

## **Datura metel is deleterious to the visual cortex of adult wistar rats**

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### **ABSTRACT**

*Datura metel is a commonly abused natural plant throughout the world for its hallucinogenic effects. It is also known to produce other effects such as euphoria. Some of its effects were studied in this work using the biochemical estimation of activities of LDH and G6PDH as well as histological examination of visual cortex. Eight adult Wistar rats constituted a group, each of which was treated with 300mg/kg of aqueous extract of Datura metel and the equivalent volume of phosphate buffered saline daily for twenty-one (21) consecutive days. The levels of LDH and G6PDH in the homogenate of visual cortex are higher in the animals treated with Datura metel relative to those of animals in the control group. There is a statistically significant difference in the mean value of LDH of animals treated with Datura relative to the control group animals ( $p < 0.05$ ) but no statistically significant difference in the mean value of G6PDH. Histological findings showed vacuolations and perinuclear spaces in the sections of visual cortices of the animals administered with 300mg/kg/day of the plant extracts for 21 days. The findings in this study suggested that Datura metel altered carbohydrate metabolism by elevating the levels of LDH and G6PDH in the tissue homogenate and altered the cellular and functional integrity of the visual system. It is concluded that the hallucinogenic plant has deleterious effects on the functional integrity of cells of the visual system of Adult Wistar Rats.*

**Keywords:** Datura metel, Visual Cortex, Wistar rats.

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### **INTRODUCTION**

Hallucination is the subjective perception of an object or event when no such stimulus or situation is present. In a stricter sense, it is defined as a perception in a conscious and awake state in the absence of external Stimuli, which have qualities of real perception located in external objective space. It can occur in any sensory modality: visual, auditory, olfactory, gustatory, tactile, proprioceptive, equilibrioceptive, nociceptive, thermoceptive and chronoceptive [1]. A mild form of it is called disturbance, and can occur in any of the senses. Hallucination may be benevolent (i.e. telling someone good things about himself or malicious (i.e. cursing someone) and occurs in both healthy and sick individuals [2]. The most common modality of hallucination is visual which includes the phenomena of seeing things which are not present. Different causes of visual hallucination have been classified as psychobiochemical, (a disturbance of neurotransmitters), psychophysiological (a disturbance of brain structure) and psychological/psychodynamic (e.g. meaningful experience intruding into consciousness [3].

A lesion of any nature and from any source on the visual system, especially involving the visual cortex will result in visual hallucination. Studies have shown that drugs and plant extracts have association with visual hallucination.

One of the important plants which have been attributed with visual hallucination is *Datura metel* [4]. *Datura metel* belongs to the family Solanaceae, the nightshade, which include some 2,400 species [5]. It is one of the most interesting plants with hallucinogenic property [4] and despite having this reputation as one of the darker hallucinogens, it has widely been used by societies historically in both old world and the new, and continues to be today [6]. Local findings have shown that all the different parts of the plants are, either in the fresh form or in the sun-dried powdered form, used for its psychoactive property in South-Western Nigeria. Literatures have also shown that *Datura metel* is one of the most commonly abused local plants all over the world. Report of Drug Abuse in Nigeria by the United Nations Office [7] on Drugs and Crime in 2007, showed 0.4% use of *Datura metel* out of the various narcotic and psychotropic substances of use in Nigeria.

It was first documented in Sanskrit literature by the Arab Physician Avicenna in the 11th century [4]. It has numerous names, as it is found throughout Africa, Asia, America, Australia, and Europe as either a native or an adventive plant [8]. Some of its common names are raving nightshade, thorn apple, devil's apple, stinkweed, Jimson weed and angels' trumpet [6, 9]. It is popularly called "gegemu" or "ewe ikan" in Yoruba language. Its use has a very wide array because of its hallucinogenic property and it differs from one continent to another. It is also known to have a wide array of uses especially medicinally. The plant was used all over the world from historical times. Folk uses include cure for cancer, local analgesic for burns, sedatives in epilepsy, influenza, cough remedy treatment of Asthma, healing of wounds and treatment of acne. It is used as ritualistic herb and for inebriation purposes because of its hallucinogenic effects.

