British Biotechnology Journal
4(12): 1253-1262, 2014
ISSN: 2231–2927

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Effects of Methanolic Extract of *Dioclea reflexa* Hook F Seed on some Haematological and Kidney Function Parameters in Rats following Single or Repeated Carbon Tetrachloride Intoxication

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Authors' contributions

This work was carried out in collaboration between both authors. Author SEA designed the study wrote the protocol and the manuscript, while author UDI managed the analyses and the literature searches and performed the statistical analysis. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2014/13836
Editor(s):
(1) Samuel Peña-Llopis, Developmental Biology and Internal Medicine, Oncology Division, University of Texas Southwestern Medical Center, USA.
Reviewers:
(1) Anonymous, Helwan University, Egypt.
(2) Yousif Y. Bilto, Dept. Biol and Biomed Sciences, The University of Jordan, Amman, Jordan.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=800&id=11&aid=6726

Original Research Article

Received 6th September 2014
Accepted 7th October 2014
Published 30th October 2014

ABSTRACT

Objective: To investigate the effect of methanolic extract of *Dioclea reflexa* Hook F seed on male rats following single (acute) or repeated (chronic) carbon tetrachloride intoxication.
Methods: Male albino rats were divided into groups of six each consisting of plant extract only, plant extract + carbon tetrachloride, solvent only, solvent + carbon tetrachloride, vitamin E only, vitamin E + carbon tetrachloride and untreated control. The rats in the

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acute experiment received the extract (5mg/kg) by intraperitoneal injection for two days, and 1 hour after administration of CCl_4 (0.6ml/kg body weight) on the third day, while those in the chronic experimental model received 2.5mg/kg for 10 consecutive days with 72 hourly administration of CCl_4 at 0.3ml/kg.
Results: 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_4 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_4 control group with concomitant reduction in the levels of bilirubin.
Conclusion: 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.

Keywords: Dioclea reflexa; haematological effect; kidney damage; CCl_4 intoxication; acute toxicity; chronic toxicity.

1. INTRODUCTION

Spices, condiments and herbs are recognized as sources of natural antioxidants and phytochemicals that could play an important role in the chemoprevention of diseases that have their etiology and pathophysiology in reactive oxygen species [1-3]. Thus, it has been recommended that adequate intake of antioxidant through the consumption of antioxidant rich foods can prevent the development of oxidative stress [4-6]. In line with our earlier work on the assessment of the beneficial effects of some Nigerian foodstuffs and herbs [7-9,5,10], the effects of dried seeds of *Dioclea reflexa, an* Eastern Nigeria condiment on some haematological and kidney function parameters were investigated following single or repeated intoxication with carbon tetrachloride.

Also known as “Ukpo” in Igbo and “Agbaarin” in Yoruba, *Dioclea reflexa* Hook F. (*Papilionaceae*) is an annual climber crop that can be cultivated more than once a year. The plant is high yielding, bearing pods that contain between three and four seeds [11], which are usually dark brown to black, sometimes speckled, depending on variety. 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 [12]. Both its seed and leaf have high economic values in culinary and pharmaceutical industries [13].

Dioclea reflexa seed oil has acid value, saponification value, iodine value, ester value and iodine number to be 8.69mg KOH/g, 251mg KOH/g, 72.8mg I/g, 242 and 27.9, respectively. The fatty acid composition include unsaturated fatty acid, especially oleic acid, while the saturated fatty acids include palmitic acid and stearic acid. The cake and seed flour of *Dioclea reflexa* has appreciable levels of protein that could serve as important protein source in livestock production and in human foods [14].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Methanol, petroleum ether, vitamin E as tocopherol acetate carbon tetrachloride, corn oil, and assay kits were purchased from sigma chemical Co, Ltd (USA) and are of analytical grades.

2.2 Plant Collection and Authentication

Dioclea reflexa seeds and plant parts were collected from Achalla village in Awka North Local Government of Anambra State, Nigeria in June of 2009. It was authenticated at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria where voucher number 1286 was assigned.

2.3 Sample Preparation and Extraction

The *D. reflexa* seeds were dried in the laboratory at room temperature and pulverized using laboratory mortar and pestle. Pulverized material (35g) was placed in the thimble of soxhlex extractor and extracted first, using petroleum ether (300ml) for 8 hours each and then methanol (300ml), three times for 5 hours each. The methanol extracts were combined and dried *in vacuo* at 45℃ using a rotary evaporator (Büchi Labortechnik AG, Switzerland). The methanol extracts were combined and dried *in vacuo* at 45℃ using a rotary evaporator.

