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Datura Stramonium Leaf Extract Toxic Effects on Testis in Swiss Albino Mice *Mus musculus*

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ABSTRACT

The notorious weed, jimson weed *(Datura stramonium L.*) is a hallucinogenic plant that is both toxic and medicinal. The presence of tropane alkaloids, which contain a methylation nitrogen atom (N-CH3) and inhibit neurotransmitters in the brain, is thought to be responsible for the plant's neurotoxicity. Toxic symptoms have been linked to the recreational use of *D. stramonium*, according to ethnomedicine.

This investigation has been designed to examine the toxicity and describe the possible changes in the structural function of vital organs, following the orally intubation of non-lethal doses of *D. stramonium* leaves crude aqueous extract. Through preliminary trials, crude aqueous extract. Of 200 mg leaves per kilogram body weight was established as a tolerable non-lethal dose. Three doses 0.36, 0.7, and 4 mg/kg were orally weekly, administered to the male mice in a 0.1 ml volume.

Acute toxicity studies were accomplished through oral intubation of three dosages in each case. Observation and mortality were reported for 24 .48, 72 hours.

Prolonged toxicity was performed through the administration of weekly, single doses oral for 40 days. The observation was made on the mice's testis weight and histological abnormality of a testis organ.

1 Introduction

All nightshades and agricultural plants, including potato, tomato, coffee, and pepper, belong to the genus Datura (Solanaceae). Genetic markers are frequently used in the classification of distinct species within the Datura genus, implying that this genus has a lot of variety owing to mutation.

Humans and other mammals are poisoned by all portions of Jimsonweed. Anticholinergic alkaloids are present in high concentrations in the plants. (block neurotransmitters) and have been used for its psychoactive effects. The concentration of toxins varies greatly from plant to plant making the risk of fatal overdose high (Mukhtar et al., 2019).

D. stramonium is used to treat ulcers, wounds, inflammation, rheumatism and gout, sciatica, bruising and swellings, fever, asthma and bronchitis, toothache, and other human illnesses in Ayurvedic medicine (Kirtikar et al., 1999). *D. stramonium* is used in a variety of folk medicine therapies. The medicinal uses of D. stramonium have been eclipsed by its acute toxic consequences in modern medicine (Gaire et al, 2013). The central nervous system is affected by significant doses of *D. stramonium*, resulting in symptoms such as confusion, odd behavior, hallucinations, and amnesia. Though death by *D. stramonium* poisoning is rare, recovery may take several days (Norton, 2008).

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Therefore, rigorous knowledge and understanding of the acute toxicity effects of this plant is undoubtedly need to be accounted for.

Tropane belladonna alkaloids, which have powerful anticholinergic effects, are the poisons in Jimson weed. Hyoscyamine (leaves, roots, seeds), hyoscine (roots), atropine (d,l-hyoscyamine), scopolamine (l-hyoscine), sitosterol, and proteins are all alkaloids.

1.1 Intoxication and Mode of Action

Consumption of *D. stramonium* interferes and obstructs the action of neurotransmitters in the nervous system. Datura toxins pass through the blood-brain barrier and block acetycholine production (The main neurotransmitter used by the parasympathetic nervous system). Atropine poisoning is very dangerous for children, and the prognosis is usually deadly. The State Chemical Laboratories in Agra, India, evaluated 2,778 deaths caused by consuming Datura from 1950 to 1965. Symptoms likely to be produced by tropane alkaloids such as scopolamine, hyoscyamine, and atropine include urinary retention, Dizziness, convulsions, fever, euphoria, hallucinations, short-term memory loss, and delirium are all symptoms of delirium.

Leaf alkaloids range from 0.25 to 0.45 percent, whereas seed alkaloids range from 0.47 to 0.65 percent. Hyoscine content in leaves is 0.1 percent, 0.05 percent in stems, and 0.1 percent in roots; and hyoscyamine content in leaves is 0.4 percent, 0.2 percent in stems, and 0.1 percent in roots (Kaur *et al*., 2020).

Sperm generation is controlled by the testis, which is the primary male reproductive organ. It is held in place by the scrotal sac's bilateral chambers. The production of spermatozoa from testis stem cells is a time-consuming process that takes approximately 64 days in humans and 48 to 53 days in rats.

Sertoli cells are important for spermatogenesis, but leydig cells are the main source of androgen synthesis. Both types of cells can be harmed by toxins and chemical medications.

A change in the functional integrity of these cells could induce a harmful disruption in hormonal balance, or disrupt the spermatozoa growth process, resulting in reduced male fertility (Adekomim, 2011).

The objective of this experiment is to evaluate the toxicity of *D. stramonium* to testis male Swiss albino mice and this has been achieved by conventional LD50 biochemical function tests in addition to the histo-

physiology approach. Changes in behavior, physical activity, and body weight during the period of study have also been observed, Furthermore, the relative weight of testis.