#### MATERIALS AND METHODS

Fresh leaves of *Datura metel*, plucked from the garden of the department of Botany of the University of Ilorin were sun-dried for 5 days to make 400g of dried *D. metel* leaves. The 400g dried leaves were grinded with local mortar and pestle into powder. 100g of the powder was soaked in 100mls of distilled water for 72 hours and filtered afterwards with Whatman's No 1 filter paper. 800mls of filtrate was obtained and the residue discarded. The filtrate was oven dried at a temperature of 40<sup>o</sup>c for 10days to form a dark green paste of 14 g which was made to dissolve in 70mls of phosphate buffered saline to make a 200mg/ml aqueous solution of *Datura metel*.

Sixteen adult Wistar rats with average weight of 200 g were procured from the animal house of the Department of Zoology, University of Ilorin. Standard rat diet was purchased from Bendel Feeds, Taiwo Road, Ilorin. The animals were reared in the animal holdings of the Faculty of Basic Medical Sciences of University of Ilorin. They were made to acclimatize for two weeks before the commencement of administration of plant extracts. They were fed with standard rat diet which was purchased from Bendel feeds at once to avoid change in diet composition and also given tap water ad-libitum. They were given free access to food and water. They were kept in standard laboratory wooden cages in groups of four with male and female animals kept separately. They were generally cared for under standard laboratory conditions of good lighting, moderate temperature and adequate ventilation, and in a neat environment. They were weighed routinely everyday using digital weighing balance (Saltun® EK5055Max).

The animals were divided into two experimental groups A and B. Each group of animals had four male and four female rats which were kept in separate unit of cages to prevent mating and pregnancy by the female animals. Animals in group A were treated with 300 mg/kg (0.3ml) body weight/day of *Datura metel* for 21 days while group B animals received phosphate buffered saline in equal volume of the dose of the extract (i.e. 0.3 ml) daily for 21 days. Administration of the extract and phosphate buffered saline was done orally with the aid of oro-gastric feeding tube at 07.00 hour each day.

24 hours after the last administration, the animals were sacrificed by cervical dislocation, brain tissues were carefully excised from their skulls and the left occipital cortices were quickly excised for tissue homogenate preparation to quantify the activities of LDH and G-6-PDH histochemically according to the method of Kind and King [10] and King and Jagatheeson [11]. The histochemical activities of these enzymes were read spectrophotometrically.

#### Statistical Analysis

Data were statistically evaluated using the student's t-test with SPSS/14.0 software (SPSS Inc, Chicago, USA) and Excel 2007 (Microsoft Corporation, USA) and were expressed as Mean  $\pm$  Standard error of mean (SEM). A value of  $p < 0.05$  was considered to indicate a significant difference between groups.

## RESULTS

The animals were closely observed throughout the period of the investigation. During acclimatization, all animals appeared presumably normal with smoothly laid hairs and pinkish eyes. They ate well throughout this investigation. From the first day of administration of the plant extract, animals in the treatment group appeared restless for a few hours after administration. They had hairs on their back standing erect with pinkish-red and radiating eyes. They appeared docile and inactive for about 6 hours and abstained from feed and water. On approaching the end of administration, the animals in the treatment groups, especially appeared more docile and less active. The animals in the control group appeared normal when compared with the animals in the treatment groups.

The body weights of the animals in both the treatment and control groups were monitored and documented at 0700 hour of every day. Prior to the administration of the plant extract, all animals in both the experimental and control groups were steadily gaining weight. From the third day of administration, animals in the treatment group began to lose weight. Animals in the control group however continued to have a steady weight gain. Statistical analysis of the weight changes indicated that there is no statistically significant difference in the body weight changes of the animals in the treatment group relative to those in the control group at  $p < 0.05$ . The mean, standard error of mean (SEM) and the level of significance at 95% confidence interval were presented in tables 1, 2 and 3.

**Table 1: Statistical presentation of the body weight on 1d, 8d, 15d and 22d of administration.**

Group Days	A	B
Day 1	212	197
Day 8	204	202
Day 15	194	207
	192	210

**Table 2: Mean and Standard Error of Mean of body weight changes**

	Mean	SEM
Group A	198	15
Group B	199	13

**Table 3: Statistical Significance of Weight Changes**

Pairs	Sig (2-tailed)
A & B	0.13

There was an increase in the homogenate level of LDH in the treated animals, compared to control animals with a statistically significant difference in the average level of LDH. The results of the average LDH level with the Standard Error of Mean (SEM) and level of significance are represented in the tables 4 and 5.

