



Aqueous leaf extracts of *Albizia lebbeck* induce histological changes of reproductive organs of alloxan induced diabetic albino rats.

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Abstract

Background: The frequent use of herbal preparations for treatment of infections and diseases have led to the need to investigate possible side effects that could be associated with these plants. **Objective of study:** This study was designed to investigate the effects of aqueous leaf extracts of *Albizzia lebbeck* on the histology of the male and female reproductive organs of alloxan induced diabetic Albino rats.

Materials and methods: The rats (n = 40), weighing 100 – 150g were randomly assigned into five treatments groups (A-E). The rats were induced with diabetes using Alloxan (100mg/kg) administered interperitoneally (Groups B, C, D). Treatment groups were treated with distilled water (Group A), and aqueous leaf extract of *Albizzia lebbeck* (100mg to group C and E, 200mg to group D) administered orally via orogastric tubes.

Results: All the rats were fed with pellets and clean water ad libitum. Cage side examination was done daily to observe for behavioral signs. It was observed that *Albizzia lebbeck* leaf extract in female rats had no effect on the histology of the uterine endometrium, but, the ovaries were found to have a decreasing number of follicles indicating a possible arrest of oogenesis. The male rat's testes showed a parallel decrease in germinal epithelium in all groups from group A to group E indicating a decrease in spermatogenesis. It was also noted that *Albizzia lebbeck* caused a decrease in feed intake and animal weight in all treatment groups during the course of this study.

Conclusion: Findings from The aqueous extract of *A. lebbeck* leaves causes spermatogenic and oogenic arrest in male and female albino rats respectively. However, *Albizzia lebbeck* should be subjected to further analysis in order to unravel the active ingredients responsible for their antifertility actions.

Keywords: Antifertility; *Albizzia lebbeck*; Histology

1. Background

Albizzia lebbeck (L.) Benth (Mimosoideae), commonly called Indian Siris or East Indian walnut, is one of the most promising fodder trees for semi-arid regions (Gupta et al., 2004). The tree is used in folk remedies in bolus, enemas, ghees or powders for abdominal tumors. Reported to be pectoral astringent, rejuvenant and tonic, the siris tree is a folk remedy for boils, cough, flu and eye and lung ailments. The seed oil is used for leprosy and the powdered seed in scrofulous swelling. The ethanolic extracts of *A. lebbeck* leaves exhibited anticonvulsant activity (Kasture et al., 2000). *Albizzia julibrissin* Durazz is reported to have sedative activity (Kang et al., 2000). The total alkaloidal fraction of *Albizzia inopinata* leaves has been shown to act on the central nervous system (Assis et al., 2001). The studies on the male antifertility effects of various medicinal plants have aroused much interest (Seetharam et al., 2003).

Male reproduction anatomy is a complex structure that involves the testes, epididymis, accessory sex glands and associated hormones (Saalu, 2016). Testes perform two highly organized and intricate functions, called spermatogenesis and steroidogenesis, which are crucial for the perpetuation of life. Spermatogenesis, a highly dynamic and synchronized

process, takes place within the seminiferous tubules of the testis with the support of somatic Sertoli cells, leading to the formation of mature spermatozoa from undifferentiated stem cells (Hess and de Franca, 2008). The interstitial compartment, which comprises Leydig cells, is the site of steroidogenesis in the testis (Osinowo, 2006). Several plants are reported to enhance reproductive processes in laboratory animal models.

In male rats the methanolic extract of *Albizzia lebbbeck* pods causes spermatogenic arrest and brought about a significant decrease in sperm motility and density. There was a marked reduction in the numbers of primary spermatocytes, secondary spermatocytes and spermatids (Gupta *et al.*, 2004). Further, administration of saponins isolated from *Albizzia lebbbeck* L. (50 mg kg⁻¹ b.wt. day⁻¹) for 60 days caused a significant decrease in the weights of reproductive organs of rats. The population of various spermatogenic cells in seminiferous tubules decline significantly (Gupta *et al.*, 2005). In contrast, there is paucity of data in regards to the effects of *Albizzia lebbbeck* on rats female reproductive tissues. Hence, this study was designed to investigate the possible effects of aqueous leaf extracts of *Albizzia lebbbeck* on the histology of the male and female reproductive organs of alloxan induced diabetic Albino rats.

2. Materials and methods

2.1 Preparation of plant extract

The plant materials were identified and authenticated by a botanical taxonomist of the Biological Science Department, University of Jos, Nigeria. The analysis and description was in consonance with those found in various literatures. Fresh *Albizzia lebbbeck* leaves were air dried at room temperature for three weeks and pulverized with pestle and mortar. 50g from the coarse powder was macerated with 500mls of distilled water and the sample was left for 48 hours, mixture was filtered and concentrated in a vacuum at 40°C to yield the brown extract of *Albizzia lebbbeck*. The dried extract was transferred into a sample container and stored in desiccators. The extract obtained was weighed and percentage yield determined. For working treatment 1.0g of extract was dissolved in 5mls for the 200mg solution, and 1.0g of extract was dissolved in 10mls for the 100mg solution.

2.2 Experimental rats

Forty (40) weaned albino Rats were used for the study. The Rats were housed and maintained under standard conditions (12 hours' light and dark cycles). Food (pellet feeds and clean

water) was administered ad libitum daily with left over feed and water being recorded before every daily feeding session. The rats were randomly divided into five groups of four rats each.

2.2.1 Grouping of experimental rats.

Group A: Negative control group – This group received no treatment for the duration of the experiment. Distilled water was administered as a placebo.

Group B: Positive control group – This group was administered 100mg/kg body weight Alloxan to induce diabetes but received no other treatment.

