Original Research Article

Evaluation of Methanol Extract of *Napoleonaimperialis* Seeds on Some Biochemical Indices, Fertility status and Organs of Albino Rats

ABSTRACT

The present study evaluated the *in vivo* toxic effect of methanol extract of *Napoleonaimperialis* seeds on some biochemical indices and organs of albino rats. The phytochemical constituents as well as the acute toxicity test of the extract were evaluated using standard methods. Twenty five experimental rats were divided into five groups of five rats each, group 1 was the control. They were administered the extract orally for 28 days, on the 29th day after an overnight fast, blood was collected and organs harvested after sacrificing the animals. The biochemical parameters; malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD),reduced glutathione (GSH) and catalase (CAT) activities were analyzed, caudal epididymal and testicular sperm cells were counted, histological examination of the testis, liver and kidney was also performed. The results showed that methanol extract of the seeds contain phenols, flavonoids, carotenoids, carbohydrates, saponins, alkaloids, hydrogen cyanide, phytates, tannins and oxalates. LD_{50} was not calculated since no death of mice was recorded in both phases. The extract increased MDA concentration and CAT activity at 600 mg/kg with no significant ($p > 0.05$) difference in the activities of GPx and SOD of the experimental groups compared to the control. GSH concentrations also showed no significant ($p > 0.05$) difference when compared to control. The extract reduced caudal epididymal and testicular sperm counts non-significantly ($p > 0.05$) . Photomicrograph images of the liver, kidney and testis showed no histological changes. These results demonstrate that the seed extract may not be toxic to the vital organs at the doses administered although mild elevations were observed in catalase and little reduction of sperm counts. The extract. may induce lipid peroxidation due to significantly increased concentration of MDA.

Keywords:*Napoleonaimperialis* anti-oxidant enzymes, phytochemistry , Toxicity, vital organs.

INTRODUCTION

Evaluation of toxicity levels is generally important in screening of natural products with nutritional, pharmacological and medicinal potentials. Toxicity is defined as a science that defines the limits of safety of chemical agents for human and animal populations [1]. Toxicity tests for bioactive compounds are mostly used to examine specific adverse biological events or specific end points such as cancer, cardio toxicity, and skin/eye irritation. Toxicity testing also helps calculate the No Observed Adverse Effect Level (NOAEL) dose and is helpful for clinical trials [1]. Toxicity can be measured by effects on the target such as the organism, organ, tissue or cell. Because individuals have different levels of response to the same dose of a toxin, a population-level measure of toxicity is often used which relates the probabilities of an outcome for a given individual in a population [2].

The plant, *N. imperialis* belongs to the family *Lecythidaceae* which is a small tropical family that grows in all the regions of Nigeria and other parts of West Africa [3]. The plant is commonly known as ntum in the Ikwuano dialect of Ibo language in Nigeria..I It is a wild plant with potentials as an alternative livestock feed source {4}. In the rural communities, the trunks and branches serve for firewood. The leaves are cut and fed to livestock as forage or used as green manure while the epicarp (flesh) and seeds are discarded after the pulp is eaten by humans. Studies [5] have confirmed the presence of anti-nutritional factors in raw *N. imperialis* seeds and the resulting negative performance of animals fed these seeds.There is therefore need to evaluate the extent of toxicity caused by ingesting seed of *N. imperialis* on organs such as heart, liver, kidneys and testes.This study therefore aimed to evaluate the toxicological effects of methanol extract of *N.imperialis* seeds on some biochemical parameters and organs of albino rats.

MATERIALS AND METHODS

Materials

Plant material

Fresh fruits of *N. imperialis* were harvested from a habitat in Aku, Igbo-Etiti Local Government Area of Enugu State, Nigeria. The plant materials were identified and authenticated at Bioresources and Development Centre, University of Nigeria, Nsukka, A specimen was deposited at the herbarium of the Department for reference purposes.

Animals

The animals used for this work were adult wistar albino rats and mice. They were obtained from the Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were acclimatized for seven days under standard environmental conditions and were maintained on a regular feed and clean water.

Preparation of the Plant Extract

Seeds of the ripe and mature fruit were removed from the pods and washed with clean water. The seeds were air-dried at room temperature and decoated. The dried and decoated seeds were pulverized into coarse powder. The pulverized powder (1 kg) was soaked in 2.5 litre of methanol for 72 hours at room temperature with occasional stirring. The suspension was filtered using a mesh followed by Whatman No. 4 filter paper and concentrated using water bath at 40˚C. The extract was refrigerated till further use. Percentage yield was calculated as follows;

Qualitative Phytochemical Analyses of the Extracts

The preliminary phytochemical screening of the methanol extract of *N. imperialis* seeds was carried out according to the methods of [7], [8] and [9].