**Table 4: Mean and Standard Error of Mean for Homogenate Lactate Dehydrogenase**

	Mean	SEM
Group A	7 930	20
Group B	5 940	10

**Table 5. Statistical Significance of Homogenate**

**LDH Levels**

Pairs	Sig (2-tailed)
A &B	0.03

There was also an increase in the homogenate level of G6PDH in the animals in experimental group when compared with those in the control group. However, there was no statistically significant difference in the average level of G6PDH of animals in treatment and control groups ( $p < 0.05$ ). The results of the average G6PDH levels, standard error of mean (SEM) and level of significance were presented in the tables 6 and 7.

**Table 6. Mean and Standard Error of Mean for**

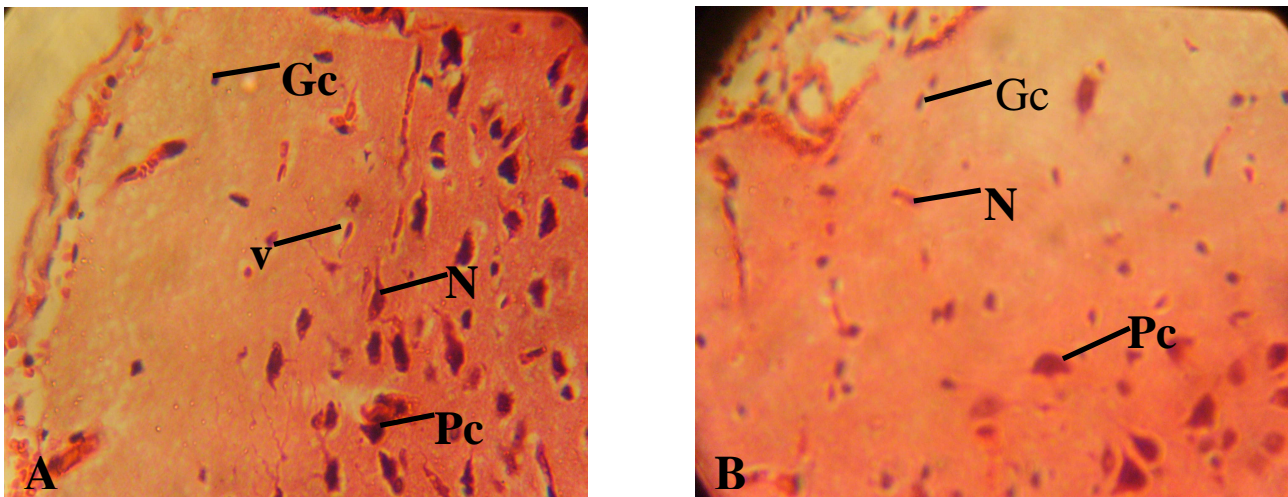
**G6PDH**

	Mean	SEM
Group A	0.859	0.02
Group B	0.724	0.06

**Table 7. Statistical significance of G6PDH values**

Pairs	Sig (2-tailed)
A & B	0.16

**NEUROHISTOLOGY**



**Fig 1: Sections of visual cortex showing vacuolation of neurons, glial cells and pyramidal cells in plates A (treated group) and preserved histological outline in plate B (control group). (H&E, x520).**

*Legends: Gc = glial cells, v = vacuolation, N = neuron, Pc = pyramidal cell*

Sections of the visual cortices were examined under the light microscope and were studied carefully. Histological sections (Fig. 1) stained with H & E obtained from the treated animals showed vacuolations in neurons, glial cells and pyramidal cells indicating that there was cell death in the neurons of the animals. Although the particular pattern of cell death observed can not be ascertain for now histochemically, this is left for further study. Also, histochemically, the sections obtained from these animals were sparsely stained indicating that there were few neurons to take up the stain (H & E). Using the stereological grid according to the method of Caroline *et. Al* [12], there were wider perinuclear space and reduced neuronal proportions.

## DISCUSSION

The plant extract appeared to have an initial psycho stimulating effects on the animals which makes them to be restless and more aggressive few hours after administration. Several hours after administration of the plant extract, the animals were docile and inactive. It is suggested that the sedative effects of the extract began after the psycho stimulating effects and was more pronounced. The plant extract also appeared to have weight loosing effect. The average body weights on 1d, 8d, 15d and 22d (Table 1) showed a marked reduction in the treated animals, though the average weight loss when compared between the treated and control animals was not statistically significant. The plant extract also appeared to increase the activities of G6PDH and LDH in the neurons of the visual cortex respectively.