Group C: Test group I - This group was administered Alloxan and treated with 100mg/kg body weight of *Albizzia lebbeck*.

Group D: Test group II- This group was administered Alloxan and 200mg *Albizzia lebbeck* leaf extract.

Group E: Treatment control group - This group was administered 100mg of *Albizzia lebbeck* leaf extract only

2.3 Animal treatment

2.3.1 Alloxan induction

All rats in groups B, C and D respectively were treated intra-peritoneally with a single dose of 100mg/kg body weight of Alloxan. The rats used had a mean weight of 130g. This was done three days before beginning treatment with *Albizzia lebbeck* after diabetes had been established using blood glucose levels.

2.3.2 Albizzia lebbeck.

All rats in groups C and E were treated with an oral dose of 100mg/kg body weight of *Albizzia lebbeck*. Group D was treated with a double dose of 200mg/kg body weight of *Albizzia lebbeck*. This treatment was done daily for a period of eight days.

Animals were weighed at the beginning, and after every five days till the end of the experiment. Also food and water intake was monitored daily by weighing left over feed and water before every daily feeding session. Physical examination of fur appearance, Anemia (eye appearance, agility, initial and subsequent sugar levels were also recorded every daily.

There was a baseline sacrifice at the beginning (day 1), a midterm sacrifice after induction of diabetes with Alloxan to confirm diabetes (day 4) and the final sacrifice of all animals at the end of the treatment duration (day 12).

2.4 Histological analysis

All tissue sections used was 5 microns thick. Sectioned tissues were stained by Haematoxylin and Eosin staining technique by progressive method and examined microscopically for photomicrography.

3. Results

3.1 General observations

Table 1: Behavioral observations after treatment with *Albizia lebbbeck*.

Groups	Treatments	Physiological and behavioral Observations
I	Control (Distil Water) (day 1 – day 18)	Smooth fur appearance, rats were active. Eye color was normal but pale after bleeding during the course of the experiment.
ii.	Alloxan (100mg/kg) (day 8 – day 10)	Rough fur appearance, rats were active. Eye color was normal but pale after bleeding during the course of the experiment.
iii.	Alloxan +AL (100mg/kg) (day 11 – day 18)	Rough fur appearance, rats were active. Eye color was normal but pale after bleeding during the course of the experiment.
iv.	Alloxan +AL (200mg/kg) (day 11 – day 18)	Rough fur appearance, some rats were sluggish. Eye color was normal but pale after bleeding during the course of the experiment.
v.	<i>Albizia Lebbeck</i> (200mg/kg) (day 11 – day 18)	Rough fur appearance, rats were active. Eye color was normal but pale after bleeding during the course of the experiment.

Key:• AL- *Albizia lebbbeck*

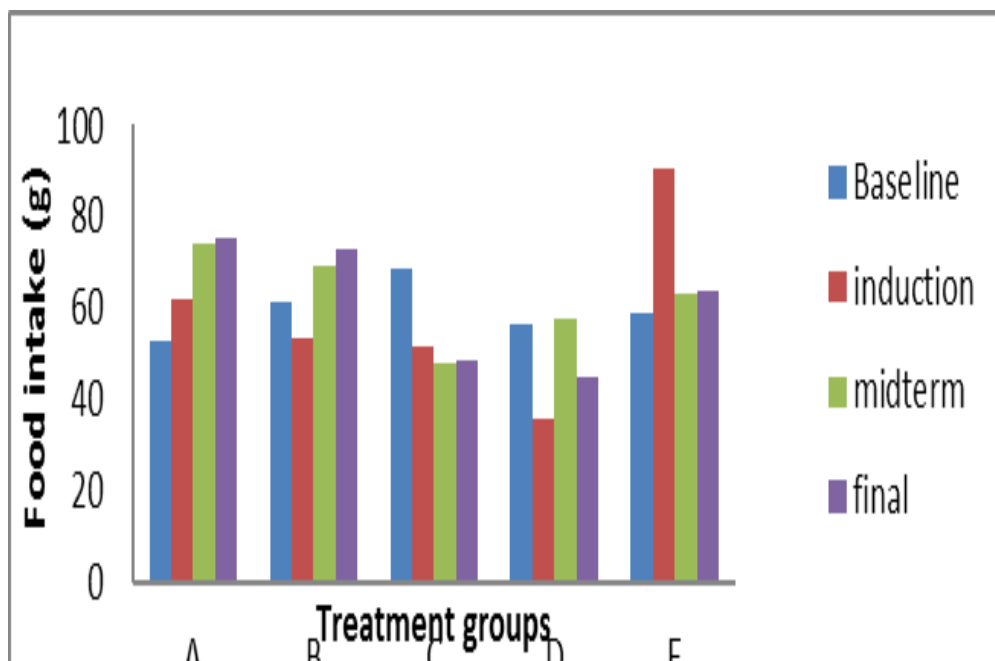


Figure 1: Bar chart showing food intake over duration of experiment in various treatment groups.

