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Deleterious Effects of Datura Metel Leaf Extract on the Liver and Kidney of Sprague Dawley Rats

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ABSTRACT: For many centuries, the potentials of *Datura metel* as a traditional herb are well documented. The widely reported traditional uses include the use of the plant as narcotic and local anesthetic drug in many societies. Despite having a reputation as one of the 'darker' hallucinogens, it has been widely used by societies historically in both the "Old and New World", and continues to be today. All parts of *Datura* plants contain dangerous proportion of tropane alkaloids (highly poisonous) and may be fatal if ingested by humans or other animals, including livestock and pets. The aim of this study was to elucidate some of the effect of the aqueous leaf extract of *Datura metel* on the cytoarchitecture and morphology of the kidney and liver of adult Sprague Dawley rats following 21 days administration of aqueous leaf extract of *Datura metel*. At the termination of administration, it was observed that the consumption of aqueous leaf extract of *Datura metel* has degenerative, deleterious and necrotic effects on the kidney and liver of adult Sprague Dawley rats. Furthermore, a marked statistical significant decrease in the body weight of the animals in the treatment group when compared to the control group ($p<0.05$).
The results obtained from this study suggests that *Datura metel* has compromising and deleterious effects on the cytoarchitecture and histology of the kidney and liver of adult Sprague Dawley rats, with a marked reduction in the body weight of the treated animals.
Key words: - *Datura metel,* Kidney, Liver, Cytoarchitecture, Degenerative effects.

Introduction

Indeed the use of plants in their natural unprocessed and unrefined form undoubtedly began when the first "intelligent animal" observed that certain plants altered particular body function (Katzung, 2004). Medicinal plants are plants that have at least one of their biochemical components and /or structural parts (leaves, stem, barks or roots) used for therapeutic purposes (Bruneton, 1993). Recently, medicinal plants have become of immense use in the treatment of different disease conditions, such as diabetes, malaria, anemia (Fola, 1993). The availability and relatively cheaper cost of medicinal plants in sub-Saharan Africa, makes them more useful as therapeutic agents when compared to 'modern' medicines (Agbor and Ngogang, 2005; Agbor et al., 2005). The importance of medicinal plants, and the contribution of phytomedicine to the clinical well-being of a significant number of the world's population, has attracted interest from a variety of disciplines (Prosper-Cabral *et al.*, 2007).

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Datura metel (Family; Solanaceae) is an annual herb that grows to about five feet in height. The flowers are violet on the outside but slightly whitish on the inside. The fruit has a spiny capsule measuring about 1.25 inches in diameter. In a study conducted by Anozie (1986) it was observed that the seeds of *Datura metel* have the highest alkaloid content compared to the flowers, stem, immature fruits and leaves.

Some other plants with narcotic properties in this family Solanaceae are mandrake (*Mandrogora*), belladonna (*Atropa*), henbane (*Hyoscyamus*), and tobacco (*Nicotiana)*. They are appropriately called the "paradoxical plants" by Heiser (1969); this family also includes such common food plants as the tomato, potato, and eggplant (Safford, 1922). Conklin (1976) classifies herbaceous Datura into five sections, while the older citation by Avery *et al* (1959) contains only four. In any case, this genus contains about ten different herbaceous species, the most important ones being *D. stramonium*, *D. inoxia*, *D. metel*, and *D. ceratocaula* (Schultes and Albert, 1979).

According to Wong (1976), *Datura metel* was observed to be one of the 50 fundamental herbs used in traditional Chinese medicine, where it is called yáng jīn huā. All parts of Datura plants contain dangerous proportions of tropane alkaloids (highly poisonous) and may be deleterious to Man or other animals if ingested. In some places it is prohibited to buy, sell or cultivate Datura plants (Preissel and Preissel, 2002).

Van (2001), states that the normal disease-free kidneys are reddish brown in color and are positioned against the posterior wall of the abdominal cavity at the levels of the twelfth thoracic and the third lumbar vertebrae. The right kidney is usually 1.5 to 2.0 cm lower than the left because of the large area occupied by the liver on the right side. The kidneys are *retroperitoneal*, which means that they are located behind the parietal peritoneum.

