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Some effects of the aqueous leaf extract of *Datura metel* on the frontal cortex of adult Wistar rats (*Rattus norvegicus*)

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SUMMARY

Datura has been documented as a plant with hallucinogenic properties. Although it has a reputation as one of the 'darker' hallucinogens, historically it has been widely used by societies in both the Old and New World, and indeed continues to be used today. It is one of the drugs of abuse in Nigeria; young people add the decoction (of the leaves or the fruit) of the plant to their drinks in order to get "high". This is because it is cheap and readily available in comparison with to marijuana. The alkaloids present in the plant have been in demand in the past and its application as a subject for botanical and medical research is vast.

The aim of this study is to highlight some of the effects of aqueous leaf extracts of *Datura metel* on the frontal cortex of adult Wistar rats. Twenty wistar rats were used for this study. The treatment groups consisted of 3 subgroups designated A, B, and C and these were given 200 mg/kg, 150 mg/kg and 100 mg/kg bwt of the extract respectively, while the control group, designated D, received equal volumes of phosphate buffered saline (PBS). Administration was performed once daily over seven days using an orogastric tube. Twenty-four hours after the last administration, all the animals were sacrificed by cervical dislocation. The brains were carefully extracted from the skulls of the animals and fixed in 10% formol calcium for histological examination. Special staining techniques such as Cresyl fast violet (CFV) and Feulgen DNA were employed followed by routine hematoxylin and eosin (H&E) stain. It was observed from this study that the administration of aqueous extracts of *Datura metel* (at the doses administered) had deleterious effects on the frontal cortex of adult albino Wistar rats. There were vacuolations in the stroma of the brains of the rats in the extract treatment group and the degree of vacuolation was dose-dependent.

Key words: *Datura metel* – Cresyl fast violet – Frontal cortex – Vacuolations – Alkaloids

INTRODUCTION

Datura metel belongs to the family *Solanaceae*, also known as the nightshade family. This plant is widely distributed all over the world (Duke, 1985; Hussein, 1985; Zamora and Pola, 1992). The genus *Datura* consists of five species. These include *D. arborea*, *D. fastuosa*, *D. innoxia*, *D. metel* and *D. stramonium*.

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The alkaloids content of the different parts of the Datura plants has been studied by investigators studying the chemical composition of the plant. In a study conducted by Berkov and Zayed (2004), it was observed that the leaves, fruits and root of *D. innoxia* contain about 38 alkaloids. According to Checke and Shull (1985), Xu et al. (1985) and Shonle and Bergelson (2000), phytochemical analysis of all species of the genus Datura reveals atropine, hyoscyamine and scopolamine (hyoscine) as the main tropane alkaloids but their concentrations depend on the species and on the part of the plant used. These tropane alkaloids are of great importance in the pharmaceutical industry.