Key:

- A – Control (Day 1 - 18)
- B – Alloxan(100mg/kg, day 8 - 10)
- C- Alloxan(100mg/kg)+ *Albizia lebbeck*(100mg/kg, day 11- 18)
- D - Alloxan(100mg/kg)+ *Albizia lebbeck*(200mg/kg, day 11 - 18)
- E - *Albizia lebbeck*(100mg/kg, day 11 - 18)

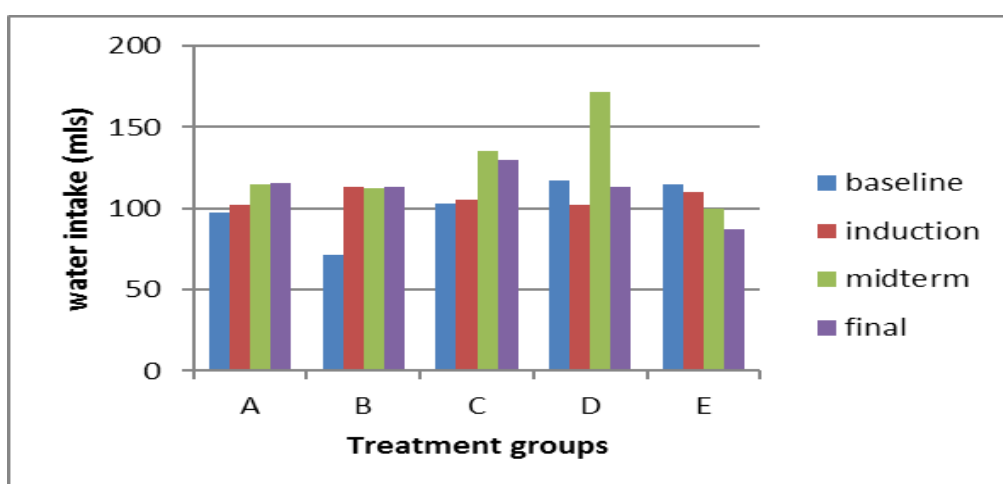


Figure 2: Bar chart showing water intake over duration of experiment in various treatment groups.

Key:

A – Control (Day 1 - 18)

B – Alloxan(100mg/kg) (day 8 - 10)

C- Alloxan(100mg/kg)+ *Albizia lebbeck*(100mg/kg, day 11- 18)

D - Alloxan(100mg/kg)+ *Albizia lebbeck*(200mg/kg)(day 11 - 18)

E - *Albizia lebbeck*(100mg/kg) (day 11 – 18)

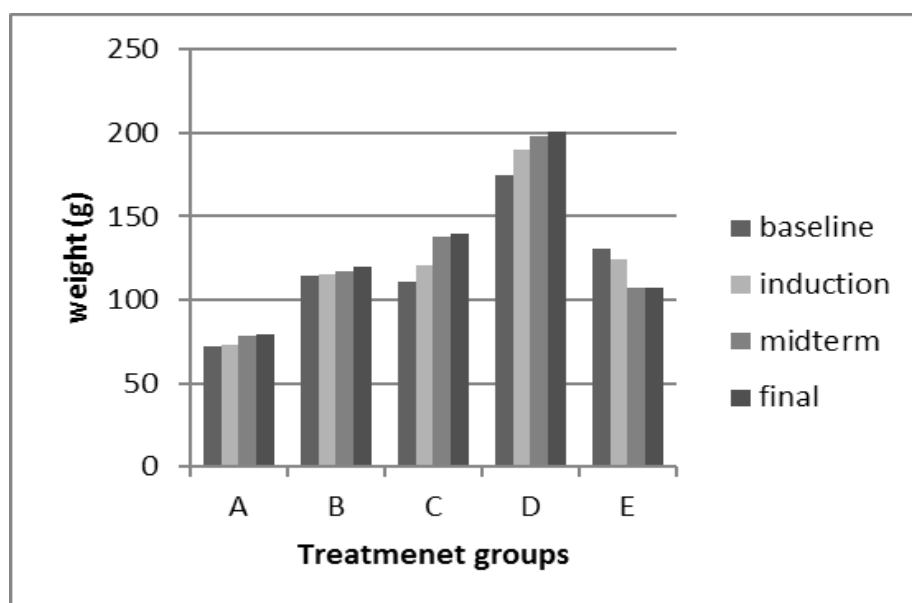


Figure 3: Bar chart showing rat weight (g) over duration of experiment in various treatment groups.

Key:

A – Control (Day 1 - 18)

B – Alloxan(100mg/kg) (day 8 - 10)

C- Alloxan(100mg/kg)+ *Albizia lebbeck*(100mg/kg, day 11- 18)

D - Alloxan(100mg/kg)+ *Albizia lebbeck*(200mg/kg)(day 11 - 18)

E - *Albizia lebbeck*(100mg/kg) (day 11 – 18)

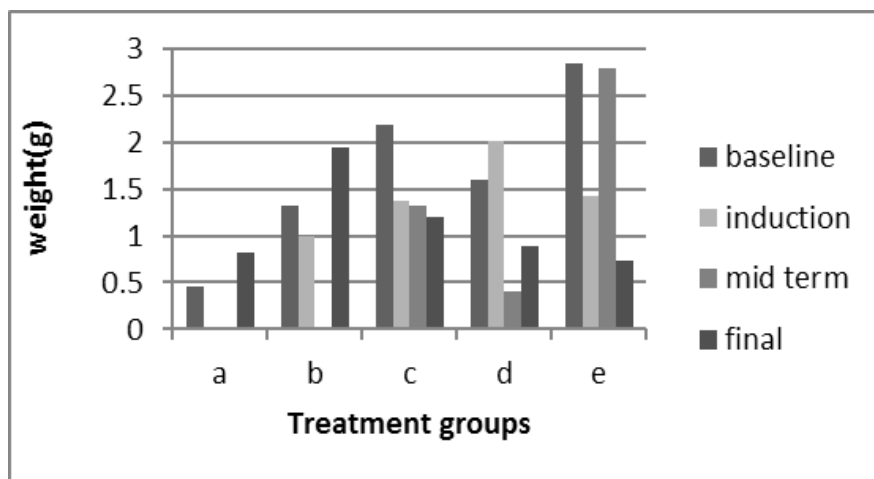


Figure 4: Bar chart showing Female reproductive organ weight over duration of experiment in various treatment groups.