The kidneys remove a wide range of waste substances that are either ingested or produced by the process of metabolism, including urea, uric acid, creatinine and many toxins (drugs). It is also known that the kidney performs multiple crucial functions in physiological homeostasis (Guyton and Hall, 2000) this include acid-base regulation and/or balance, electrolyte concentrations balance, precise regulation of blood volume, and blood pressure. At a slightly alkaline pH of 7.4, the blood plasma is maintained by the kidney (Guyton and Hall, 2000).

There are about one million nephrons per kidney, each of which is made up of five main functional segments (i.e. the glomerulus, the proximal convoluted tubules, the loops of Henle, the distal convoluted tubules, the collecting ducts) (Crook, 2006).

The liver is the largest gland in the human body. In some way the liver functions as the 'gatekeeper' to the blood. As the blood from the hepatic portal vein passes through the liver, it removes poisonous and toxic substances and detoxifies them (Mader, 2004). The liver functions as a vast biosynthetic chemical factory. It synthesizes large complex molecules from substances brought to it in the blood, most importantly substances recently absorbed by the intestine and transported by a portal blood system (Stevens and Lowe, 2005).

All the biochemical attributes of the liver are carried out by the epithelial parenchymal cells of the liver, the hepatocytes, and are dependent on close interrelationships between the vasculature (branches of the hepatic artery and portal vein, sinusoids and central vein); the hepatocytes, and; the bile drainage systems (the canaliculi and intrahepatic bile ducts) (Stevens and Lowe, 2005). This present study was undertaken to elucidate some of the effects of the aqueous leaf *Datura metel* extract on the liver and kidney of Sprague Dawley rats after 21 days of administration.

Materials and Methods

Animal Care

Sixteen (16) Sprague Dawley rats weighing between 208 and 235g were purchased from the Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria and randomly assigned into two (2) experimental groups identified as; A and B, (n=8). The animals in the group designated as **A** serves as the treatment group while the animals in the group designated as **B** serves as the control. The body weights of the experimental animals were obtained using a digital weighing scale (Saltun® EK5055Max) and their cages were cleaned daily.

The animals were allowed to acclimatize at the Animal Holding Unit of the University of Ilorin, Ilorin, Kwara State, Nigeria for 24 hours (12 hours of light and 12 hours of darkness,) (Yakubu *et al*, 2008), were treated in accordance with 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).

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

Preparation of Plant Extracts

Fresh leaves of *Datura metel* were fetched along the road leading to the Federal Polytechnic, Ede, Osun State and were authenticated at the Department of Plant Science University of Ilorin, Nigeria. The leaves of the plant were air-dried in the herbarium of the Osun State University, Osogbo, Osun State, Nigeria for a period of thirty-seven days (37d). The dried plant material was weighed using Gallenkamp (FA2104A, England) electronic weighing balance and grinded with Miller III, (model MS - 223, China).

Sixty grams (***100***g) of the grinded plant sample was later soaked in 1000 ml of phosphate buffered saline (**PSB**) for 24 hours (Emeka and Clement, 2005) at room temperature, and then filtered through silk cloth (Mostofa *et al*, 2007), after which the filtrate was again filtered with Whatman's #1 filter paper. The final filtrate was however concentrated using a rotary evaporator (Rotavapor® R-210). The residue of the extract obtained was kept in a capped sample bottle and stored in a refrigerator until used (Mostofa *et al*, 2007).

Animal Treatment

The animals in the treatment group (**A**) were administered with **300mg/kg** body weight, of the aqueous leaf extract of *Datura metel* once daily (07:00–07:45 h) using plastic syringes attached to a flexible plastic or rubber oropharyngeal cannula (Yakubu *et al*, 2008), while the animals in the control group (**B**) were administered with equal volume of phosphate buffered saline (**PBS**) for twenty-one (***21***) days respectively.

Twenty-four hour after the last administration, all the experimental animals were sacrificed using cervical dislocation (Eweka and Adjene, 2008). Laparatomy was performed, the quadrate lobe of the liver and the left kidneys of the sacrificed animals were carefully excised, trimmed free of fat (Akinola *et al*, 2009). The livers were fixed in 10% formol saline while the kidneys were fixed in Bouin's fluid for routine histological study.