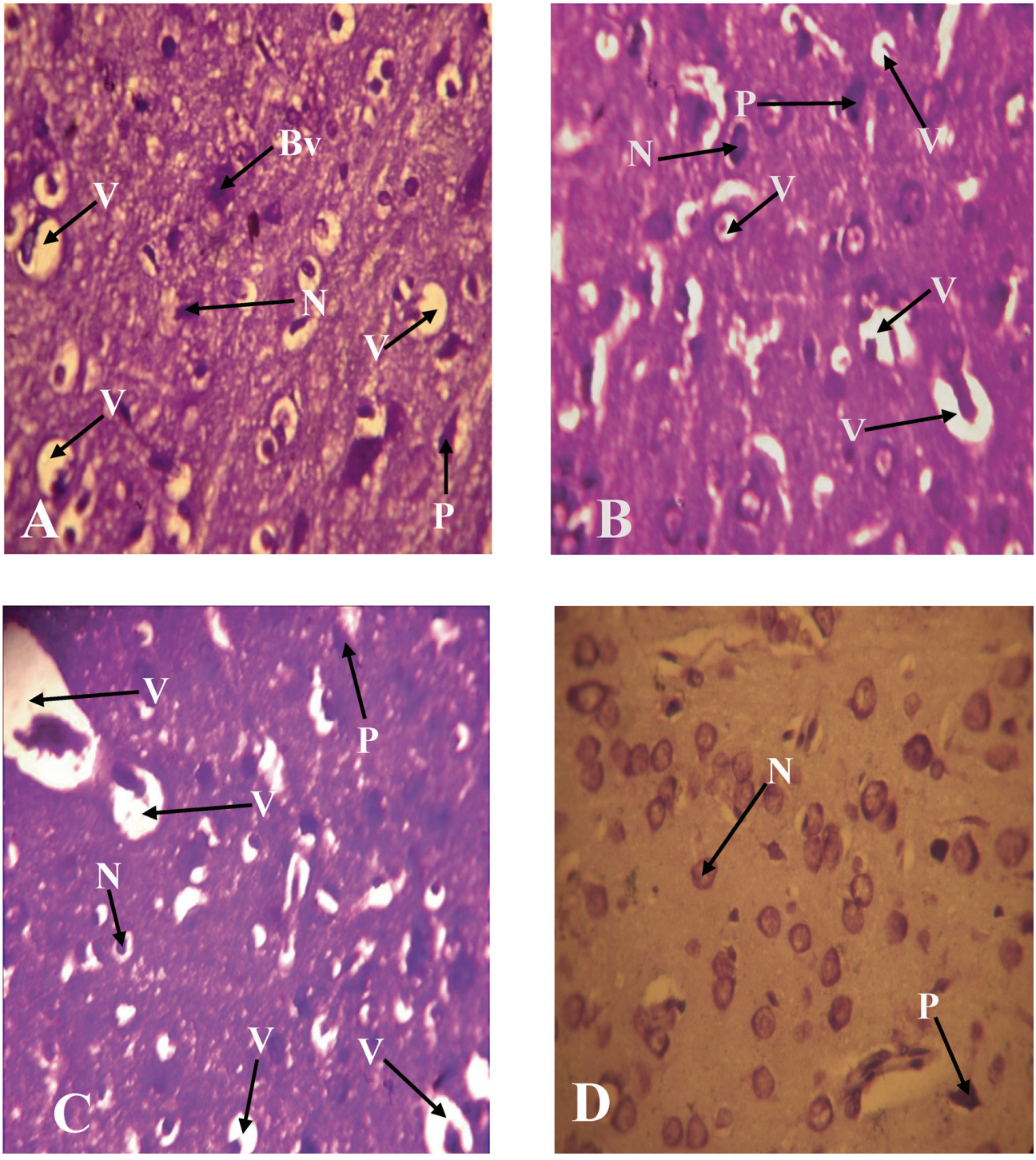

Fig. 1. Images of the frontal cortex of the animals in groups **A**, **B**, **C** and **D**. (CFV, x1920). V= vacuolations, P= pyramidal cells, Bv =Blood vessels, N= neurons. Note the extent and degree of vacuolations in the treatment groups **A**, **B**, **C** compared with the control group **D**. The distortion in the arrangement and shape of the neurons in the sections of the treatment group compared vis-à-vis the control.

Medicinal folklore use of the flowers of Datura plants as a source of analgesic mixtures by the Ancient Chinese (Xu et al., 1985), Indians (Duke, 1985) and Mexicans (Zamora and Pola, 1992) has been documented. The contents of the Datura plant have been employed in treating clinical conditions relating to the nervous system as well as other systems. These have been outlined by several authors such as Perry, (1980); Duke, (1985); Hussein, (1985); Weiss, (1996); Al-Gadi, (1997); Henry and Wiseman, (1997); Guarrera, (1999); Rojas et al., (1999) and Peredery and Persinger (2004). Such clinical deviations include: Parkinson's disease, paralysis, psychological and sleeping disorders, epilepsy, headache, spasms, shock, motion sickness, asthma, tuberculosis, breast cancer, glaucoma, arthritis, rheumatism, intestinal parasitic infestation, hydrocele, hemorrhoids, boils, hair loss, ringworm and depression of sexual desire. Induced diabetes mellitus in rodents can also be treated with seed extracts of *D. metel* (Krishna et. al., 2004).

The brain is the center of the nervous system in all vertebrates and most invertebrate animals (Shepherd, 1994). In vertebrates, the brain is located in the head, protected by the skull and close to the primary sensory systems of vision, hearing, balance, taste, and smell. Brains are extremely complex in structure and function.

The brain controls the other organ systems of the body, either by activating muscles or by causing the secretion of chemicals such as hormones. This centralized control allows rapid and coordinated responses to changes in the environment. Some basic types of responses are possible without a brain: even single-celled organisms may be capable of extracting information from the environment and acting in response to it (Gehring, 2005). Sponges, which lack a central nervous system, are capable of coordinated body contractions and even locomotion (Nickel, 2002).