Key:

a – Control (Day 1 - 20)

b – Alloxan(100mg/kg, day 8 - 10)

c- Alloxan(100mg/kg)+ *Albizia lebbeck*(100mg/kg, day 11-18)

d - Alloxan(100mg/kg)+ *Albizia lebbeck*(200mg/kg, day 11 - 18)

e - *Albizia lebbeck* (100mg/kg, day 11 - 18)

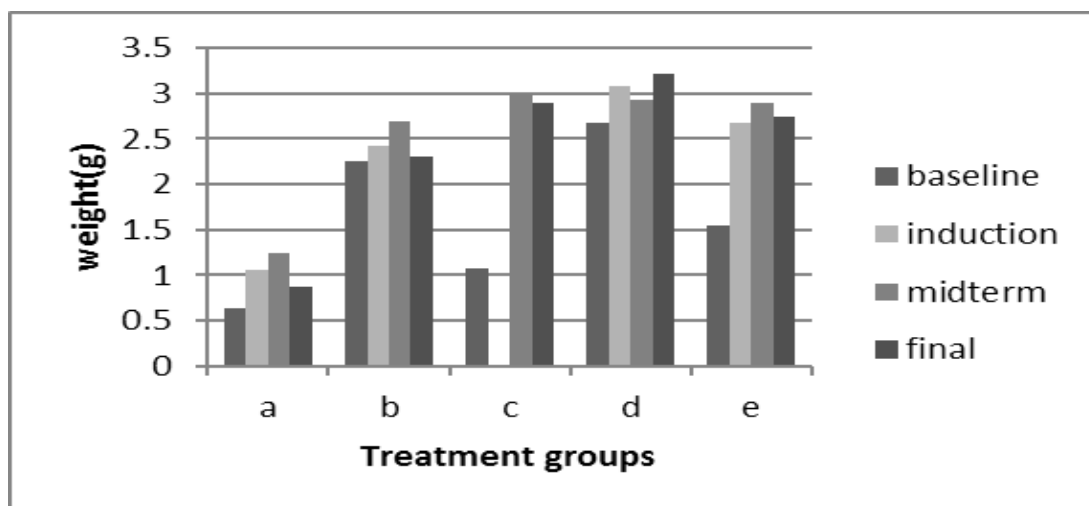


Figure 5: Bar chart showing Testis weight over duration of experiment in various treatment groups.

Key:

a – Control (Day 1 - 18)

b – Alloxan (100mg/kg) (day 8 - 10)

c- Alloxan (100mg/kg)+ *Albizia lebbeck* (100mg/kg, day 11- 18)

d – Alloxan (100mg/kg)+ *Albizia lebbeck* (200mg/kg)(day 11 - 18)

e - *Albizia lebbeck* (100mg/kg) (day 11 - 18)

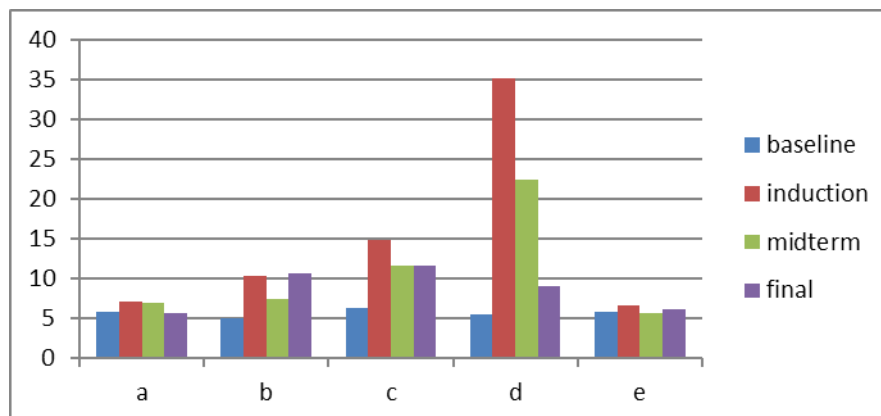
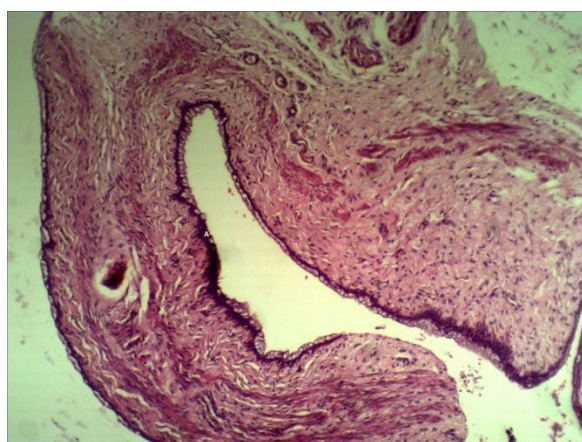


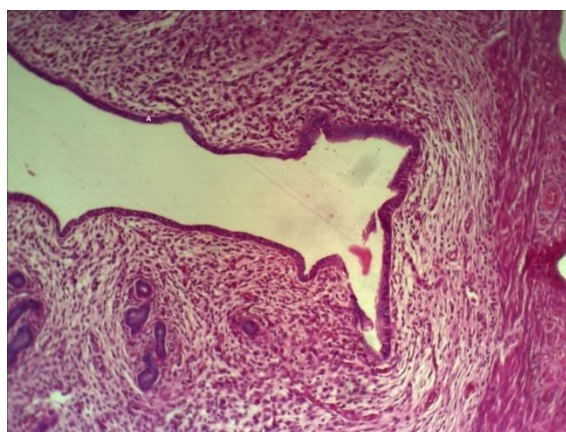
Figure 6.0: Bar chart showing blood glucose levels (mmols/l) over duration of experiment in various treatment groups.