Histological Procedure

After fixation in the respective fixatives for eighteen (18) hours, the histological study for the organs of study (i.e. liver and kidney) was carried out using the method of Carleton (Carleton, 1967). The studied organs were separately dehydrated through graded alcohol solutions, cleared in xylene, infiltrated and embedded in molten paraffin wax. Tissue blocks of 5µ thickness were sectioned on a Leitz rotary microtome (Leitz 1512 Microtome). The tissues were subjected to Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS) staining procedures and the histological examination was done with the aid of the Olympus binocular light research microscope (XSZ-107BN, No. 071771).

The permanent photomicrograph of each slide was taken with a Samsung Digital Camera (Digimax i6 PMP, Samsung #11 PMP) for subsequent histological analysis (Akinola *et al*, 2009).

Statistical Analysis

Data obtained from the study were statistically evaluated using the student's t-test with SPSS/17.0 software (SPSS Inc, Chicago, USA) and Excel 2007 (Microsoft Corporation, USA) and were expressed as Mean ± Standard error of mean (SEM). A value of $p<0.05$ was considered to indicate a significant difference between groups.

Results

Body Weight

Following the administration of the aqueous leaf extract of *Datura metel* on the experimental animals in the treatment group (**A**), their average body weight (Fig. 2) were calculated on day **21** of experimental procedures and compared with that of the animals in the control group. Using t-test analysis technique at 95% confidence interval, it

was observed that there was a significant decrease in body weight between day 1 and 21 in the treatment group (**A**) when compared to the control group (**B**).

Morphological Observations

No morphological alterations were seen in the gross outline of the kidneys and livers of the animals in both the treatment and the control group sacrificed twenty-four hours (24hrs) after the termination of administration of the aqueous leaf extract of *Datura metel*. The kidney and the liver of the animals in both the treatment and control groups appeared morphologically normal.

Microscopic Observations of the Kidney

When the processed histological kidney slides of the animals in the treatment group were viewed under the microscope, it was observed that the sections of the tubular system of all the animals in the treatment group does not conform to normal histological outline; there were observable necrosis, degeneration, distortion and derangement of the cells lining the convoluted tubules when compared with the control. Even with the distinct morphological appearance of the kidney, within the renal parenchyma is a gradual pattern of cell death. Furthermore, there was shrinkage of the glomerulus with an enlarged Bowman's capsule (Fig. 1-2).

Microscopic Observations of the Liver

The histological outline of the liver sections obtained from the animals in the treatment group (**A**) following the oral administration of the aqueous leaf extract of *Datura metel* shows vast array of necrotic foci in the liver parenchyma. These necrotic foci are found virtually in all segment of the liver parenchyma of the animals in the treatment group when compared with the animals in the control group (Fig.3).

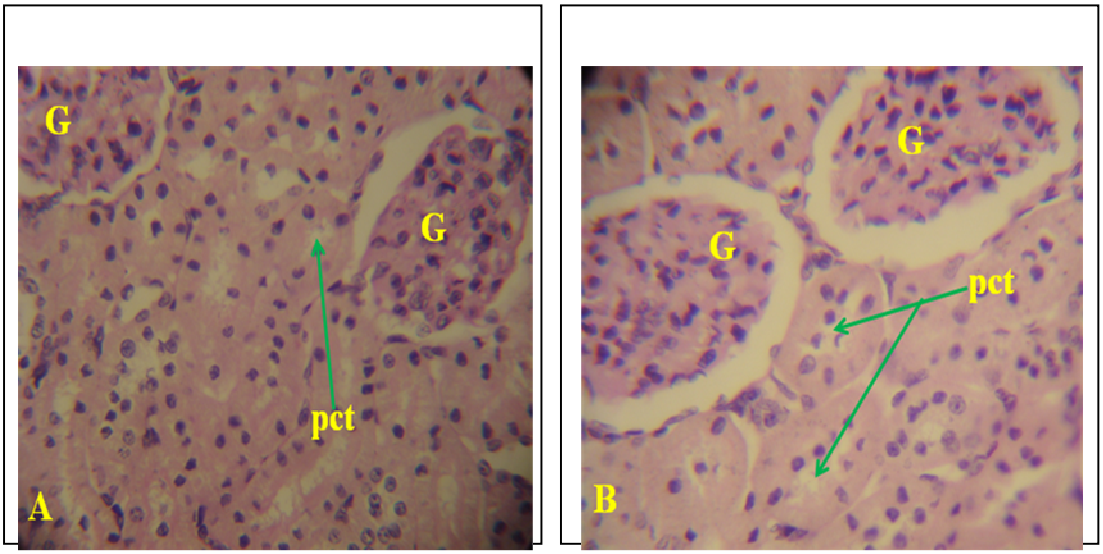

Figure 1: Photomicrograph of the left kidney of the animals in groups **A** and **B**. Legends: **pct**= proximal convoluted tubule, **G**= glomeruli (**PAS x1200**).