The increased level of G6PDH suggests the generation of oxidative stress following administration of the plant extracts. G6PDH is a cytoplasmic enzyme that plays a protective role during oxidative stress in eukaryotic animals since they provide co-enzymes and substrates to the primary antioxidant enzymes [13]. By virtue of its ability to produce NADPH along with glutathione reductase, G6PDH is conventionally regarded as a supporter of the antioxidant system [14]. The antioxidant enzymes regulate free radical reactions by scavenging, repairing, quenching and chain-breaking reactions [15, 16].

Oxidative stress occurs when the generation of free radicals increase or the capacity to scavenge free radicals and repair oxidatively modified macromolecules decreases or both [17]. This imbalance leads to accumulation of oxidatively modified molecules predominantly end-products of superoxide and hydroxyl ion [18, 19]. The reactive O<sub>2</sub> species (ROS) has been shown to mediate cell injury. Protection from ROS requires the maintenance of endogenous thiol pools, most importantly, reduced glutathione (GSH) by NADPH. G6PDH affects the production of reduced form of cytosolic nicotinamide adenosine dinucleotide phosphate co-enzyme (NAPDH), by controlling the step from glucose-6-phosphate to 6-phosphogluconate in the pentose pathway (which involves direct oxidation of glucose) [20]. Salvemini *et. al.* [21] demonstrated that G6PDH expression is enhanced by oxidative stress induced by agents that either increase the intracellular oxygen concentration or decrease glutathione pool. Elevated oxygen consumption and intracellular concentration during increased metabolic activity increases the electron leakage from the mitochondrial transport system and causes an increase in oxidative stress and generation of the free radicals with increased vulnerability to cellular damage [22].

Following this arrangement, the plant extract, serving as oxidative stress induced agents, have been shown to mediate cell injury by either increasing the intracellular oxygen concentration and so producing more of the ROS or decreasing glutathione pool, response to which G6PDH levels (a supporter of the antioxidant system) increased. Though higher, there is no statistically significant difference in the mean value of G6PDH in the animals in the extract treated group (0.859mmol/L) when compared with the 0.724mmol/L in the control.

LDH is present in almost all tissues of the body and is used to detect tissue alterations in the body [23]. It is responsible for the conversion of pyruvate to lactate and is the key enzyme in anaerobic breakdown of glucose to pyruvate during glycolysis. Because of its wide distribution throughout the body, cellular damage causes an elevation in the total serum and tissue levels of LHD, such that when there is an injury to the tissue, the cells increase in LDH and thus releasing it into the bloodstream, where it is identified in higher than normal values [24].

Elevated oxygen consumption during increased metabolic activity with increased electron leakage from mitochondrial transport system and increased oxidative stress and generation of free radicals which damage cellular components has increased the levels of LDH in the tissue homogenates of animals in the experimental group in this work. The mean LDH level of the *Datura* treated group of 7930u/L is higher than that of the control (mean LDH of 5940u/L) and is also significant statistically.

In this study, increase in the G6PDH and LDH levels in the experimental groups suggests that some amount of cellular damage has occurred in the visual cortex of the animals in the group. The increased levels of the enzymes also suggest that neurons in the visual cortex metabolized carbohydrate via both the pentose phosphate pathway (PPP) which is an aerobic pathway and the glycolytic (anaerobic) pathway. The rate of metabolism appeared to be more via the aerobic PPP. The increased G6PDH activities in the animals treated with the plant extract suggest there is production of ribose – 5 –phosphate in the PPP, which is used in the synthesis of nucleotides and nucleic acid. A high rate of destroyed cells indicates an increased level of LDH activity and since there was significant alteration in LDH, it is inferred that the extract caused tissue breakdown.

### CONCLUSION

From this study, it has been demonstrated that aqueous leaf extract of *Datura metel* has deleterious effects on the visual cortex of adult Wistar rats. Histochemically, the leaf extract alters carbohydrate metabolism, caused cellular damage through mediation of oxidative stress and release of reactive oxygen species. Also the plant extract altered the activities of glucose-6-phosphate dehydrogenase (G-6PDH) and lactate dehydrogenase (LDH) in the visual cortices of the treated animals. Histologically, sections of the visual cortex showed vacuolations and enlarged perinuclear spaces, with evidenced reduced population of cells and altered structural integrity of the DNA in groups of animals treated with *Datura*. Hence, the use of this plant for its hallucinogenic effect and for other application by Man should be done with great cautions, as there is a high vulnerability of toxicity.

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