3.2 Photomicrographs

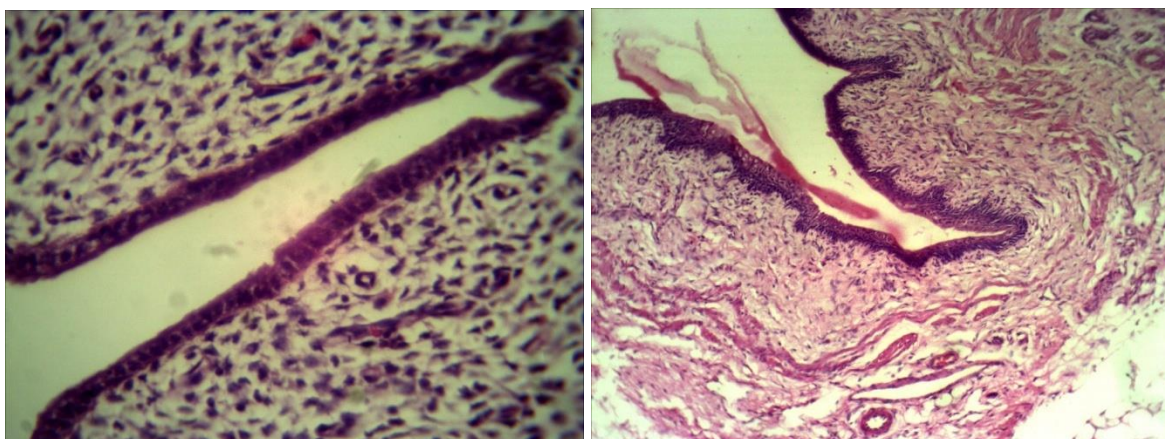
3.2.1. Histological appearance of Uterus



A

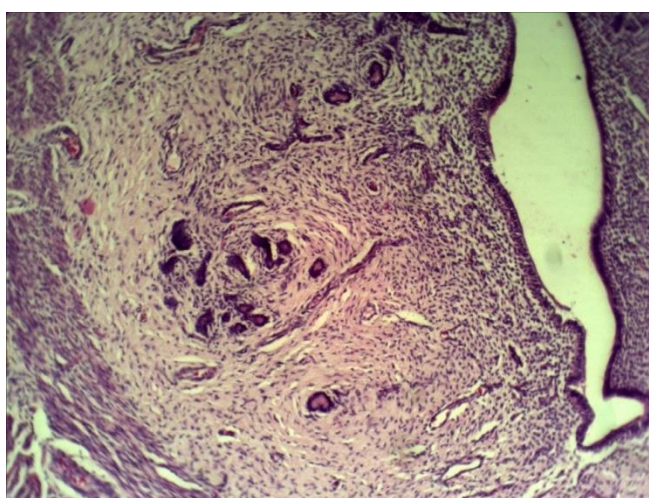


B



C

D



E

Slide A: Histological section of the uterus showing a well defined Oestrus endometrium (A), magnification x100, H&E staining technique, (Control).

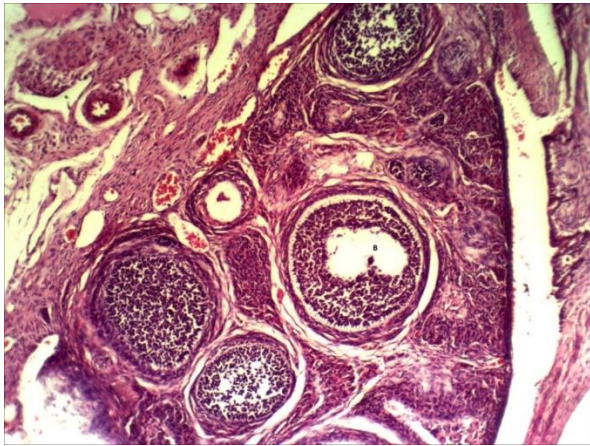
Slide B: Histological section of Uterus showing well defined endometrium (A). Magnification x 100, H&E staining technique, (Alloxan 100mg/kg)

Slide C: Histological section of Uterus showing well defined endometrium. Magnification x400, H&E staining technique, (Alloxan100mg/kg + *Albizia lebbeck*100mg/kg)

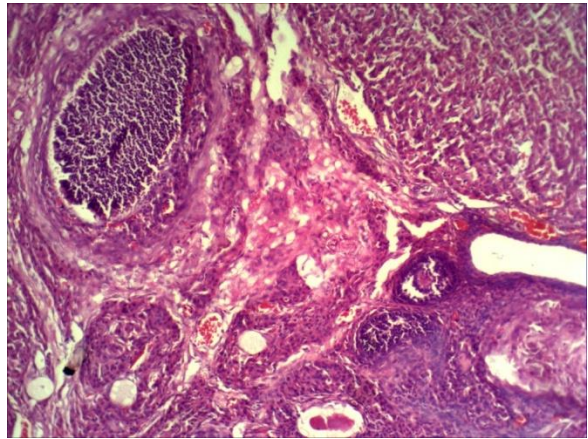
Slide D: Histological section of Uterus showing well defined endometrium. Magnification x100, H&E staining technique (Alloxan 100mg/kg + *Albizia lebbeck* 200mg/kg)

Slide E: Histological section of Uterus showing well developed endometrium. Magnification x100, H&E staining technique (*Albizia lebbeck* 100mg/kg)

