

# Toxicological study of the matured seeds of dioclea reflexa on some biochemical and haematological parameters and histopathology of liver and kidney in mice

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## Abstract

This study was carried out to test the potential toxic effects of the consumption of the seeds of *D. reflexa* on haematological and biochemical parameters and hence, histological examination of the vital organ of the kidney and the liver was carried out. Thirty matured Wistar rats of both sexes were treated with oral repeated doses (2g, 3g and 4g /kg body weight) once daily of the matured seed flour of *D. reflexa*. The result showed that *D. reflexa* seed flour did not negatively impact on the haematological and biochemical parameters. Histological examination of the liver showed that the liver tissue components were normal in all the doses administered. However, there was haemorrhage of blood into the tissue component of the kidney above 2g/kg/body weight while at 4g/kg/body weight, apart from haemorrhage of blood into the tissue component of the kidney, eosinophils materials could also be observed and the kidney functions were seriously impaired and performed below normal. It was concluded that *D. reflexa* possessed no toxicological property at low doses of 2g/kg/body weight as all the animals survived throughout the experiment. The seed flour could be considered safe on acute oral administration at this low dose where it did not impact negatively on the haematological and biochemical parameters.

**Keywords:** *Dioclea Reflexa*; *Histological Examination*; *Islet of Langerhans*; *Seed Flour*; *Haemorrhage*; *Haematological and Biochemical Parameters*; *Histopathology*.

## 1. Introduction

Plants are being used as valuable sources of food and medicine for the prevention of illness and maintenance of human health (Eugeniusz, Bożena et al, 2017). Plant seeds are important sources of oils of nutritional, industrial and pharmaceutical importance. *Dioclea reflexa*, belonging to the legume family called Fabaceae, is native to West-central tropical Africa, South tropical Africa and Southern America. In Nigeria it is referred to as “Agbaarin” by the Yorubas and “Ufor” by the Igbos.

*Dioclea reflexa* is a vigorous, evergreen climbing shrub with twining, woody stems. Older stems can be up to 5cm thick. The mature pods are 10 - 15 (occasionally to 20)cm. long, around 5cm wide, and around 2cm thick. They contain 3 - 4 hard biconvex seeds, 26 - 29mm in greatest diameter. They are usually tan, brown, or dusky coloured, and rarely mottled (Vaclavic & Christian, 2008). *Dioclea reflexa* (DR) called marble vine is a legume belonging to the sub-family papilionoideae is grown in the tropics. An oil is obtained from the seed. It has both unsaturated fatty acids (especially oleic acid) and saturated fatty acids (especially palmitic acid and stearic acid). The reddish-brown seeds are nearly spherical and slightly flattened with one side rounded, the other straight. They are worn as pendants and used in necklaces in the Old and New World tropics. The seeds are used like marbles in a traditional child's game in Nigeria. The plant is harvested from the wild for local use as a food, medicine and source of materials. The plant has potential for a wider use of its seed as human food, and also as a source of an effective larvicide. The seeds of many species in this genus are likely to be used as an emergency food in times of need (Ajatta et al, 2021).

This is not unusual - the seeds of many commonly eaten legumes (including the various *Phaseolus* species) also often contain anti-nutritional substances -these are largely removed in the cooking or other preparatory process (such as sprouting). *Dioclea reflexa*, with its protein content which range between 15 and 30% is highly desirable as a protein supplement to cereal-based diets (Akoja & Coker, 2019).

The seed is a potential food source. It contains around 14% protein, 8% fats and 58% carbohydrates, but there needs to be further work carried out to see if there are any anti-nutritional substances. The endosperm, which is rich in gum is pulverized and used as thickener in many traditional food preparations, while there are several reports on its suitability in processed foods, including use as a rheology modifier. The cake and seed flour has appreciable levels of protein that could serve as an important protein source in human foods (Mbah et al, 2015).

Extract of DR seed has been shown to boost hematological parameters and antioxidant activities which protect the kidney and blood from oxidative and related injuries under acute and chronic toxicological challenges. A decoction of the root is used to alleviate coronary pain.

A methanolic extract of the seed has shown the capacity to boost haematological parameters and protect the kidney and blood from oxidative and related injuries under acute and chronic toxicological challenges. A decoction or poultice of the leaves is applied topically to treat aches and pains, skin rashes and sores ( Omotoso, et al, 2020; Atawodi & Iliemene 2014).