2.4 Management of Experimental Animals

Male albino rats (7-8weeks old and weighing about 120-150kg) were purchased from the animal house of National Research Institute for Chemical Technology, Zaria, Nigeria. They were acclimatized for two weeks prior to commencement of experiment. They were kept at room temperature and were maintained *ad libitum* on tap water and growers mash (Vital feeds, Jos, Plateau State Nigeria) except in the last 15 hours before termination of the experiment. They were weighed prior to commencement and termination of the experiment.

2.5 Grouping of Animals

In the chronic experimental model, rats were divided into 7 groups of six rats each. Group 1 was administered the plant extract only, group 2 was administered plant extract and carbon tetrachloride, group 3 was administered solvent only, group 4 was administered solvent and carbon tetrachloride, group 5 was administered vitamin E only, group 6 was administered vitamin E and carbon tetrachloride, group 7 was untreated control. The CCl_4 was administered at a dose of 0.6ml/kg body weight before the administration of the first extract or vitamin E, and subsequently every 72 hours until 10 days before termination of the experiment. After the first day, extract and vitamin E were administered daily at a dose of 2.5mg/kg body weight for 10 days. The animals were sacrificed 24 hours after the last administration of extract [9,10].

In the acute experimental model, rats were divided into 7 groups with each group containing six rats**.** Group 1 was administered the plant extract only, group 2 was administered plant extract and carbon tetrachloride, group 3 was administered solvent only, group 4 was administered solvent and carbon tetrachloride, group 5 was administered vitamin E only, group 6 was administered vitamin E and carbon tetrachloride and group 7 was the untreated control. The rats received 24 hourly administration of the extract at 5mg/kg for three days by intraperitoneal injection. On the third day, CCl_4 (0.6ml/kg) was administered 1hr after the extract administration, and the animals sacrificed 24 hr later [9,10].

2.6 Collection and Storage Blood

At the point of sacrifice, blood from each rat was withdrawn from carotid artery at the neck and collected in previously labeled test tubes and allowed to stand for 3 hours. Clear serum

were collected from the blood in eppendoff tubes and stored under - 20℃ for biological assay.

2.7 Determination of Packed Cell Volume (PCV) and Hemoglobin Concentration

In determining the packed cell volume and hemoglobin concentration, whole blood samples were collected into heparinized capillary tubes, filled up to 2/3 the length during animal sacrifice, sealed with plasticine and centrifuged at 3000 × g for 10 minutes. Packed cell volume was determined using hematocrit reader and expressed as percentage erythrocytes content of the blood, while the hemoglobin concentration was calculated using the formula: packed cell volume level divided by 3 and expressed as percentage (%) hemoglobin content of the blood.

2.8 Determination of Bilirubin Concentration

Conjugated, unconjugated and total bilirubins were estimated colorimetrically at 560nm following the principle described by Sherine and Safinaz [15] using Randox assay kit, while the value of the total bilirubin (mg/dl) was obtained by multiplying the absorbance by a factor of 10.8. In the case of direct bilirubin, the absorbance was read at 530 nm, and the value of direct bilirubin (mg/dl) was obtained by multiplying the absorbance by a factor of 14.4, while indirect bilirubin was estimated by difference.

2.9 Determination of Urea

Urea in serum is hydrolysed to ammonia in the presence of urease, and the ammonia is then measured spectrophotometrically at 540nm by Berthelot's reaction utilizing analytical kits based on the principle described by Stephen and coworkers [16]. Urea level was calculated from sample absorbance relative to the absorbance of standard of known concentration (mg/dl).

2.10 Assessment of Creatinine Levels

Creatinine level was assayed colorimetrically at 510nm following 30 sec and 2 min incubation with picric acid reagent by utilizing Randox reagent kit based on the principle described by Stephen and co-workers [16]. Creatinine level was calculated as change in the sample absorbance divided by the change in the absorbance of the standard multiplied by the concentration of the standard.

2.11 Statistical Analysis

Results are expressed as mean ± SD. Analysis of variance(ANOVA) was used for statistical analysis using the Statistical Package for Social Sciences (SPSS) version 20 software. A value of $P<0.05$ was used to denote statistical significance.