Acute Toxicity Test

The median lethal dose (LD_{50}) was determined using Lorke's method[6]. This method has two phases which are phases 1 and 2 respectively. **In phase 1, n**ine mice were used in this phase. The nine animals were divided into three groups of three animals each. Each group of animals was administered different doses (10, 100 and 1000 mg/kg) of the extract orally. The mice were placed under observation for 24 hours to check for any mortality. In phase 2, four mice were used, they were distributed into three groups of one mice each. The mice were administered higher doses (1500, 2250, 3500 and 5000 mg/kg) of the extract and then observed for 24 hours for behavior as well as mortality.

Experimental Design

A total of twenty five male albino rats (age) were divided into five groups of five rats each and treated as follows;
Group 1: Normal control (1 ml/kg of distilled water)
Group 2: 100 mg/kg of methanol extract of *N. imperialis* seeds
Group 3: 200 mg/kg of methanol extract of *N. imperialis* seeds
Group 4: 400 mg/kg of methanol extract of *N. imperialis* seeds
Group 5: 600 mg/kg of methanol extract of *N. imperialis* seeds
The rats were administered the extract orally for 28 days. On day 29, after an overnight fast, blood samples were collected from ocular vein for haematological and biochemical analyses after which the rats were humanely sacrificed by suffocation with mild chloroform and the liver, kidney and testis harvested for histopathology. One of the testis and the epididymis were further crushed to release sperm cells for sperm count

Determination of Biochemical parameters

Malondialdehyde (MDA) concentration was measured spectrophotometrically [10]. Glutathione peroxidase (GPx) activity was assayed spectrophotometrically according to the method of [11]. Superoxide dismutase, (SOD),Catalase activities, Glutathione (GSH) concentration were assayed spectrophotometrically according to the method of International Federation of Clinical and Applied Chemistry [12] as outlined in the RANDOX.was carried out according to the method outlined in Randoxkits.The testicular and caudal epididymal sperm reserves were determined using the standard hemocytometric method [15].

Histological Examination

This was carried out as described by Bancroft and Stevens (2002).

Procedure: At the end of the experiment the liver, kidney and testicles from group 1 and 5 were collected for histopathology.The organs were fixed in 10% formal saline and dehydrated in ascending grades of (70%, 90% and 100%) ethanol respectively. Thereafter, the tissues were cleared in chloroform overnight, infiltrated and embedded in molten paraffin wax. The blocks were later trimmed and sectioned at 5 – 6 microns. The sections were de-paraffinized in xylene, taken to water and subsequently stained with Haematoxylin and Eosin (H and E) for light microscopy.

Statistical analysis

The data obtained were analyzed using one-way analysis of variance with the help of a software known as IBM statistical product and service solution (SPSS) package, version 20.0 and the results were expressed as mean ± standard deviation followed by LSD and Duncan test for level of significance. The acceptance value of significance was $p < 0.05$ for all the results.

RESULTS

Percentage yield of the Extract

The percentage yield of the extract was 5.4%

Qualitative and quantitative phytochemical Analysis

The qualitative phytochemical composition of *N. imperialis* seed extract showed the presence of hydrogen cyanide in high amount. Phenols, flavonoids, carotenoids, carbohydrates and alkaloids in low amounts while saponins was present in moderate amount.

Quantitatively, the amount of phenols was found to be 0.81±0.11 g/100g, flavonoids, (0.79±0.20) carotenoids, (1.25±0.47),carbohydrates, (0.99±0.18) and alkaloids, (0.56±0.10)g/100g (table 1).

Table 1: Qualitative and quantitative Phytochemical composition of methanol extract *Napoleonaimperialis* seed extract

Phytochemicals	Qualitative composition	Quantitative Composition (g/100mg)
Phenols	+	0.81±0.11
Flavonoids	+	0.79±0.20
Carotenoids	+	1.25±0.47
Carbohydrates	+	0.99±0.18
Saponins	++	1.99±0.64
Alkaloids	+	0.56±0.10
HCN (mg/kg)	+++	18.74±3.57
Phytates	+	1.56 ±0.31
Tannins	+	1.35±0.61
Oxalates	+	0.85±0.19

Results are expressed in mean ± SD (n = 3)

Key:
+ Present in low amount
++ Present in moderate amount
+++ Present in high amount

Median Lethal Dose (LD_{50}) of Methanol Extract of *Napoleonaimperialis* Seeds

As shown in table 2, no mortality was observed in the mice treated with different doses of the extract in both phase one and phase two. Hence LD_{50} was not calculated.

