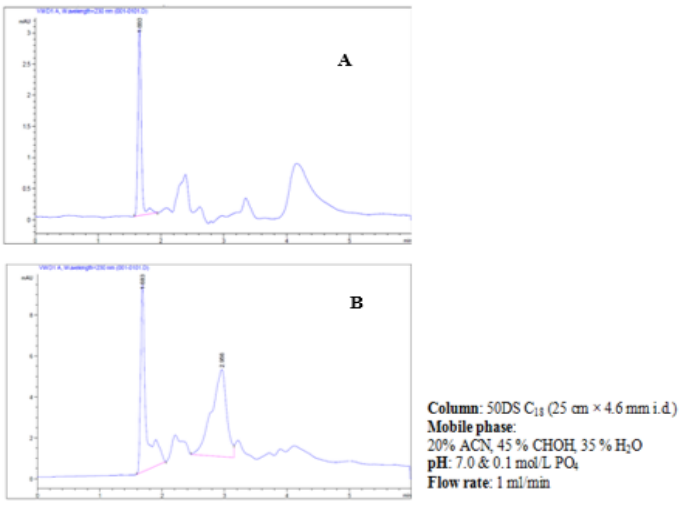

Figure 1: HPLC chromatogram of L – hyoscyamine standard powder (A) and hyoscyamine fraction of *D. stramonium* seeds (B).

Committee for Scientific Research Purposes.

The synchronization and phase determination of the oestrus cycle

The female rats were injected with an equivalent bodyweight of *Zoladex*® (3.6 mg AstraZeneca) intraperitoneally to synchronize their oestrus cycle. Twenty-four after the synchronization, vaginal smears were collected by vaginal lavage [23] using a 1 ml plastic pipette filled with 10 µL of normal saline (NaCl 0.9%). The tip of the pipette was gently but superficially inserted into the rat vagina [24]. The vaginal fluid was carefully aspirated and placed on a cleaned glass slide. A different glass slide was used for each rat, and unstained material was observed under a light microscope, without the use of the condenser lens, at 10 x objective lenses [24]. Round and nucleated ones are epithelial cells; irregular ones without a nucleus are the cornified cells, and the little round ones are the leukocytes were recognized. The proportion among them was used for the determination of the oestrous cycle phases [24].

Experimental design

Acute toxicity study: The acute oral toxicity test was performed following Lorke's method [25]. The study was carried out in two phases using 12 rats. During the first phase, 9 rats were randomly selected into three groups each. Their weights were taken and received 10, 100 and 1000 mg/kgbwt for the 1st, 2nd and 3rd groups respectively. The animals were left to observe for neurotoxicity symptoms. Twenty – four hours (24 hrs) later, the second phase of the study was undertaken using three (3) different sets of animals, as no mortality was recorded in the previous phase. During this phase, doses of 1600, 2900 and 5000 mg/ kgbwt were given to the three rats (one rat per dose) and further observed for neurotoxicity symptoms. Neurotoxicity symptoms were observed, but no mortality was recorded even at the highest dose, therefore considered it the hyoscyamine fraction was considered safe for the study.

Animals mating and grouping

The animals were randomly selected and divided into two (2) groups; control and treatment. Each group contained a total of eight (8) rats in the ratio of 1:1 adult male to virgin female. Animals in each group were allowed to mate freely and evidence of mating was established by the presence of sperm tails in the vaginal smears collected and viewed under a microscope after 24 hrs (Figure 2). Abdominal palpation was carried out to avoid error due to pseudopregnancy. The pregnant dams detected, were isolated and transferred to maternity cages. Animal from the same group was kept closely but separately together in different cages. The control and treated groups received an equivalent bodyweight dose of normal saline and 200 mg/kgbwt of hyoscyamine fraction of *D. stramonium* seeds respectively orally from gestational day (GD) 15-20.