Phytochemical analysis of the extracts of *Dioclea reflexa* showed the presence of alkaloids, tannins, phenols and glycosides. The seed of this species (and many other members of the genus) contain lectins that have a range of actions within the body that are of interest to the pharmaceutical industry. Many have the potential as the basis of new drugs to treat a range of diseases including cancer. The seeds are used in Africa as an insecticide and arachnicide (Kebede et al, 2016).

Alabi (2016) examined the Nutritional, anti-nutritional composition and in vitro protein digestibility of tropical edible pulse bean '*Dioclea reflexa*' – an under-utilized legume. The nutritive composition of three tropical locally cultivated *Dioclea reflexa*, were investigated using standard methods in order to assess the numerous potentials of the pulse seeds. *Dioclea reflexa* could serve as a good source of protein and also revealed the potentials of different types of cultivar to produce essential and bio-available protein.

Omotoso et al (2020) highlighted the potential of three common and under-utilized tropical leguminous seeds. These seeds (their plants inclusive) are valuable sources of food and medicine for the prevention of illness and maintenance of human health. The medicinal properties of these seeds include antimicrobial, anti-inflammatory, anti-oxidant and immuno-stimulant.

Atawodi and Iliemene (2014) examined the effects of methanolic extract of *Dioclea reflexa* seeds on some Haematological and Kidney Function Parameters in Rats following Single or Repeated Carbon Tetrachloride Intoxication. In the acute model, the level of total bilirubin and conjugated bilirubin were significantly ( $P<0.05$ ) reduced in *D. reflexa* seed extract pre-treated rats compared to the CCl<sub>4</sub> control, while in the chronic model, the level of packed cell volume (PCV) and hemoglobin were significantly ( $P<0.05$ ) boosted in extract treated group compared to the CCl<sub>4</sub> control group with concomitant reduction in the levels of bilirubin. These results indicate that the seed of *D. reflexa* possess capacity to boost haematological parameters and protect the kidney against acute and chronic toxicological challenges.

Adepoju & Balogun (2014) investigated the anti-diabetic effect of the seed flour of *Dioclea reflexa* on alloxan-induced diabetic rats. The result showed that *D. reflexa* seed flour possessed potent oral hypoglycaemic property probably mediated via increased peripheral utilization of glucose. Alloxan induces diabetes by destroying the beta cell of the islet of Langerhans and impairing renal functions (Benattia et al, 2019). The use of traditional medicines in many African communities in particular and other regions of the world is still paramount. Also, the improvement in technology has improved the detection of minute quantities of carcinogenic and toxic chemicals and recognizes potentially hazardous effects of some of the herbs used in traditional medicines (Yusuf &, Lasisi, 2006).

Unless there is clear information that the seeds of this species are completely safe to eat, then it would be unwise to eat them raw; or to consume large quantities of them in one meal; or to consume them on a regular basis over a long period of time. Moreover, recent studies on laboratory animals have shown that many plants that are used as medicinal substances have potential toxicity on blood parameters and histopathology of internal organs .(Akande et al ,2010). Hence, investigating the potential toxic effects of these plants is not out of place as they all have their limitations. It is as a result of this that this study is being carried out to investigate the histo-pathological effect of *D. reflexa* on the vital organs of the liver and kidney of alloxan-induced diabetic rats.

## 2. Materials and methods

### 2.1. Plant material

Samples of the seeds of *D. reflexa* (Fabaceae) were identified and validated at the Taxonomy section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The specimen was issued a Voucher Number FH1108765. The seeds were shade-dried at room temperature before cracking. Cracking was to remove the pericarp and the seed coat. The cracked seeds were further shade-dried for ten (10) days before crushing and milling in a mechanical grinder to fine powder of mesh 40. The phytochemical screening confirmed that of Akinyede et al. (2016) as containing tannins, proteins, flavonoids, terpenoids and sugars.

### 2.2. Animals

Thirty Wistar rats of both sexes weighing 85 to 125 g were purchased from the Animal House, Pre-clinical Department, College of Medicine, University of Ibadan, Nigeria. They were fed with adequate ration of standard rat feed and water for 30 days under standard laboratory conditions. The rats were randomly assigned into six groups of equal membership. The rats have not participated in any experimental study before. All experimental investigations were done in compliance with the " Guide on the care and use of Laboratory Animal Resources " National Research Council and in accordance with the Guideline and approval of Nigeria Medical Ethical Association for Accreditation of Laboratory Animal Care. Ethical approval for the animals used for the study was obtained from the University Medical Faculty Institutional Review Board Ethical Approval Number EC/22/ 0265.