Van de Graaff (2001) observed that the frontal lobe is responsible for responses relating to memory, emotions, reasoning, judgment, planning, and verbal communication. Owing to the significant functions of the frontal cortex, any structural alteration in this structure is vital to health.

This present study was undertaken to observe some of the effects of aqueous leaf extracts of *Datura metel* on the frontal cortex of adult Wistar rats.

Materials and Methods

Collection of plants and preparation of plant extracts

Fresh leaves of *Datura metel* were collected along the Agbo-Oba axis, Ilorin, Kwara State and were authenticated at the Department of Plant Science University of Ilorin, Nigeria. The leaves of the plant were air-dried at 50°C. The dried plant material was weighed using Gallenkamp (FA2104A, England) electronic weighing balance and ground with Blender/Miller III, (model MS -223, China). Sixty grams (60 g) of the ground plant sample was then soaked in 600 ml of phosphate buffered saline (PSB) for 24 hours at room temperature with constant shaking (Stuart Scientific Orbital Shaker, UK), and then filtered through silk cloth. The filtrate was concentrated using a rotary evaporator (Rotavapor® R-210) at 42-47°C. The residue of the extract obtained was kept in a capped sample bottle and stored in a refrigerator until use.

Animal care and experimental design

Twenty (20) male albino Wistar rats of the first filial generation were randomly assigned to three (3) treatment groups identified as; A, B, and C (n=15), and a control group D (n=5). The body weights of the animals were recorded on daily basis using a digital weighing scale (Saltun® EK5055Max).

The animals in the treatment groups designated as A, B, and C were administered orally (through an orogastric tube) with 200 mg, 150 mg and 100 mg per kilogram body weight of the aqueous extract of *Datura metel* for seven days respectively, while the animals in the control group (group D) were administered an equal amounts of phosphate buffered saline (PBS).

All the animals were housed in clean cages with dimensions of 33.0 x 20.5 x 19.0 cm under in well ventilated standard housing conditions (temperature: 28-31°C; humidity: 50-55%) (Yakubu et al., 2008). Their cages were cleaned daily. All animals were checked for illness, abnormal behavior, and morphological anomalies.

All experimental procedures followed the recommendations provided in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and Published by the National Institute of Health (NIH, 1985).