Table 2: Acute toxicity studies of methanol extract *N.imperialis* seed extract

Phase 1

S/NO.	Dose (mg/kg b.w)	Mortality
1	10	0/3
2	100	0/3
3	1000	0/3

Phase 2

S/NO.	Dose (mg/kg b.w)	Mortality
1	1900	0/1
2	2600	0/1
3	5000	0/1

Effect of methanol Extract of *N. imperialis* Seed Extract on malondialdehyde and Antioxidant parameters of Rats

Table 3 shows non-significant ($p > 0.05$) difference of MDA in the test groups (2- 4) compared to the control except group 5 rats that were administered the highest dose of 600mg/kg b.w extract which was significantly ($p < 0.05$) higher compared to group 1.

GPx activity was observed to decrease non-significantly ($p > 0.05$) in groups 2-4 compared to the control and increased non-significantly ($p > 0.05$) in group 5 compared to the control.

There was non-significant ($p > 0.05$) increase of SOD in all the test groups compared to the control as shown in table 3. A non-significant ($P > 0.05$) increase of catalase activity was observed in all the treated groups compared to the control except group 5 rats that were administered 600mg/kg b.w extract where the value was found to be significantly ($p < 0.05$) higher compared to the control, as shown in table 3.From table 3, GSH could also be seen to decrease non-significantly ($p > 0.05$) in group 2 and 3 and increased non-significantly ($p > 0.05$) from group 4 down to group 5 compared to the control.

Table 3: Effect of Methanol Extract of *N.imperialis* Seeds on the pro- and antioxidant parameters of Rats

Treatment Group	**MDA and Anti-oxidant Indices**				
	MDA Conc. (mg/dl)	**GPx Activity (IU/L)**	**SOD Activity (IU/L)**	**Catalase (IU/L)**	**GSH Conc. (mg/dl)**
Group 1	3.60 ± 1.54^{a}	22.90 ± 3.55^{c}	11.61 ± 1.59^{d}	3.32 ± 1.71^{e}	3.38 ± 1.43^{g}
Group 2	5.74 ± 1.14^{a}	20.91 ± 3.28^{c}	12.38 ± 2.17^{d}	3.77 ± 2.17^{e}	$1.44\pm.49^{g}$
Group 3	3.68 ± 2.08^{a}	21.94 ± 2.4^{c}	12.34 ± 2.21^{d}	4.23 ± 1.58^{e}	3.33 ± 2.33^{g}
Group 4	5.03 ± 2.14^{a}	21.41 ± 1.94^{c}	12.95 ± 2.25^{d}	3.60 ± 1.28^{e}	4.43 ± 2.43^{g}
Group 5	9.94 ± 2.58^{b}	23.31 ± 4.05^{c}	13.32 ± 5.29^{d}	7.95 ± 4.00^{f}	5.18 ± 3.67^{g}

Results are expressed in Means ± SD (n = 5)

Mean values with different letters as superscripts down the column are considered significant at $p < 0.05$

Group 1 = Normal Control
Group 2 = 100 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds
Group 3 = 200 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds
Group 4 = 400 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds
Group 5 = 600 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds

Effect of methanol extract of *N. imperialis* seeds on Caudal Epidydermal and testicular Sperm Counts

Caudal epidydermal sperm count in all the test groups compared to the control showed non-significant ($p > 0.05$) decrease as shown in table 4.

A non-significant ($p > 0.05$) decrease of testicular sperm count in the treated groups compared to the control was also observed from groups 2, 3 and 4 and increased non-significantly ($p > 0.05$) in group 5 rats that were administered 600 mg/kg b.w.

Table 4: Effect of Methanol Extract of *N. imperialis* Seeds on Sperm counts of Rats

Treatment	**Sperm Count**

Group	Caudal Epidermal (10^6/ml)	Testicular (10^6/ml)
Group 1	107.66 ± 5.37^a	42.00 ± 6.00^b
Group 2	103.20 ± 4.41^a	41.26 ± 2.47^b
Group 3	102.60 ± 2.49^a	42.80 ± 1.38^b
Group 4	95.00 ± 1.39^a	40.62 ± 2.81^b
Group 5	101.88 ± 7.68^a	44.46 ± 2.57^b

Results are expressed in Means ± SD (n = 5)

Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$

Group 1 = Normal Control

Group 2 = 100 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds

Group 3 = 200 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds

Group 4 = 400 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds

Group 5 = 600 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds

Photomicrographs of Methanol Extract of *Napoleonaimperialis* Seeds on some Metabolic Organs liver, kidney and testis of rats.