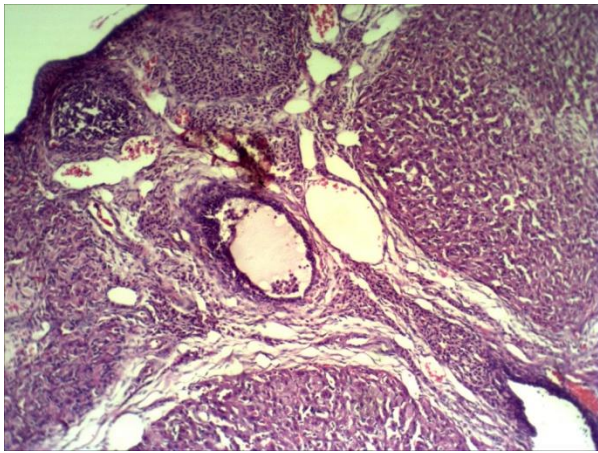
3.2.2 Histological appearance of ovaries



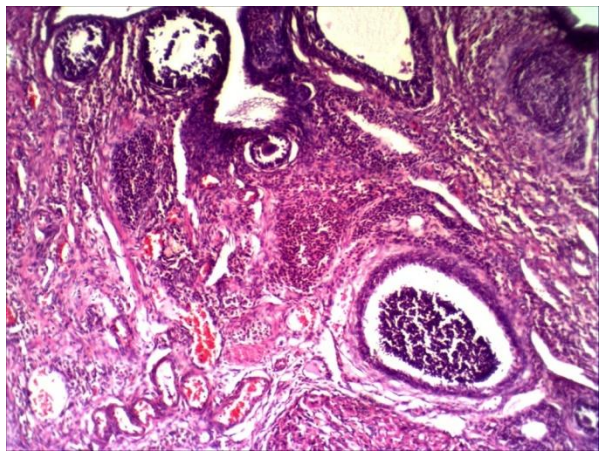
A



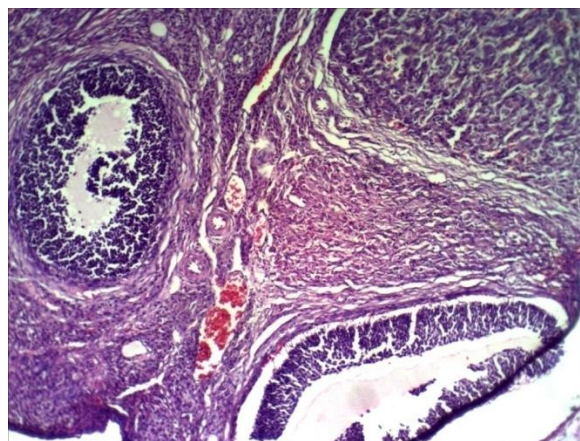
B



C



D



E

Slide A: Histological section of an Ovary with multiple follicles of different sizes. Several large follicles are seen with one showing having ovulated. Magnification x100. H&E staining technique, (Control).

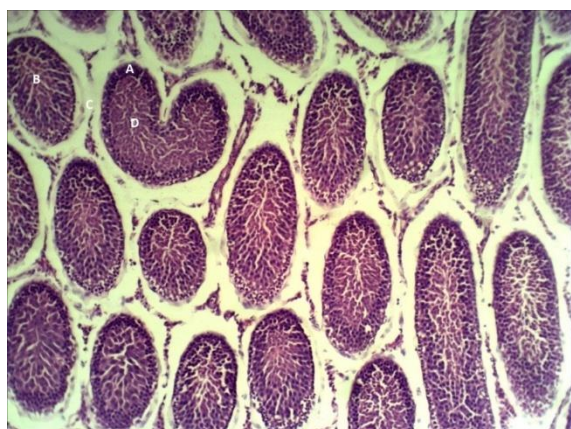
Slide B: Histological section of Ovary shows fewer follicles compared to the control group, one large follicle seen. Magnification x100, H&E staining technique (Alloxan 100 mg/kg)

Slide C: Histological section of Ovary shows few follicles compared to the control group, empty follicles seen. Magnification x100, H&E staining technique (Alloxan 100mg/kg + *Albizia lebbbeck* 100mg/kg)

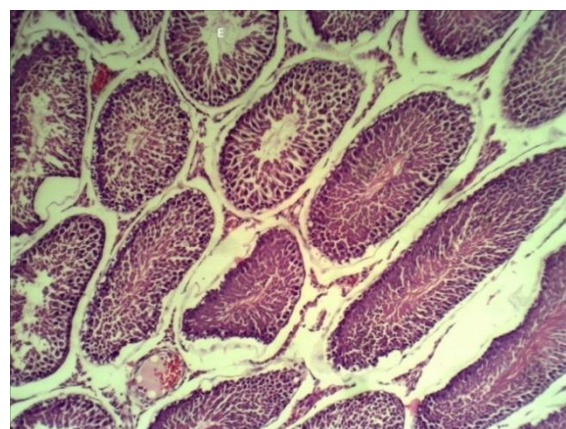
Slide D: histological section of Ovary shows few viable follicles and some empty follicles seen. Magnification x100, H&E staining technique (Alloxan 100mg/kg + *Albizia lebbbeck* 200mg/kg)

Slide E: Histological section of Ovary showing a decrease in oogeneis, few viable follicles seen. Magnification x100, H&E staining technique (*Albizia lebbbeck* 100mg/kg)