### 2.3. Haematological and biochemical parameters

The animals were fasted for 24 h before the administration of the respective group doses as follows:

Animals were assigned to four groups with each group containing five rats .

Group 1: (Normal saline group): Rats were treated with normal saline by injection.

Group 2: The animals had 2g/kg body weight *Dioclea reflexa* seed flour per day p.o for 10 days.

Group 3: The animals had 3g/kg body weight *Dioclea reflexa* seed flour per day p.o for 10 days.

Group 4: The animals had 4g/kg body weight *Dioclea reflexa* seed flour per day p.o for 10 days.

At the end of the experiment, after 24hours of the end of the administration of the seed flour , the animals anaesthetized using diethylether were sacrificed by decapitation. The blood sample needed for the bioassay was collected by cardiac puncture using sterile syringe and needle to examine changes in the haematological and biochemical parameters.

### 2.4. Statistical analysis

The data obtained from the study were calculated and expressed as mean  $\pm$ standard error of the mean (SEM). A one-way analysis of variance (1-way ANOVA) was used for the analysis. The percentage change relative to the Normal Saline group (Control) was calculated. The statistical level of significance of the difference between the mean of the control and the treated groups was at  $p<0.05$ .

## 2.5. Results

**Table 1:** Shows the Effects of the Administration of the D. Reflexa Seed Flour of on the Animals

Group	Test/Control	Urea $\pm$ SEM	Creatinine $\pm$ SEM	AST $\pm$ SEM	ALT $\pm$ SEM	Cholesterol $\pm$ SEM	PCV	WBC	Platelet $\pm$ SEM	Bleeding Time
1	Control	30.8 $\pm$ 3.6	11 $\pm$ 0.2	87.4 $\pm$ 19.0	40.2 $\pm$ 9.6	94.8 $\pm$ 4.6	24 $\pm$ 6.2	4960 $\pm$ 433.4	92000 $\pm$ 23537.2	18.4 $\pm$ 537.2
2	2g/kg	51.2 $\pm$ 3.9	1.76 $\pm$ 1.4	89.6 $\pm$ 6.3	38 $\pm$ 6.0	76.2 $\pm$ 14.01	25 $\pm$ 1.2	7380 $\pm$ 817.6	196400 $\pm$ 39559.6	22.8 $\pm$ 3.0
3	3g/kg	46 $\pm$ 10.8	1.4 $\pm$ 0.41	78.2 $\pm$ 13.8	34.6 $\pm$ 6.2	98 $\pm$ 12.8	24.4 $\pm$ 2.54	63800 $\pm$ 1502.13	412000 $\pm$ 42591.1	29.8 $\pm$ 2.2
4	4g/kg	55.2 $\pm$ 9.1	1.74 $\pm$ 0.4	72 $\pm$ 9.5	43.8 $\pm$ 4.4	86 $\pm$ 10	19.8 $\pm$ 5.0	6660 $\pm$ 2529.7	290000 $\pm$ 79498.4	36.8 $\pm$ 0.97

## 2.6. Discussion on the haematological and biochemical effects

Table 1 shows the effects of the administration of the seed flour of D.reflexa on haematological and biochemical parameters of the animals. There was no statistically significant difference in the PCV between the Normal Saline (Control) group and the Group 2 that was fed with 2g/kg body weight D. reflexa flour for 10 days ( $p < 0.05$ ). This shows that D. reflexa has no significant effect PCV despite the 33% high iron content of the agent (Vaclavic & Christian ,2008) , it still didn't increase the PCV significantly at this dose. The slight increase observed might be as a result of the normal physiologically erythropoietic function of the bone marrow. However, significant increases were observed with the higher doses ( $p < 0.05$ ) except at 4g/kg body weight group (Group 4) where there was a statistically significant decrease in the PCV.

Also, there was no significant increase in the white blood cell count ( $p < 0.05$ ). However, a slight increase was observed which might have been due to increased production of eosinophils and lymphocytes. Lymphocytic increases might have been due to the increased production of antibody to counter the antigen (D. reflexa) introduced into the body (Omotoso, 2018). Overall, there was a slight increase in the white blood cell in all the groups when compared with the control group and as the doses increased. This might be due to the normal defence mechanisms of the body against antigens.