Physical observation and weighing the bodyweight

Each pup was physically observed and recorded daily for the appearance of developmental landmarks i.e. the appearance of pigmentation, bilateral ear detachment, hair growth, eye-opening, and teeth eruption starting from PND 4 – PND 75 [26]. The weights of the rats in each group were weighed and recorded weekly using LB – 1000 Compact bowl digital scale (China) from PND 8 to PND 84.

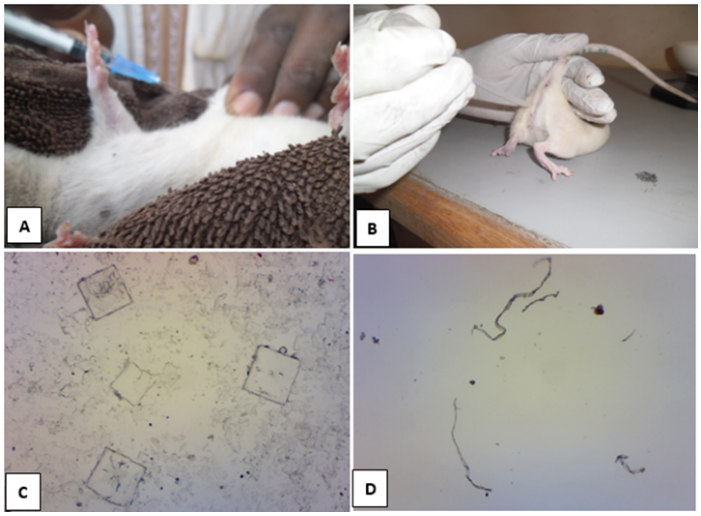

Figure 2: Synchronization and determination of the animals' oestrus cycle.
A: Synchronization of the adult female virgin Wistar rat cycle with Zoladex, **B**: Collection of vaginal smears for cytology, **C**: Proestrus phase in the female virgin Wistar rats, **D**: Remnant of the sperm tails collected early morning after overnight mating with the adult male Wistar rats.

Statistical analyses

Data obtained were expressed as mean ± SEM. Pearson's chi-square test and General Linear Model (GLM) repeated measures ANOVA followed with Fisher's multiple comparisons post-hoc were carried out to find the association and mean the differences between the variables in the groups using *Minitab* 17 (LLC., U.K.) statistical package software. $P<0.05$ were considered statistically significant. All figures and charts were constructed using GraphPad Prism 8.

RESULTS

Acute toxicity study

No mortality was recorded in the first phase. However, muscarinic symptoms such as restlessness (hyperactivity), laboured breathing, piloerection, abdominal cramps, stooling (diarrhoea), and urination, were observed especially at the highest dose. The symptoms later disappeared and the animals became calm, weak and quiet. Similar symptoms were observed with higher intensity in all the treated groups during the second phase. No mortality was also recorded even at the highest dose.

Observation of physical growth indices

Table 1 shows associations between exposure to hyoscyamine fraction of *D. stramonium* seeds and physical growth indices in Wistar rat pups. The table indicated no significant association ($p>0.05$) between the groups. The table shows that bilateral ear detachment was not observed before PND 3 of the neonatal pups, however, from the PND 4 onwards, mild association in the bilateral ear detachment was observed between the groups. Also, before PND 7 the groups did not show elements of hair growth, however, at PND 7 the hairs growth was visibly observed in the control group whereas, the appearance of hair growth was observed at the PND 8 in the treated group. There was a slight difference between the groups in the days of eyes-openings. However, no significant association ($p=0.747$) was observed after birth as a result of the ingestion of the hyoscyamine fraction. This shows that the pups' eye did not open until the early third-week postnatal development,

Table 1: Association Between Exposure to Hyoscyamine Fraction of *D. stramonium* Seeds and Physical Growth Indices in Wistar Rat Pups.