2. Materials and Methods

2.1 Test Animals:

A total of 16 adult male and female Swiss albino mice Mus muscullus were brought, from the animal house of the faculty of Medicine, to the Zoology Department, University of Benghazi. The animals were reared in the laboratory.

2.2 The chemicals:

D. stramonium Hematoxylin, eosin and all other chemicals used in this study were of a technical grade with known structures and functions.

2.3. The experimentation

LD50 determination (Acute toxicity study):

The acute Toxicity of *D. stramonium* was evaluated through using five treatments 200, 100, 50, 25 and 12.5 mg of leaf extract per kg mice body weight. In addition to control treatment receiving only saline water.

Each treatment was replicated two times with four mice per replicate. Mice between the ages of 50 and 70 days were chosen at random for each treatment based on their body weight. The calculated doses (mg/kg) were orally delivered in a 0.1 ml solution through the mouth intubation. Observations were made on the behavior while other symptoms and mortalities were recorded on 24, 48, 72 and 96 hours post treatment.

2.4. Prolonged toxicity study:

A total of 36 male mice were used in this study. The animals were divided into three treatments each treatment contained three replicates and each replicate having three animals. The fourth treatment having 9 mice in three replicates formed the control .Treatment animals were intubated with 0.1 ml solution containing the specified treatment concentration 0.36, 0.7 and 4 mg/kg leaf extract per kg mice body weight, whereas, control mice were orally intubated with 0.1 ml of saline water .

2.5. Tissue Sample:

Testis of each animal were dissected out and weighted. After washing with normal physiology salin solution, the organs were transferred into glass

containers containing formalin –acetic acid-alchol (FAA) fixative solution and kept for weighting and histological studies.

3. Results

In the acute toxicity symptoms were found to follow dose dependent acute toxicity to male mice receiving an orally single dose of various concentrations .

After 72hr of exposure no mortality was reported for the 200, 100, 50, 25, 12.5 mg/kg body weight .

Percent organ weight / body weight:

The effect of *D. stramonium* extract on the testis organ were measured through the relative organ weight per 100g body weight in each treatment and compare that with control treatment after 40 days of exposure.

Testis Weight

The relative weight mg testis weight/ 100 g body weight of control and D.stamonium treated male mice revealed a significant differences between control and treated mice (t-test $p \leq 0.5$). Control mice had significantly lower weight values compared to all treated mice . The mean±SD of testis weight were 0.71±0.28 for C1 ,0.60±0.21 for C2, 0.76±0.25 for C3 compared to only 0.36±0.11 for control.

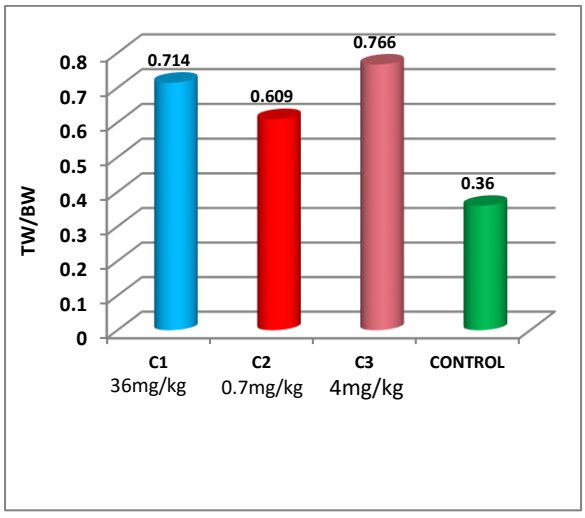

Figure 1: The relative weight mg Testis weight /100g body weight of control and *D. stramonium* treated male mice for 40 days of exposure.

Micromorphology of the organs :

A photomicrograph of the testis of the control group revealed some microstructural alterations due to multiple exposure of adult male mice to the given dose of *D. stramonium* leaves crude aqueous extract (Figure 1).

The seminiferous tubules with dense nuclei spermatogonia, (Figure 2). The portion of tubule in control group showed boundary tissue, spermatocytes, spermatids and spermatozoa, note the intact lining epithelium (Figure 3).

The histological changes in C2 and C3 treatments showed interruption of lining epithelial in somniferous tubule (Figure 4, 5 and 6).