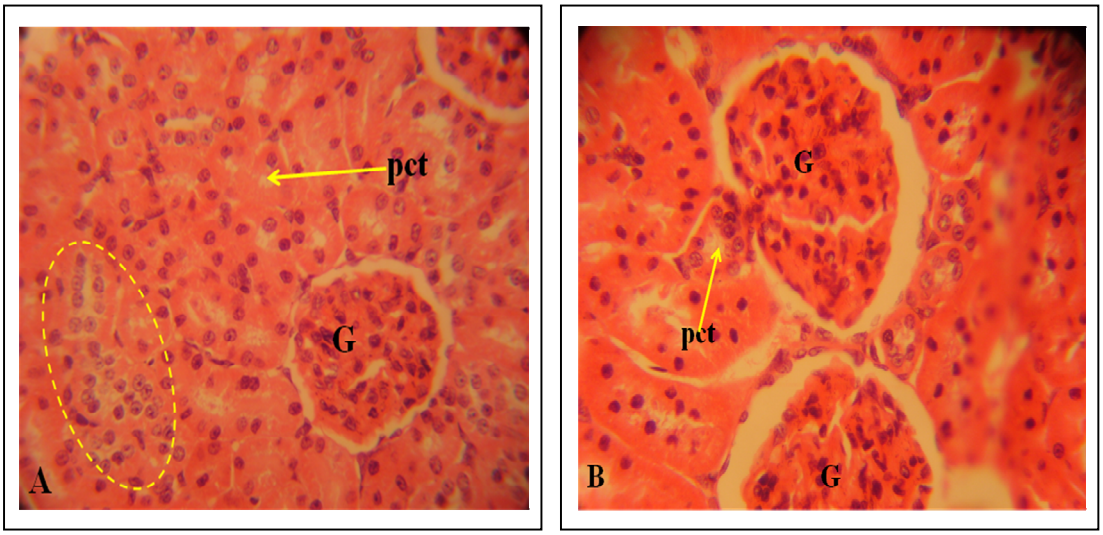

Figure 2: Photomicrograph of the left kidney of the animals in groups **A** and **B**. Legends: **pct**= proximal convoluted tubule, **G**= glomeruli. The circular segment on the slide is pointing to area of cell death. (**H&E x1200**).