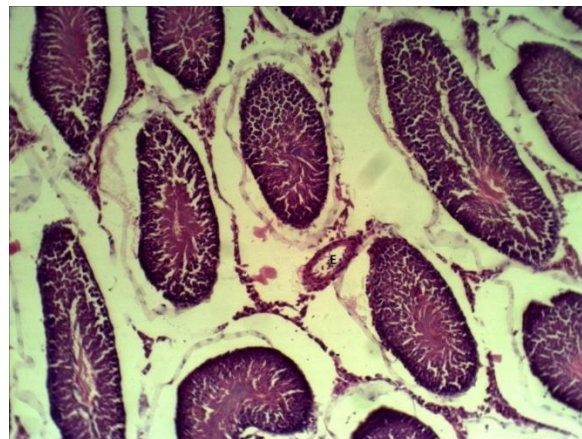
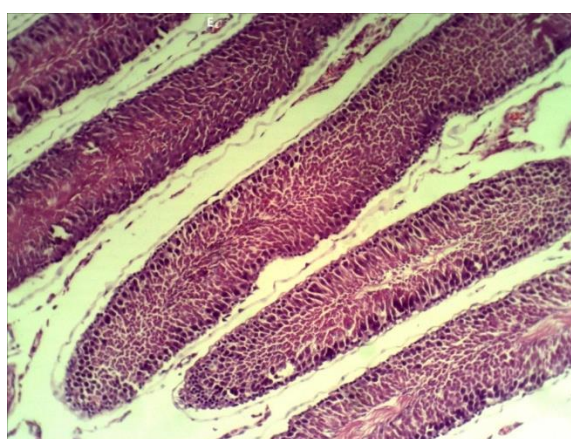
3.3.3 Histological appearance of Testes



I

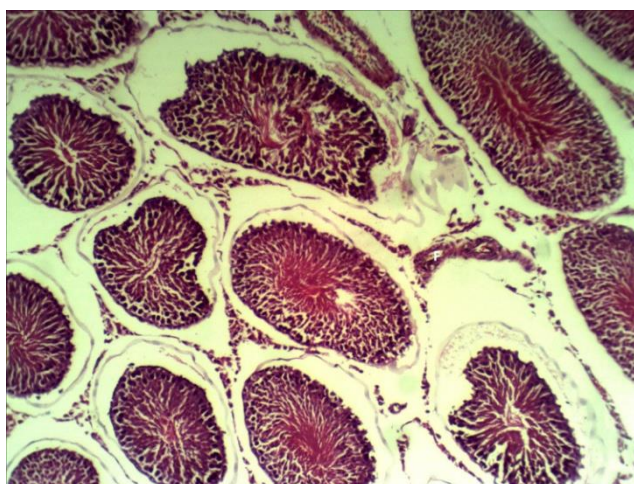


II



III

IV



V

Slide I: Histological section of Testicular tissue showing numerous seminiferous tubules with well defined germinal epithelium (Spermatogonia) (A) and Spermatids (B) in development. The seminiferous tubules are separated by minimal interstitial tissue (C). Magnification x100, H&E staining technique (Control)

Slide II: Histological section of Testicular tissue showing seminiferous tubules with increased spermatogenesis. Many mature spermatocytes (E) with tails lined within the tubules are seen. Germinal epithelium is not as pronounced as in control. Magnification x100, H&E staining technique (Alloxan 100mg/kg)

Slide III: Histological section of Testicular tissue showing larger seminiferous tubules with many well developed spermatocytes in the lumen. Germinal epithelium is thinner indicating a reduction in spermatogenesis. Magnification x100, H&E staining technique, (Alloxan 100mg/kg + *Albizia lebbeck* 100mg/kg)

Slide IV: Histological section of Testicular tissue showing larger seminiferous tubules with many well developed spermatocytes in the lumen. Germinal epithelium is thinner indicating a reduction in spermatogenesis. Sperm duct (E) containing few spermatids are seen amidst seminiferous tubules. Magnification x100, H&E staining technique, (Alloxan 100mg/kg + *Albizia lebbeck* 200mg/kg)

Slide V: Histological section of Testicular tissue showing seminiferous tubules with many well developed spermatocytes in the lumen. Germinal epithelium is thinner indicating a reduction in spermatogenesis. Numerous spermatids seen and artery is present. Magnification x100, H&E staining technique (*Albizia lebbeck* 100mg/kg)

4. Discussion

There was no mortality recorded in all groups over the course of the experiment. This showed that a dosage as high as 200 mg/kg body weight does not constitute lethality.

At the start of experiment all rats had a smooth fur appearance, normal eye color (indicating there was no anemia) and were active. As experiment progressed, after induction of diabetes and subsequent treatment with *Albizia lebeck* (day 8 - 18) furs in treatment groups (groups B-E) were seen to become rough while control group maintained a smooth fur appearance. Rough fur appearance is often as a result of an underlying problem especially dehydration. Eye color in all groups including control became paler likely due to intermittent bleeding (indicating anemia) as experiment progressed. Rats remained active in all groups over the course of the experiment (day 1 - 18).

Food intake (Figure 1) was seen to increase progressively all through the experiment in the control group. Groups induced with diabetes (groups B, C, D) showed a decrease in feed intake during the period of induction (Day 8- Day 11). An average of 7% decrease was observed between these groups compared to control that had a 3% increase. This decrease in feed intake on days (8 -10) might be as a result of stress on the rats during induction. As experiment progressed groups treated with *Albizia lebeck*, (C, D, E) still continued to have a reduced food intake, though group IV (Alloxan + *Albizia lebeck* 200mg/kg) showed a slight increase between days 11 to day 14 before dropping again. This decrease in food intake was most obvious in Group V (*Albizia lebeck* (100mg/kg) which was not induced with diabetes but treated with the plant extract. The aqueous leaf extract of *Albizia lebeck* is responsible for this decrease in food intake, as it has been found to reduce feed intake in non ruminant animals (Cornell University, 2008). Group B (Alloxan 100mg/kg) which was induced with diabetes but not treated showed an increase in food intake which continued progressively till the end of the experiment (day 8- 11). This increased food intake in the diabetes only group supports the findings of (Havel *et al.*, 2000) which noted an increased food intake in induced diabetic rats.