Features	Response	Test	Control	Treatment	*p*-value
Ear-detachment	No	(Observed)	3.00	3.00	1.000
		(Expected)	3.00	3.00	
		Contribution to χ^2	0.00	0.00	
	Yes	Yes (Observed)	18.00	18.00	
		(Expected)	18.00	18.00	
		Contribution to χ^2	0.00	0.00	
		Total	21	21	
Hair-growth	No	(Observed)	7.00	8.00	0.747
		(Expected)	7.50	7.50	
		Contribution to χ^2	0.03	0.03	
	Yes	(Observed)	14.00	13.00	
		(Expected)	13.50	13.50	
		Contribution to χ^2	0.02	0.02	
		Total	21	21	
Eye-opening	No	(Observed)	13	14	0.747
		(Expected)	13.50	13.50	
		Contribution to χ^2	0.02	0.02	
	Yes	(Observed)	8.00	7	
		(Expected)	7.50	7.50	
		Contribution to χ^2	0.03	0.03	
		Total	21	21	
Teeth-eruption	No	(Observed)	18.00	18.00	1.000
		(Expected)	18	18	
		Contribution to χ^2	0.00	0.00	
	Yes	(Observed)	3.00	3.00	
		(Expected)	3.00	3.00	
		Contribution to χ^2	0.00	0.00	
		Total	21	21	

Table 2: Comparisons of Bodyweight Measurements in Wistar rats treated at Gestation.

Week	Dose (mg/kgbwt)	Group	Mean ± SEM	*t* – value	*p*-value
1st	Normal saline	Control (n=5)	12.68 ± 0.30	-0.66	0.513
	200	Treated (n=6)	10.60 ± 0.31		
2nd	Normal saline	Control (n=5)	20.96 ± 0.58	-1.28	0.203
	200	Treated (n=6)	16.90 ± 0.40		
3rd	Normal saline	Control (n=5)	29.86 ± 0.61	-2.21	0.030
	200	Treated (n=6)	22.87 ± 0.66		
4th	Normal saline	Control (n=5)	44.40 ± 1.30	-3.41	0.001
	200	Treated (n=6)	33.58 ± 0.69		
5th	Normal saline	Control (n=5)	59.80 ± 1.40	-4.85	<0.001
	200	Treated (n=6)	44.43 ± 0.77		
6th	Normal saline	Control (n=5)	74.04 ± 1.50	-4.62	<0.001
	200	Treated (n=6)	59.40 ± 1.00		
7th	Normal saline	Control (n=5)	93.28 ± 1.80	-6.19	<0.001
	200	Treated (n=6)	73.65 ± 1.20		
8th	Normal saline	Control (n=5)	95.24 ± 1.80	-5.04	<0.001
	200	Treated (n=6)	79.25 ± 2.20		
9th	Normal saline	Control (n=5)	110.56 ± 2.50	-4.19	<0.001
	200	Treated (n=6)	97.27 ± 2.40		
10th	Normal saline	Control (n=5)	122.12 ± 3.20	-3.10	0.002
	200	Treated (n=6)	112.30 ± 3.70		
11th	Normal saline	Control (n=5)	133.38 ± 3.10	-2.52	0.013
	200	Treated (n=6)	125.40 ± 3.80		
12th	Normal saline	Control (n=5)	140.62 ± 3.50	-2.60	0.011
	200	Treated (n=6)	132.40 ± 4.70		

although the control preceded the treatment group by a day. There was also no significant difference in the teeth-eruption time (p=1.000) between the groups. This implies that the teeth eruption was not observed in both control and treated groups until after the PND 18.

Bodyweight

Comparisons of bodyweights between the control and prenatally exposed hyoscyamine fraction Wistar rat pups from the early postnatal period of 1st-12th week were shown in Table 1. There was a continuous increment in the bodyweight between the groups from the first week to adulthood. The growth pattern plateaued at the seventh week with the mean bodyweight of 93.28 ± 1.80 and 73.65 ± 1.20 grams for the groups respectively. Although the patterns remained the same in both groups, however, a significant difference (p<0.001) was observed between them. The difference was steeply maintained from the third week until the end of the study (PND84). The mean bodyweights were not significantly different in the first two weeks of birth between the groups. However, from the fourth week to twelfth week (adulthood), the bodyweights of the treated groups significantly decreased [$F(1,108)=137.73$, p<0.001] when compared to the control groups. There were no observed physical malformations in the growing and adult animals of either group during the study. This trend was maintained throughout the study.