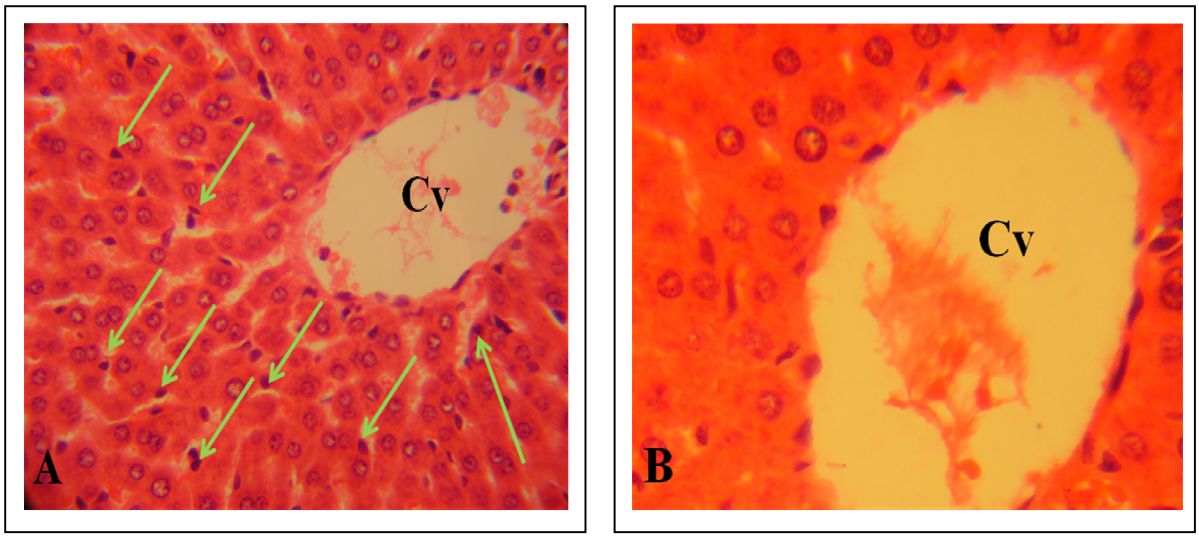

Figure 3: Photomicrograph of the quadrate lobe of the liver of the animals in groups **A** and **B**. Legends: **Cv**= central vein. The arrows are pointing to the necrotic foci (**H&E x1200**).

Table 1. Statistical representation of the average weight on days 1, 8, 15 and 22 of administration of extracts

Days	Group A	Group B
Day 1	235	208
Day 8	231	214
Day 15	227	219
Day 22	219	227

Table 2: Average weight changes in experimental animals

	Mean ± SEM
Group A	204 ± 12
Group B	199 ± 13

Discussion

The effects of *Datura metel* on the kidney and liver of Sprague Dawley rats was investigated to elucidate some of the possible histological implications that could arise following its consumption. Using the Olympus binocular light microscope (XSZ-107BN, No. 071771), the histological observations seen in the sections of the kidneys and livers (of the treated animals) stained with Hematoxylin and Eosin (H&E) and Periodic acid Schiff (PAS) revealed that oral administration of *Datura metel* has deleterious effects on the histological outline of the kidney and liver of the animals in the treatment group as there were histological derangement, degenerative changes, and progressive cell death of the cells within the renal and liver parenchyma of the treated sections when compared with the sections obtained from the control group.

It is known histologically that the normal shape of the cells within the convoluted tubules under normal condition (disease-free-state) appears cuboidal or columnar was observed to have been distorted to become flattened and pyramidal (Fig. 1). This might have an adverse effect on the functions of the convoluted tubules in the exchange of fluids and ions, reabsorption of the components of the glomerular filtrate, control of acid-base balance and more importantly, in urine concentration (Steven and Lowe, 2005).

Damage to tubular cells impairs adjustment of the composition and volume of the urine. Impaired solute reabsorption from the proximal tubules reduces isosmotic water reabsorption. Counter-current multiplication may also be affected, and therefore the ability of the collecting ducts to respond to antidiuretic hormone (ADH) is reduced with a large volume of inappropriately dilute urine is produced (Crook, 2006).

The vast arrays of nuclei present in the glomerulus are those of capillary endothelial cells, the mesangial cells of the supporting mesangnium, and podocytes. As seen in the photomicrographs of the kidney of the animals in the treatment group (**A**), there was shrinkage of the glomerulus with enlarged Bowman's capsule. This could to lead to an impaired alteration(s) in the histological and biochemical integrity of the renal corpuscle. According to Steven and Lowe, (2005), biochemical dysfunctions may result from impaired tubular functions as a result of failure of excretion of H^+ and K^+ ions. This then makes the blood to have an increase in the concentration of H^+ ions (acidosis) and K^+ ions (hyperkalemia), coupled with the retention of nitrogenous waste materials as a result of impairment in the function of the glomerulus, which may ultimately lead to renal failure (e.g. acute tubular necrosis, acute and chronic renal failure).

The liver has essential synthetic and excretory functions and can be thought of as a large 'metabolic factory'. It detoxifies and like the kidney, excretes the end products of metabolism (Crook, 2006). The histological make up of the liver contains hexagonal lobules of cells known as hepatocytes. Rows of hepatocytes radiate from the central hepatic vein and are separated by sinusoidal spaces, along the walls of which are interspersed hepatic macrophages known as the Kupffer cells. Hypoxia and toxins that are metabolized in the liver cause damage to the centrilobular

area and may also affect the periphery of the lobule. Almost all nutrients from the gastrointestinal tract pass through the sinusoidal spaces prior to entering the systemic circulation. The hepatic architecture may be impaired when the cytoarchitecture of the liver is compromised (Crook, 2006).