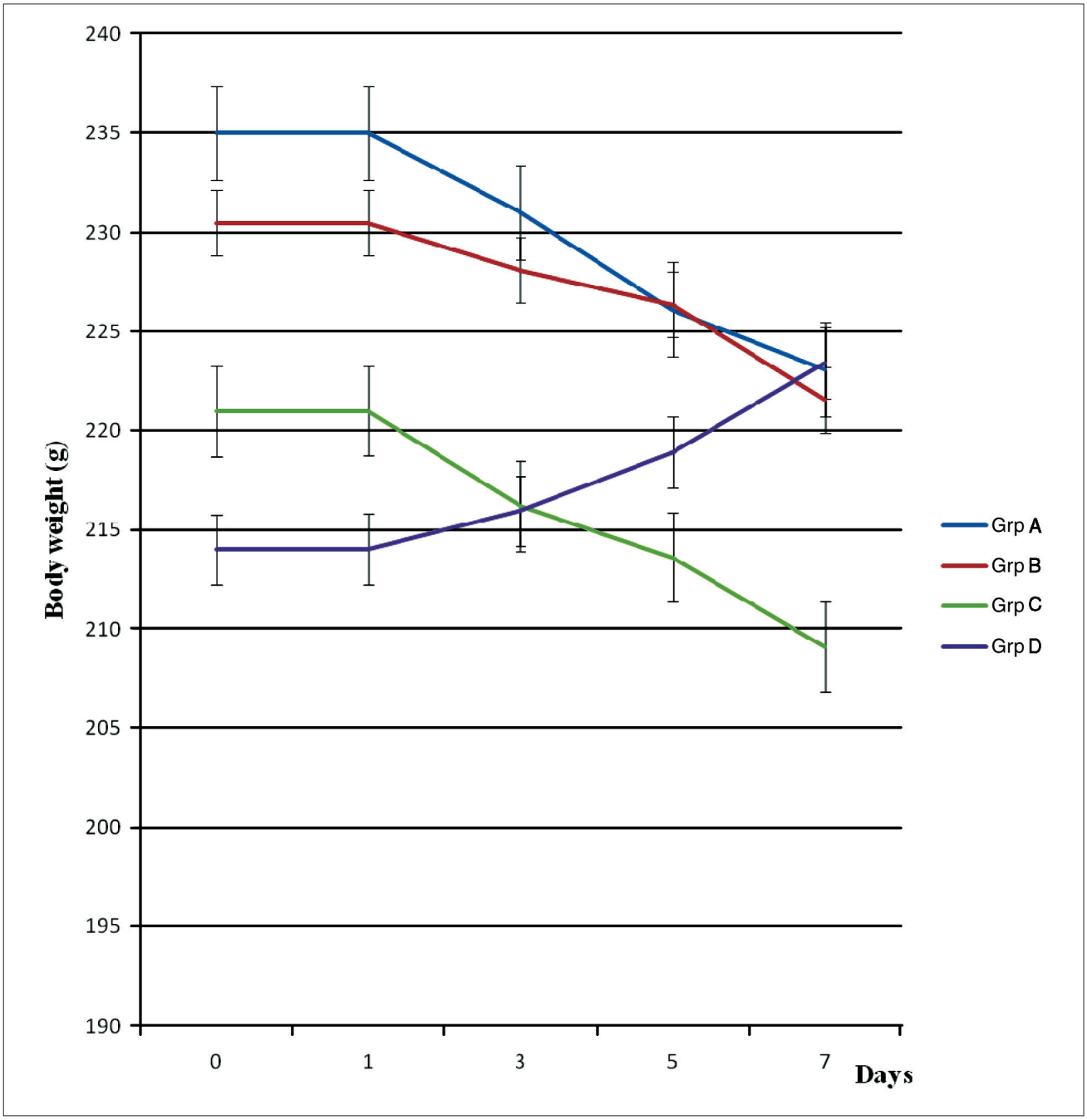

Fig. 2. Graph showing the body weight changes in grams ($p<0.05$).

The rats were fed with standard rat chow at a recommended dose of 100 g/kg as advised by the International Centre of Diarrheal Disease Research, Bangladesh (ICDDR, B) daily. Drinking water was supplied *ad libitum*.

Twenty-four hours after the last administration, all the animals were sacrificed by cervical dislocation, the brains were carefully extracted from the skulls of the animals, blotted dry on a filter paper, and placed in specimen bottles containing 10% formol calcium for histological processing.

Neurohistological parameters

After fixing the brains of both the treated and control animals, the tissues were later processed for Cresyl fast violet, Feulgen DNA and Hematoxylin and Eosin (Bancroft and Steven, 1992; Feulgen and Rossenbeck, 1924; Drury et al., 1967). After fixation, the tissues were embedded in paraffin wax; serial sections of 5 µm thick were produced using the Leitz Rotary microtome (Leitz 1512 Microtome). The sections were mounted in DPX and examined with the aid of Olympus binocular

light microscope (XSZ-107BN, No. 071771). The photomicrographs of each slide was taken with a Nikon Digital Camera DXM1200F (Nikon, Japan) for subsequent histological analysis.

Statistical analysis

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

Results

Behavioral responses of the treated animals

The animals in the treatment groups displayed aggressive and erratic resistance to handling throughout the duration of this study. The animals of extract-treated group A showed restlessness and excitation for a much longer period, followed by animals in group B and then by the animals in group C. At about 20 to 30 minutes after administration, the animals in the treatment groups began to show sign of calmness. The animals isolated themselves and avoided response to external events. Dilatation of the pupil was noted upon observing the eyes. Mydriasis is a common consequence of the anticholinergic activity of the biochemical components of the plant, as suggested by Thabet et al., (1999) and Pekdemir et al., (2004). The animals in both the treatment and control groups were frequently checked for any alteration or changes in their behavioral responses.

Body weight

Following the administration of *D. metel* extract to the experimental animals in the treatment groups, the average body weight (Fig. 2) in each of the experimental groups A, B, C, and D were calculated on day 7 of the experimental procedures. Anorexia (a reduction in food consumption and body weight) was observed in all the rats in the treatment groups administered 200, 150, and 100 mg/kg bwt of the plant extract respectively. Food intake was significantly reduced following the administration of the plant extract in a dose-dependent pattern. Since food consumption was reduced, similar reductions in body weight were observed during the study for all three treatment groups. All control group rats showed an increase in body weight.