Water intake (Figure 2) increased continuously in the control group (group A). Group B (Alloxan 10mg/kg) showed a significant increase in water intake after induction with diabetes (day 8- day 18). This increased water intake was continuous all through the duration of the experiment. Increased water intake is characteristic of diabetes, as supported by the findings of a study conducted (Carvalho *et al.*, 2003) which stated that diabetes results in increased

water intake as a result of polydipsia (abnormal thirst) and polyuria. This increase was also recorded in treatment group C (Alloxan + *Albizia lebbeck* 100mg/kg) and group D (Alloxan + *Albizia lebbeck* 200mg/kg) after induction with diabetes (day 8 - 14), but decreased as treatment with plant extract progressed. This decrease might have been as a result of the hypoglycemic activity of *Albizia lebbeck* (100mg/kg). Group E treated with *Albizia lebbeck* (100mg/kg) only, compared to the control group, showed a progressive decrease in water intake as experiment progressed, This suggest *Albizia lebbeck* causes a decrease in water intake probably as a consequence of water retention. An experiment conducted by (Mohammed *et al.*, 2012) showed *Albizia lebbeck* showed no increased diuretic activity, though it was not stated if it causes a reduction in diuresis.

Rat weights (Figure. 3) recorded weekly showed a continuous increase in group I (control), group II (Alloxan), group C (Alloxan + *Albizia lebbeck*), and group D (Alloxan + *Albizia lebbeck* (200mg/kg) all through the course of the experiment, though, in group C and group D this contradicts with the decline in food intake that recorded. As declining food intake was supposed to show a resultant decrease in weight. Group E on the other hand showed a constant decrease in weight as the experiment progressed. This weight loss was most characteristic on commencement of treatment with the aqueous *Albizia lebbeck* leaf extract (day 11-18). This relates to the decrease in food intake on commencement of treatment in this same group. This weight loss is as a caused by the aqueous leaf extract of *Albizia lebbeck*, especially its saponin content which has been found to reduce growth rate in non ruminant animals (Cornell University, 2008)

The blood glucose levels (Figure. 6) in the induced groups (Groups B, C, and D) showed a sharp increase to different extents through the period of experiment showing that diabetes had been successfully established using Alloxan in the rats in these groups. The Un-induced groups (Group A and E) showed a much lower increase in blood sugar levels. On commencement of treatment with aqueous leaf extract of *Albizia lebbeck* (day 11 - 18) group C (Alloxan + *Albizia lebbeck* 100mg/kg) and group D (Alloxan + *Albizia lebbeck* 200mg/kg) both showed a decrease in blood sugar levels compared to the high levels recorded after induction. The blood glucose levels continued to drop in these groups as the experiment progressed. This hypoglycemic property of *Albizzia lebbeck* is as a result of saponins present in the plant which give the plant antioxidant properties (Shirode *et al.*, 2012). Group D showed a greater drop in blood sugar levels than group C. Indicating that the doubling the dosage concentration increased the hypoglycemic activity of the plant. This decrease was also

observed in group E (*Albizia lebbeck* 100mg/kg) which was not induced with diabetes but treated with the leaf extract. Group B (Alloxan only) maintained the high blood sugar levels obtained at induction, while blood sugar levels within the control group continued to fluctuate within the normal range (2.774 – 8.324mmol/l)

The histological morphology of the uterus showed no morphology damage either by treatment with *Albizzia lebbeck* at varying dosages or induced diabetes. The uterus of group E (*Albizzia lebbeck* only) showed a better developed endometrium which may be as a result of the rat being older (Slide I). The control group also showed a uterus with an oestrus endometrium (Slide A)

The histology of the ovaries showed there was a progressive decrease in the number of ovarian follicles with the control group (Slide A) having the greatest number of follicles and (Slide F) group E (*Albizzia lebbeck* only) having the least. Compared to uterine morphology where group E (Slide E) had the most developed uterine endometrium which suggested the rat was developed reproductively, fewer ovarian follicles is a possible indication of a decrease in oogenesis. This raises the question if *Albizzia lebbeck* is favorable to oogenesis. Also empty follicles were observed in groups A, group C and group D (Slide A, Slide E and Slide G respectively) an indication that ovulation had taken place.

The histology of the Testis showed numerous semeniferous tubules with well-defined spermatogonia and spermatids. There was a continuous decrease in germinal epithelium observed in all groups from groups A (Slide G) to E (Slide H). This shows there was a decrease in spermatogenesis. Though thinner germinal epithelium was observed more matured spermatids are seen. This may be as a result of the rat's age with older rats having more developed spermatids. This findings support the findings of (Gupta *et al.*, 2006) which propose that *Albizzia lebbeck* causes male infertility by arresting spermatogenesis. An experiment conducted by (Rakesh *et al.*, 2007) points to the activity of triterpenes being responsible for this negative effect on the male reproductive system.

4.1 Conclusion

The histological effects of *Albizzia lebbeck* on the reproductive organs of Alloxan induced diabetic rats showed in males, it causes a reduction in spermatogenesis and in females it causes no remarkable change to the histological integrity of the organs, though a possible arrest in oogenesis is suspected. However, *Albizzia lebbeck* should be subjected to

further analysis in order to unravel the active ingredients responsible for their antifertility actions.

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