Many slowly progressive diseases destroy hepatocytes and lead to distortion of the cytoarchitecture of the liver. Majority of the inflammatory and metabolic diseases involving the liver may occur as a result of prolonged toxic damage. This may distort the hepatic architecture and may also cause regeneration of nodules of hepatocytes thereby disrupting the blood supply to the liver and may also increase the pressure in the portal vein leading to portal hypertension (Crook, 2006).

It is also known medically that liver damage severe enough to cause obvious clinical signs of impaired hepatocellular functions may be caused by severe hepatitis or advanced cirrhosis, or may occur as a result of liver toxins such as drugs (e.g. paracetamol) (Crook, 2006). Deaths of hepatocytes is often accompanied by scarring, although hepatocytes can regenerates and produce a new population of cells, their connections with the portal system and the biliary drainage are destroyed. Liver disease can affect any of its functions, and this is seen when large numbers of hepatocytes are damaged all of the liver functions may be impaired (Steven and Lowe, 2005).

Response of cell to neurotoxins has been implicated as one of the major cause of cell death and this may cause apoptotic death in various tissue cells (Waters, 1994). Genetic programme has also been implicated in cell death in that cell response to neurotoxins occurring as controlled events involving cascade and/or succession of activated enzymes (Waters, 1994). In some instances, organs (i.e. liver and kidney) may be damaged as a result of oxidized agents known as free radicals generated in the body by the oxidation of nutrients derived from food substances and other chemical reactions taking place within the cells (Anderson, 2004). Cellular degeneration in many organs of the body has been observed to be one of the major causes of cell death, which may occur either as apoptotic and/or necrotic cell death. These two forms of cellular degeneration differs from each other morphologically and/or biochemically (Wyllie, 1980).

Cell death occurring pathologically or accidentally is regarded as necrotic and could result from extrinsic implications and/or disturbances to the cell and these may include toxic or traumatic effects (Farber *et al*, 1981). Processes involved in cellular necrosis which may lead to cell death include compromise and/or disruption of the structural and functional potentials of the various membranes in and within the cell. Necrosis of the cell is not induced by intrinsic stimuli to the cells as observed in programmed cell death, but by an abrupt environmental disturbances and deviation from the normal physiological conditions, factors and functions (Ito, *et al*., 2003).

The type of cell loss and the particular part of the organ affected determines the symptoms associated with individual disease (Waters, 1994). Progressive loss of the functions of kidney and liver over time can lead to chronic kidney disease (e.g. acute tubular necrosis, acute and chronic renal failure) and impaired functions in the liver (e.g. chronic and acute hepatic failure, failure to secret bile, failure to detoxifies toxins, tumors, cirrhosis, failure to synthesis important proteins, e.t.c) (Steven and Lowe, 2005).

The histological observations recorded in this study suggest that the pattern of cell death within the liver and renal parenchyma with shrinkage in the glomerulus of animals in the treatment group (**A**) could be as a result of deleterious effect(s) of the phytochemicals substances (tropane alkaloids) present in *D. metel* on the studied organs.

Conclusion

In this study, the oral administration of the aqueous leaf extract of *Datura metel* significantly decreased the body weight of the extract treated animals. The histological outline of the aqueous leaf extract of *Datura metel* on the kidney and liver as seen in the sections obtained from the extract treated rats support this claim as the histological outline of the studied organs were compromised and distorted. This is indicative of the deleterious and toxic effect of the aqueous extract of *Datura metel* on the histology of the kidney and liver at the dose administered to the animals in this study. In conclusion, data obtained from this study showed that the oral administration of aqueous extract of *Datura metel* has no adverse effects on the kidney and liver morphology, yet the histological observations shows that *Datura metel* was hepatotoxic and as well nephrotoxic. However, further studies should be directed towards isolating the specific biological and/or chemical component(s) of the plant responsible for the deleterious effects in order to standardize the use of the plant.

It is also suggested that further studies should be carried out using higher animal models to examine the effects of *D. metel* on the kidney and liver using the electron microscopy in order to study fine structural details which might not be observable using the light microscope. Also, it is suggested that biochemical assay for the kidney and liver be carried out to actually know the pattern of degeneration and the implication of the plant extract on the functional integrity of the kidney and liver.

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