Gross observations

No gross alterations were observed in the cytoarchitecture and morphology of the frontal cortex of the animals in the treatment groups or those in the control groups twenty-four hours after the termination of the experimental procedure. The frontal cortex (with all its component parts) of the animals in both the treatment and control groups appeared morphologically normal.

Histological observations of the frontal cortex tissues

Neurohistological assessment of the right halves of the frontal lobes of the animals in the treatment groups showed varying degrees of degenerative and deleterious effects on the neurons of the frontal cortex. Also observed in the neurohistological slides were changes in the appearances of the DNA, chromatolysis of the Nissl bodies, vacuolations of the neurons in the stroma of the brain, and degeneration of the glial cells. Such damage indicates neuropathologic lesions, chromatolysis and necrosis of the neurons.

Discussion

The effects of oral administration of aqueous extracts of *Datura metel* on the frontal cortex of adult albino Wistar rats were investigated to explore the possible neurohistological implications that could result following its use. Using an Olympus binocular light microscope (XSZ-107BN, N?. 071771), the results obtained from the sections of the frontal cortex of the animals in the treatment groups stained with Cresyl fast violet (CFV) for Nissl bodies, Feulgen DNA (FDNA) for deoxyribonucleic acid, and hematoxylin and eosin (H&E) staining techniques revealed that oral administration of aqueous extracts of *Datura metel* had deleterious effects on the frontal cortex of the animals in the extract-treated groups since histological derangement, degenerative changes, and apoptosis and/or necrosis of neurons were observed within the cerebral tissues of the brains of the animals in the treatment groups when compared with the control group.

Fig. 1 A-C shows the neurohistology of the frontal cortex of the animals in the experimental groups (A, B, and C). It may be seen from the sections of the treatment groups A and B

that oral administration of aqueous leaf extracts of *Datura metel* brings about chromatolysis of Nissl bodies, which may occur as a result of trauma or other exogenous agents or factors (Lowe and Cox, 1992; Snell, 2001). However, chromatolysis usually results in a loss of normal functioning of Nissl bodies and impaired functioning, and may ultimately lead to cellular death.

DNA clustering was also observed in the extract treated groups. The DNA was also compromised, suggesting an alteration in its integrity, and this may cause impairment in the normal functioning of the frontal cortex.

The decrease in body weight of the animals in all the treatment groups as recorded in this study could have been aa result of anorexia following oral administration of the aqueous leaf extracts of *Datura metel*. Akinlolu and Shokunbi (2010) suggested that decreases in body weight could also occur as a result of the negative impacts of drugs on the normal biological, biochemical, physiological and metabolic processes, with consequent depletion of body protein in the treated animals.

The decrease in body weight of the extract-treated animals reflected the possible changes in function of the organs that are regulated by the nervous system and metabolic activities. The active constituents of the aqueous leaf extract, mainly tropane alkaloids, prevent the action(s) of the essential neurotransmitter (acetylcholine) in the brain through blockade of its receptors (Henry and Wiseman, 1997). Brain tissue has two subtypes of acetylcholine muscarinic receptors: M4 and M5 (Yasuda et al., 1993). Atropine and other related alkaloids are effectively functional on the muscarinic receptors, and have limited effects on nicotinic receptors (Bloom, 1992; Brown, 1992). Nielsen et al. (2004) demonstrated the high affinity of aqueous extracts of *Datura ferox* leaves to reuptake the transport protein of the neurotransmitter serotonin, leading to a prominent inhibition of reuptake.

Although the plant has been reported to have many beneficial and medicinal properties, its side effects (such as hallucinations, psychiatric derangement, disorientation with agitated behavior, etc.) could have been a result of the insults of the chemical components of the plant to the cells of the nervous system. It was observed that the plant has the potential to cause cerebral damage and this may lead to trauma and impaired functions of the brain.

Conclusion

This study has demonstrated some of the effects of aqueous leaf extracts of *Datura metel* in rats. People often consume herbal remedies and/or decoctions (either in aqueous form or as a tincture) with orthodox medication with no prescription either from a pharmacist or a physician. Considering some of the deleterious effects of *Datura metel* leaf extracts on the body weight and the cellular components of the frontal cortex in rats, herbal practitioners and drug abusers (particularly young people) should be *properly educated* about the use of the plant, in particular in view of the negative impact it has on body weight and the fontal cortex in the extract-treated animals in this study. Further studies should be directed towards isolating the specific component(s) of the plant responsible for the deleterious effects in order to standardize the plant preparation for maximum therapeutic benefit.

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