Effect of methanol extract of *N. imperialis* seeds on Liver of Rats

As shown in the plates below, photomicrograph images of both the liver, kidney and testis of rats appear normal, with no physical damage to them.

Plates 1 a and1 b: Photomicrograph of the liver from the experimental groups of rats untreated (U) and treated (T) with 600 mg/kg seed extract of *N. imperialis* showing apparently normal plates of hepatocytes (arrows) separated by sinusoids and radiating away from the central vein (V). H and E x 400

Effect of methanol extract of *N. imperialis* seeds on Kidney of Rats

Plates 2 a and2 b: Photomicrograph of the kidney from the experimental groups of rats untreated (U) and treated (T) with 600 mg/kg seed extract of *N. imperialis* showing the glomerulus (GL) and renal tubules (arrows) with no remarkablle histologic change. H and E x 400.

Effect of methanol extract of *N. imperialis* seeds on Testis of Rats

Plates 3a and 3b: Photomicrograph of the testis from the experimental groups of rats untreated (U) and treated (T) with 600 mg/kg seed extract of *N.imperialis* showing apparently normal seminiferous tubules (ST) separated by inter tubular connective tissue septa (white arrows). H and E x 400.

Photomicrographs of Methanol Extract of *Napoleonaimperialis* Seeds on liver, kidney and testis of rats.

Plate 1a: Untreated liver (control)

Plate 1b: Treated liver with 600mg/kg b.w

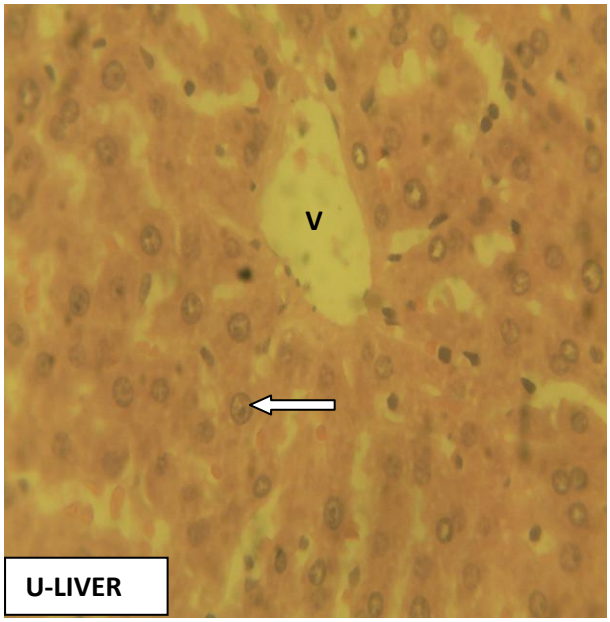


Plate 2a: Untreated kidney (control)