High magnification of the above section, showed portion of degeneration changes in lining epithelium (Figure 7)

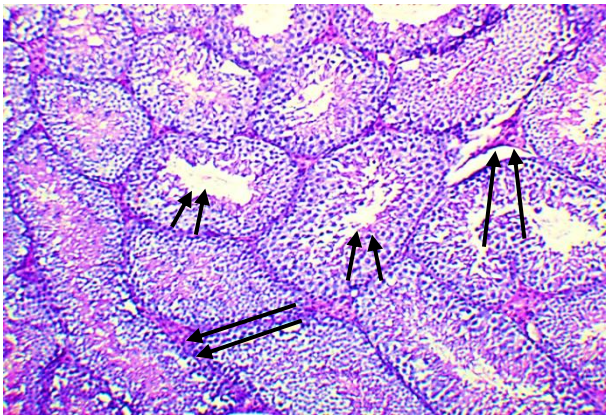

Figure 2: Lower photomicrograph of testis of control showing seminiferous tubules with dense nuclei spermatogonia (short arrow) slight amount of Interstitial connectiue tissue containg groups of leyding cells (arrows). (H&EX 100).

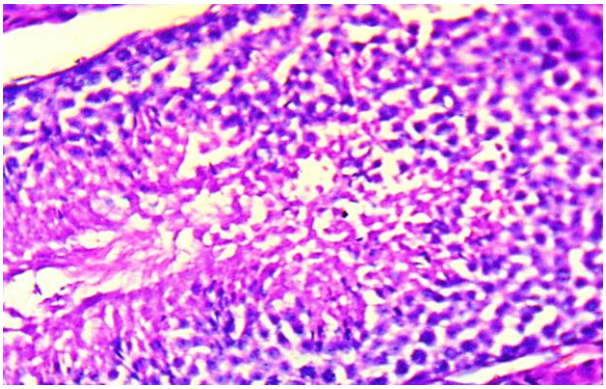

Figure 3: Photomicrograph of portion of one tubule in control group showing boundary tissue, spermatocytes, spermatids and spermatozoa in testis. Note intact lining epithelium. (H&E X400).

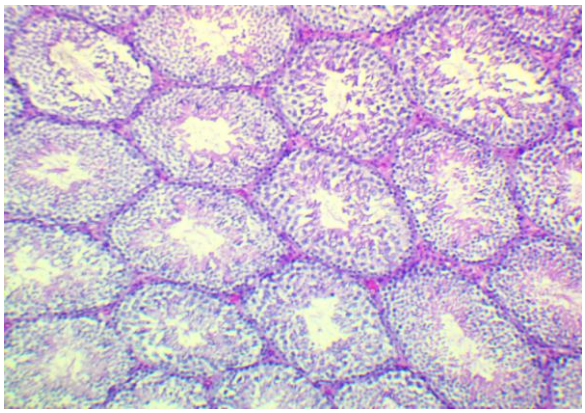

Figure 4: Photomicrograph of testis of treatment C2 showing interruption of lining epithelial in some seminiferous tubules.

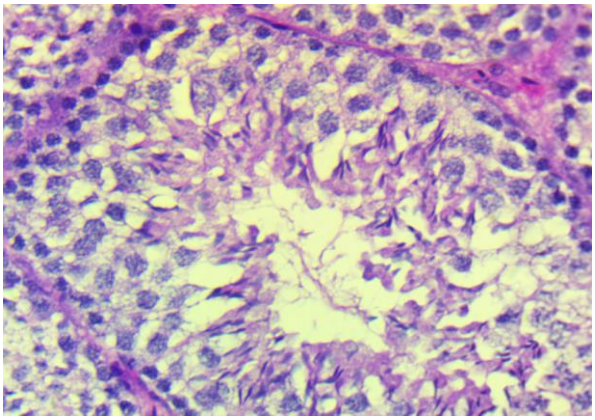

Figure 5: High magnification of the above section, showing portion of one tubule. Note marked degeneration changes in lining epithelium for testis. (H&E X 400).

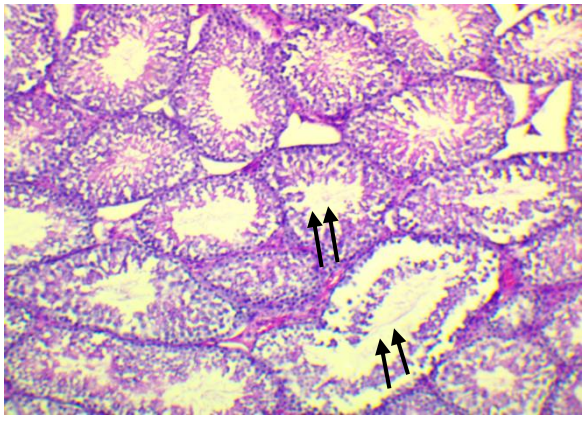

Figure 6: Photomicrograph of testis of treatment C3 showing interruption of lining epithelial in some seminiferous tubules (arrow). (H&E X 100).