DISCUSSION

Oral ingestion of hyoscyamine fraction resulted in both central and peripheral muscarinic symptoms in the rats fed with graded doses of the fraction. However, no mortality was recorded during toxicity testing and experiment itself. In a related study by Babalola *et al.* [27] reported that the median toxic dose of *D. stramonium* fed orally in dogs was at the safety margin as considered Centre for Disease Control (CDC) the United State of America, states. However, there was no published literature to make a comparison of the present study in Wistar rats. Considering the foregoing it could be assumed that oral ingestion of *D. stramonium* seeds might equally have high safety margin in Wistar rats. The clinical symptoms observed might probably not to be unconnected with the anticholinergic properties of tropane alkaloids which competes and irreversibly inhibits acetylcholine on muscarinic receptors, thereby causing both central and peripheral nervous system manifestations [28]. The central nervous system features include restlessness (hyperactivity), laboured breathing and delirium, while the peripheral symptoms observed include breathing, piloerection, abdominal cramps, stooling (diarrhoea), and urination. Similar observations were reported in patients involved in *D. stramonium* poisoning [29].

The current study showed no significant changes in the eye-opening, hair-growth, teeth-eruption and bilateral ear-detachment between the control and treated groups. This study corroborated similar finding by [30], which reported that no physical malformation observed in Wistar rats treated prenatally with ethanolic leaves extract of *Daturametel*, however, contradicted the report of [21] which said that foetal exposure to *D. stramonium* used by asthmatic mother could ultimately result in permanent damage to the foetus, that also, subcutaneous injection of scopolamine (a tropane alkaloid) resulted in malformed fetuses was observed [31]. Although it is a known fact that all part of *Datura* is toxic, short duration of exposure and dosage used could probably be considered as the possible cause of the discrepancy. In a separate finding, no adverse effects on prenatal viability or evidence of teratogenesis, and only a marginal reduction in fetal bodyweight was observed in mice treated at doses of 450 and 900 mg/kg body weight per day of tropane alkaloid [32]. Although no direct relationship between first-trimester use of atropine and birth defects was found, a study reported an increase in birth defects in the offspring of mothers who had taken belladonna alkaloids [33]. Also, it was found that no teratogenic effect of *Datura*seeds on the joint development in newborn Hampshire piglets after feeding sows 1.2 to 1.7 mg thorn-apple/kg feed (alkaloid levels not determined) [34]. The discrepancies observed probably result from the environmental factors such as plant species, rainfall, the altitude, soil composition, soil salinity, pH in addition to the phenological stage, extraction and fractionation method used [35-38]. We also conclude that single-dose used and the short prenatal period of exposure to hyoscyamine fraction of *D. stramonium* seeds does not cause physical malformation in development of Wistar rats.

No difference in bodyweights was observed between the groups at the beginning of the first two weeks of postnatal development. However, the trend changed after weaning (3rd week) up to adulthood (12th week) where a significant decrease in the bodyweight of the treated group was observed. In a related study, a significant reduction in the body weight was reported in the Wistar rat pups treated with *D. stramonium* leaves postnatally [21,39-40]. A decrease in bodyweight was also reported in Wistar rats treated with graded doses of aqueous leaf extracts of *Daturametel* [41]. In parallel experiments using broiler chickens that received analogous treatments of *D. feroxseeds* for 90 days, a significant reduction in bodyweight, especially at the higher dose levels [42] was reported. Studies in rats showed lower bodyweights and liver weights with a diet containing 0.5% ground *D. stramonium*seeds [43].

CONCLUSION

Oral ingestion of hyoscyamine fraction of *D. stramonium* seeds has a higher safety margin. This is because prenatal exposure does not cause physical malformations or delay in the appearance of physical growth indices such as ear detachment, hair growth, eye-opening and teeth eruption but a postnatal decrease in bodyweights of Wistar rat pups. Therefore, care should be taken when using hyoscyamine containing compounds during pregnancy.

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