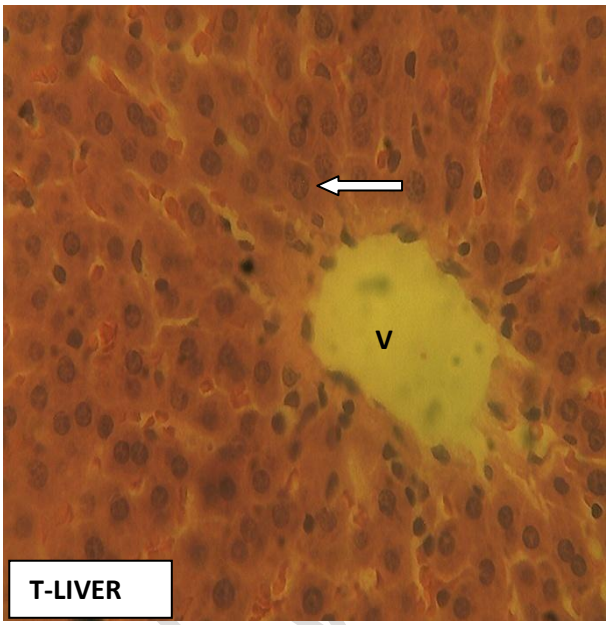


Plate 2b: Treated kidney with 600mg/kg b.w

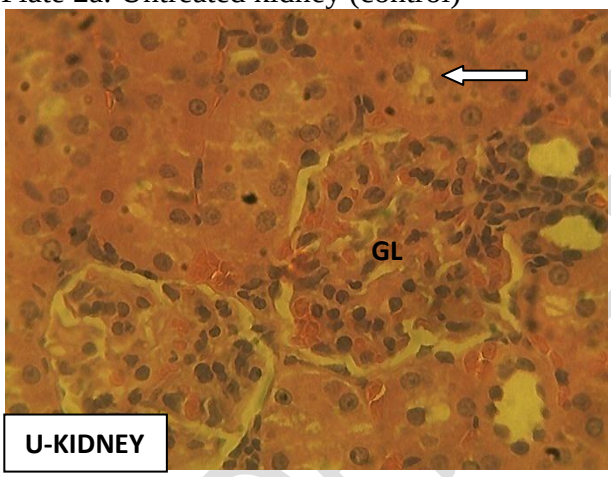


Plate 3a: Untreated testis (control)

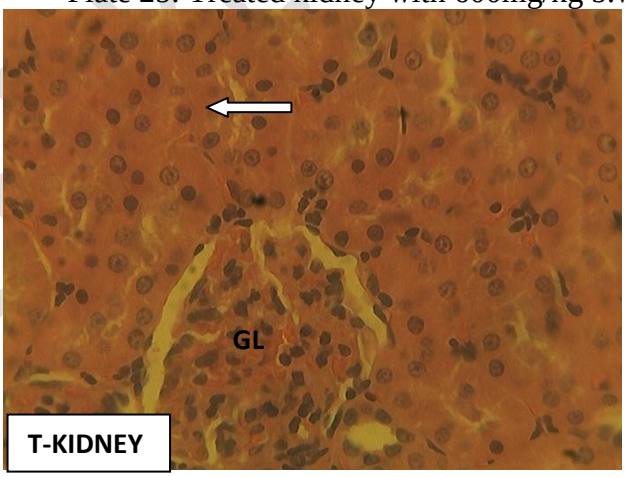
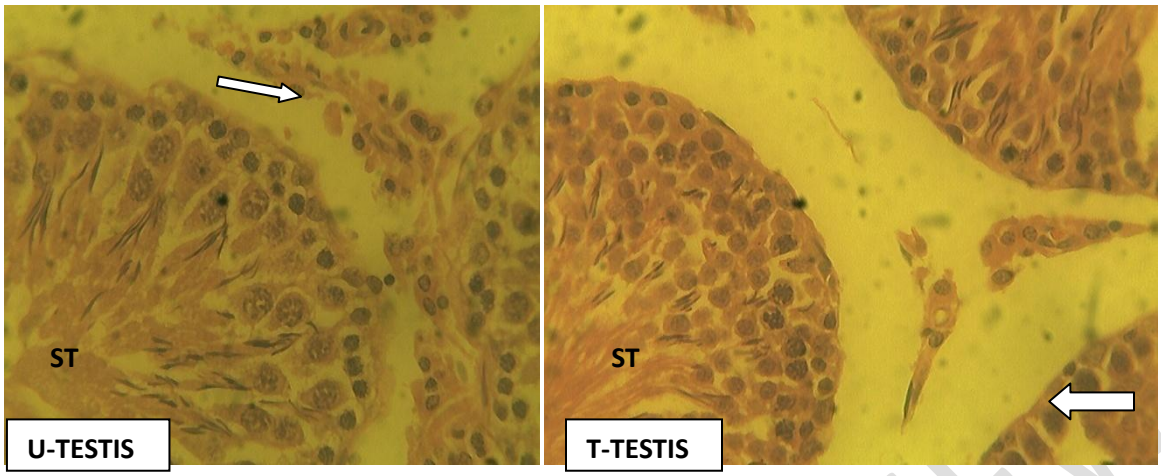


Plate 3b: Treated testis with 600mg/kg b.w



Discussion

The presence of the following phytochemicals; phenols, flavonoids, carotenoids, carbohydrates, saponins, alkaloids, hydrogen cyanide, phytates, tannins and oxalates in the seed extract corresponds with previous research findings by[17] and[18]. The 18.74 mg/kg HCN values found in raw *Napoleonaimperialis* seed was found to be much lower than those found in cowpea seeds (40 mg/kg) and was beyond the upper limit of 10 mg/kg HCN reported to be safe for human consumption [19]; [20]. The moderate amount of saponin content of the seed extract corresponded with [21] who stated that *N. imperialis* seed could be considered as a good source of haemolytic saponins.