The platelet count in the groups was found to be greater than the Normal Saline (Control) group. This was statistically significant ( $p < 0.05$ ). However, this declined in Group4 (4g/kg body weight) though still significantly higher when compared with the control group. The bleeding time was found to be statistically insignificant but, when compared with the control, there was a slight increase in the bleeding time with increases in the doses administered despite the increase in platelet count. This might be due to the formation of abnormal platelets or as a result of inadequately functioning platelet which could not mediate clotting and thereby prolonging the bleeding time (Yusuf &, Lasisi, 2006).

Cholesterol levels in all the groups did not show any significant deviation from the control group thereby indicating a less tendency of the seed flour to cause hypercholesterolemia with the administered doses despite its high fat (14%) content (Vaclavic & Christian ,2008)). The fat content in the seed flour might be that of the unsaturated fatty acids which cholesterol is not.

There was an increase in the urea levels when compared with the control. This was statistically significant. This, however did not reflect in the creatinine levels where there was no statistically significant increase when compared with the control group. Creatinine is an endogenous substance that is derived from creatine and its phosphate in the muscles. Its clearance from the body is a biomarker of renal function. The statistically significant increases in urea levels in all the groups make the seed flour a potential agent to demonstrate kidney failure. It has been demonstrated that the seed flour caused severe renal impairment at the 4g/kg body weight (Bhandari et al, 2020). This might be the reason for the slight decrease in the PCV at this dose due to the decrease in renal production of erythropoietin directly consequent upon the reduction in renal mass. Another factor contributing to this observation might be the retention of toxic substances in the blood. This suppresses bone marrow function and is responsible for the haemolytic action and the reduction in the life span of the red blood cells.

Alanine amino transferase enzyme ALT, and Aspartate aminotransferase , AST, levels were not significantly altered at all the doses . This shows that D. reflexa has a reduced tendency to cause liver necrosis, clinical jaundice that are characterized by increase in ALT and to a less extent, AST.

## 3. Figures

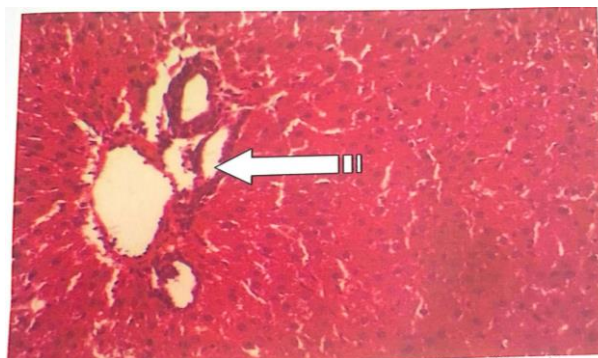


Figure iii: Photomicrograph sample of Liver of Rats of the experimental Groups that received 2g, 3g and 4g/kg/bodyweight of Dioclea reflexa for a period of ten days. The Broken Arrow is on the portal triad. The liver tissue components are normal.

Fig. 1: A.



Figure iv: Photomicrograph sample of Liver of Rats of the experimental Groups that received 2g, 3g and 4g/kg/bodyweight of Dioclea reflexa for a period of ten days. The Broken Arrow is on the hepatocytes.

Fig. 2: B.

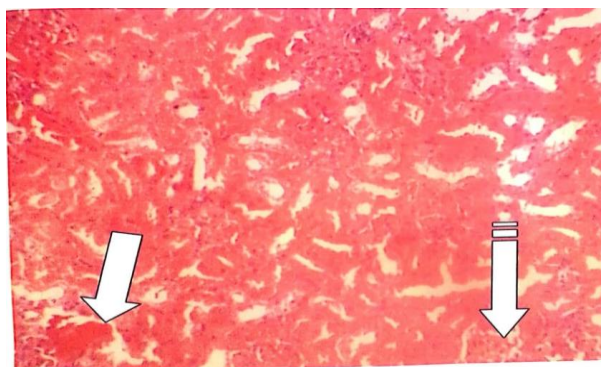


Figure v: Photomicrograph sample of Kidney of Rats of the experimental Groups that received 2g/kg/bodyweight, 3g/kg/bodyweight of Dioclea reflexa for a period of ten days. The Broken Arrow is on the collecting duct and the solid arrow is on the glomeruli. There is haemorrhage of blood into the tissue component of the kidney. Kidney functions would be below normal.

Fig. 3: C.