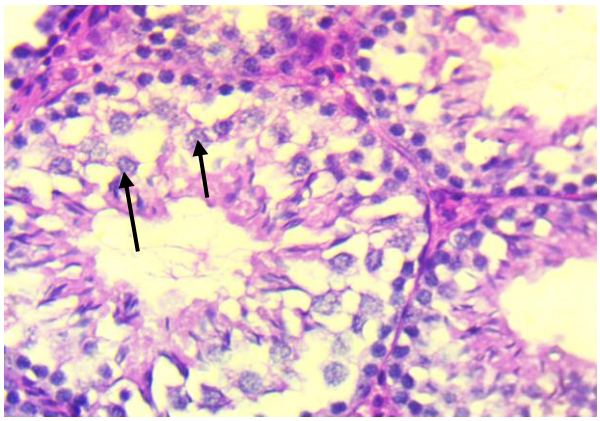

Figure 7: High magnification of the above section, showing portion of one tubule. Note marked degeneration changes in lining epithelium for testis (arrows). (H&E X 400).

4. Discussion

Close observation of the experimental animals have come in favor of optimal suitability of the laboratory conditions throughout period of the investigation. Handling of the males and females for breeding purposes have ensure the availability of the required number of males. This means that criteria such as hygienic measurements, ration, feeding and water , lighting and temperature were quite enough for well being of the males, pregnant females and growing offspring . Furthermore , males of the control treatment continued their normal activity and behavior until the end of the experiment. Males in the treatment group were given oral doses of *D. stramonium* leaves aqueous crude extract under the identical laboratory circumstances as the control group .Therefore contents of the crude aqueous extract collectively , should have stood behind the recorded changes in physical activity and behavior.

These contents should also be considered responsible for the observed deviation from normal that have been exhibited by other investigated selected parameters. Absence of mortalities in both control and extract treatment groups of animals confirmed the availability of sufficient survival requirements.

The toxicity of *D. stramonium* leaf extract was clearly observed in male mice that were exposed through oral doses . Symptoms of intoxication were easily detected . No mortality was detected in the higher used dose 200mg/kg . This result came in support of Antov *et al.*, (1991) who observed several signs of symptoms but no mortality .The toxic responses observed in the treated male mice was also supported by Bouzid *et al.*, (2002) who described similar symptoms of *D. stramonium* toxicity in humans.

The result of the toxicity obtained in this may indicate that *D. stramonium* is slightly toxic, however in reality that might not be the case when looking to previous authors who pointed the degree of this plant toxicity .

Therefore , the lake of mortality even in the higher used dose 200mg/kg leaf extract can be explained (1) most previous works were with the active ingredients of the plant , atropine and Scopolamine, whereas, our study was on the crud extract of the plant . (2) The extraction efficiency could have been low, resulting in a low percentage of the active component from the plant.(3) No organic solvent used in the extraction because the intention was on the whole plant constituents which is mostly used in the case of medication or poisoning and that synergism or antagonisms could happen in the two cases.

Furthermore, survival of the extract treatment male mice could be invested to point out to the employed tolerable non –lethal dose of the crude aqueous extract though it was repeated 4 times over 40 days weekly.

Several abnormal histological alterations seen in the testes of the animals in the treatment group, ranges from degeneration and disruption of the germ cells lining the somniferous tubules, and also the degeneration of the leydig cells. Reduction in the population of the germ cells was also seen in the histological profile of the testes obtained from the treatment group compared with the control group. This means that the number of viable sperm is decreasing, which could lead to infertility. Degenerative changes have been scientifically reported to result in cell death. There are two types of cell death, namely, apoptosis and necrosis (Cohen, 1993). Biochemically and morphologically, these two categories are distinct (Bose and Sinha, 1994). Apoptosis is a non-inflammatory response to tissue damage characterized by a series of morphological and biochemical alterations (Shen *et al*., 2002).

All parts of the plant are toxic because of their high tropane alkaloid level . Atropine and scopolamine have an approximate amount of 0.20 and 0.65 mg per flower, respectively. Because the average adult's suggested therapeutic dose of atropine plus scopolamine is 0.5 mg, it's easy to see how ingestion of just 10 blossoms can result in death. (Diker *et al*., 2007).

5. Conclusion

The weekly oral administration of non-lethal dose of D. stramonium leaves aqueous extract have been well tolerated by the male Swiss albino mice as judged by the mild transient neurotoxicity symptoms. Nevertheless, negative impacts have been observed on the body weight gain and on testis histology.

From the present study it is clear that the doses used in the acute toxicity study did not reveal in any degree of mortalities although stress symptoms were reported.

In the prolonged toxicity studies and at the designed 0.36, 0.7, 4 mg /kg concentration, significant changes in body weight between control and treatment were found.

Histological finding did reveal adverse effects on the testis.

Result of the present investigation has pointed out the necessity for systematic series of trials leading to the establishment of facts and solid information about the commonly used folk medication of this plant.

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Conflict of interest: The authors declare that there are no conflicts of interest.

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