The death of one out of three mice administered 5000 mg/kg b.w in phase 2 (acute toxicity) test may be due to the presence of HCN and other anti-nutrient compounds in the seed extract as CN is known to inhibit the energy–giving respiratory chain, causing serious, lethal energy crisis. Oxalates and phytates have been reported to inhibit some mineral absorption in the body (Auwal*et al*., 2012) thereby making them unavailable for the body's utilization. This is an indication that the seed extract might not be safe for humans and animal consumption up to that dose, (5000 mg/kg b.w).

There was a significant increase in the concentration of MDA of the rats group administered 600 mg/kg b.w of the extract (9.94±2.58) but the increase was non-significant in the other groups compared to the control (3.60±1.54). Malondialdehyde (MDA) is the end product of lipid peroxidation and measures free radical generation. This result indicates that the seed extract might induce lipid peroxidation at high doses.

Antioxidant enzymes (made in the body) and antioxidant nutrients (found in foods) can scavenge and deactivate the free radicals turning them into harmless particles [22]. Improving antioxidant status is a way of fighting degenerative diseases.

Glutathione peroxidase (GPx) is an enzyme that is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides. The result showed no significant difference in the values of GPx of the rats in the treated groups compared to that of the control group (group 1). This may be due to the fact that the administered doses of the seed extract did not stimulate the production of GPx to detoxify hydrogen and lipid peroxides at the administered doses. It may also be that the administered doses probably inhibited the activities of GPx.

There was no significant difference in the levels of SOD across the treated groups compared to control. This may be due to the fact that the administered doses of the seed extract did not

stimulate the production of SOD to detoxify superoxide radical. It may also be that the administered doses probably inhibited the activities of SOD.

SOD is the only enzyme which employs the superoxide anion as a substrate and produces hydrogen peroxide as a metabolite, this is more toxic than superoxide radical and has to be disposed by Catslase.

Catalase activity was a significantly ($p < 0.05$) higher in group 5 rats administered 600 mg/kg b.w (7.95±4.0) compared to the control group (3.32±1.7). This shows that the methanol seed extract of *N. imperialis*may scavenge the hydrogen peroxide generated by SOD.

Glutathione (GSH) is a tripeptide found in most cells and reacts with free radicals to protect cells against hydroxyl radicals, singlet oxygen and superoxide radicals [23]. Its levels decreased non-significantly in group 2 compared to the control and increased non-significantly from groups 3 to 5 compared to the control. This shows the likelihood of the seed extract to increase organ competence in detoxifying xenobiotics, as GSH is a major detoxifier in many organs such as the liver. This shows that the seed extract possesses antioxidant properties that help to stabilize the integrity of cell membranes and also prevent organ-challenged mediated free radicals.

Caudal epididermal sperm count in all the test groups compared to the control showed non-significant ($p > 0.05$) decrease. A non-significant ($p > 0.05$) decrease of testicular sperm count in the treated groups compared to the control was also observed from groups 2, 3 and 4 and increased non-significantly ($p > 0.05$) in group 5 rats that were administered 600 mg/kg b.w. This indicates that the bioactive substances in the extract did not exert a negative effect on the spermatozoa of the rats.

Photomicrograph image of the liver from the experimental groups of rats untreated and treated with 600 mg/kg seed extract of *N. imperialis* showed apparently normal plates of hepatocytes indicating no serious damage on the liver tissues at the administered dose.

Photomicrograph images of the kidney from the experimental groups of rats untreated and treated with 600 mg/kg seed extract of *N. imperialis* showed normal glomerulus and renal tubules with no remarkable histological change. This indicates that the extract did not pose serious physical damage to the kidneys.

Photomicrograph images of the testis from the experimental groups of rats untreated and treated with 600 mg/kg seed extract of *N.imperialis* showing apparently normal seminiferous tubules separated by

Conclusion

The results demonstrate that the seed extract may not be toxic to the vital organs at the doses administered although mild elevations were observed in catalase and little reduction of sperm counts. The extract may induce lipid peroxidation due to significantly increased concentration of MDA. The free radicals arising due to ingestion of the seeds may be curtailed by the body's antioxidant scavenging mechanism like GSH. photomicrograph images showed that the seeds extract had no significant damages on the liver, kidney and testis of the rats.

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