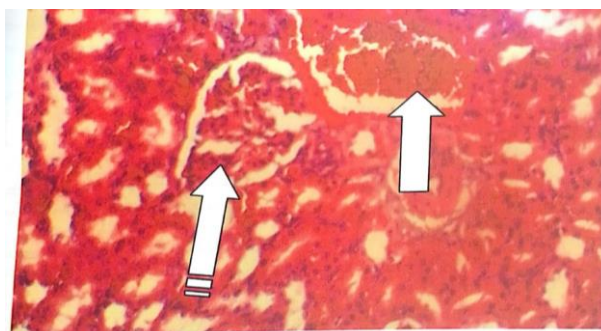


Figure vi: Photomicrograph sample of Kidney of Rats of the experimental Groups that received 4g/kg/bodyweight of Dioclea reflexa for a period of ten days. The Broken Arrow is on the collecting duct and the solid arrow is on the glomeruli. There is haemorrhage of blood into the tissue component of the kidney. Eosinophilic materials could be seen. Thickening of the glomeruli membrane could be observed. Kidney functions would be seriously impaired and below normal.

Fig. 4: D.

All the animals survived throughout the experiment. Hence, no toxicity-related mortality was recorded throughout the experiment. This seed flour could be considered safe on oral administration. Thus, Bruce (1985, 1987) and American Society for Testing and Materials (1987) showed that any chemical substance with LD50 estimate greater than 2000 to 5000 mg/kg/oral route could be considered safe and of low toxicity. Thus, lack of toxicity or associated lethality (mortality) with the administered doses of the seed was an indication of the safety of the seed flour.

### 3.1. Discussion on the histological effect on the liver and the kidneys

Figures A-D show the histological effects of the administration of the seed flour of *D.reflexa* on the liver and kidneys of the animals. Photomicrographs sample of the livers of the rats in the experimental group that received 2g, 3g and 4g/kg/ body weight of the seed flour of *D.reflexa* for a period of ten (10) days. (Figure A). The broken arrow is on the portal triad. The liver tissue components are normal. In Figure B, the broken arrow is on the hepatocytes. The liver tissue components are normal.

In Figure C, the animals received 2g and 3g/kg/body weight of the seed flour for ten days. The broken arrow is on the collecting duct and the solid arrow is on the glomeruli. /

In Figure D, the animals received 4g/kg/body weight of the seed flour for ten days. The broken arrow is on the collecting duct and the solid arrow is on the glomeruli. There is haemorrhage of blood into the tissue component of the kidney. Eosinophil materials could be seen. Thickening of the glomeruli membrane could be observed and the kidney functions are seriously impaired and below normal.

It could be observed that at 3g/kg/body weight, there was already a declining function of the kidney while it became pronounced at the 4g/kg/body weight where the kidney functions have become seriously impaired. This happened with progressive increases in the doses administered to the animals. The seed flour at 2g/kg/body weight has been found to reverse the damaged Islet of Langerhans without any severe damage to the kidneys. The seed flour was found to heal damaged B-cells of the Islets Langerhans and the hepatic functioning was intact (Adepoju & Balogun, 2014). However, in this experiment, the doses above 2g/kg/body weight p.o. for ten days showed tendencies to damage the kidneys with the liver functioning still being intact at these doses. It shows that higher doses of the seed flour cannot be tolerated.

All the animals survived throughout the experiment. Hence, no toxicity-related mortality was recorded throughout the experiment. This seed flour could be considered safe on oral administration. This is corroborated by the study of (16) that *Dioclea reflexa* seeds contain substances with potent capacity to protect the kidney and blood from oxidative and related injuries under acute and chronic toxicological conditions.

#### 4. Conclusion

It could be concluded that *D.reflexa* possessed no toxicological property at the effective dose for diabetic control as all the animals survived throughout the experiment (Adepoju & Balogun, 2014). Alanine amino transferase enzyme ALT, and Aspartate aminotransferase, AST, levels were not significantly altered at all the doses. This shows that *D. reflexa* has a reduced tendency to cause liver necrosis, clinical jaundice that are characterized by increase in ALT and to a less extent, AST. Cholesterol levels in all the groups did not show any significant deviation from the control group thereby indicating a less tendency of the seed flour to cause hypercholesterolemia with the administered doses. This lack of highly significant changes in the haematological and biochemical parameters might have been indicative of the absence of toxicity in the administration at the 2g/kg/body weight at this dose. The liver functioning is not impeded as the liver tissue components are normal and did not affect the hepatocytes in all the doses administered. The seed flour could be considered safe on acute oral administration. However, caution should be taken on the chronic dosages as the kidney functions would be seriously impaired and subnormal in their functions.

#### 5. Conflict of interest

There is no conflict of interest.

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