3. RESULTS

3.1 Serum Total, Conjugated and Unconjugated Bilirubin Concentration

In the model of chronic injury, pre-treatment with methanolic extract of *D. reflexa* seed significantly ($P<0.05$) prevented the CCl_4 induced elevation in the levels of total, conjugated

and unconjugated bilirubin (Fig. 1) with concomitant increase in the levels of packed cell volume and hemoglobin concentration (Fig. 3). Similar observations were made for the levels of total, conjugated and unconjugated bilirubin (Fig. 2) as well as packed cell volume and hemoglobin concentration (Fig. 4) in the model of acute damage.

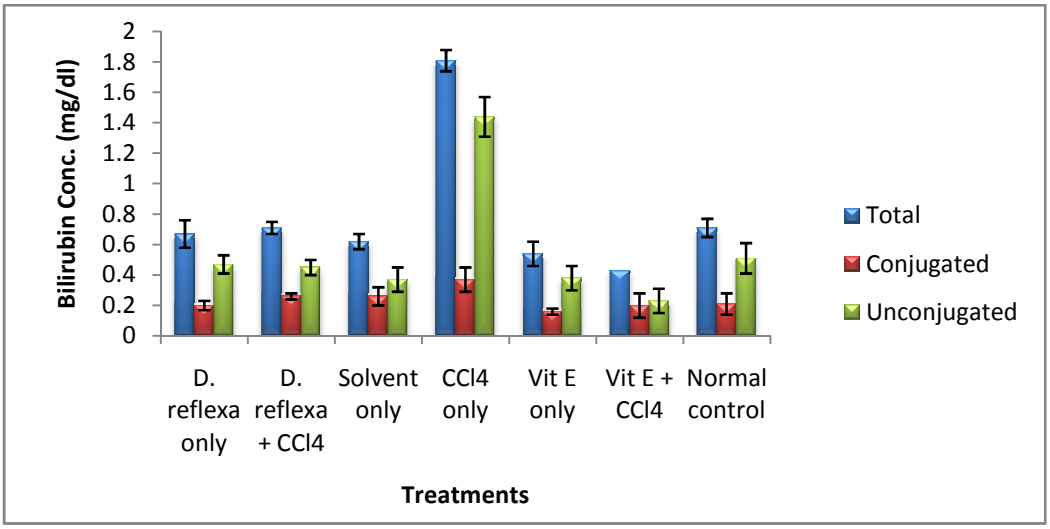

Fig. 1. Mean bilirubin concentration (total, conjugated and unconjugated) in serum after 10 days daily intraperitoneal administration of *D. reflexa* extract (2.5mg) with 72 hourly carbon tetrachloride intoxication

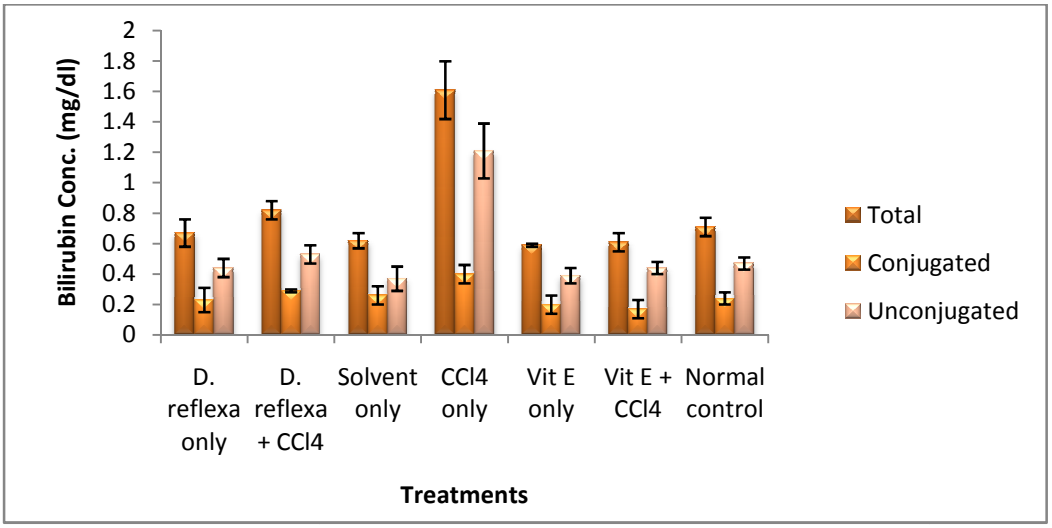

Fig. 2. Mean bilirubin concentration (total, conjugated and unconjugated) in serum of rats on intraperitoneal injection of CCl_4 following 2 days pre-treatment with methanolic extract of *D. reflexa* (5mg/kg)

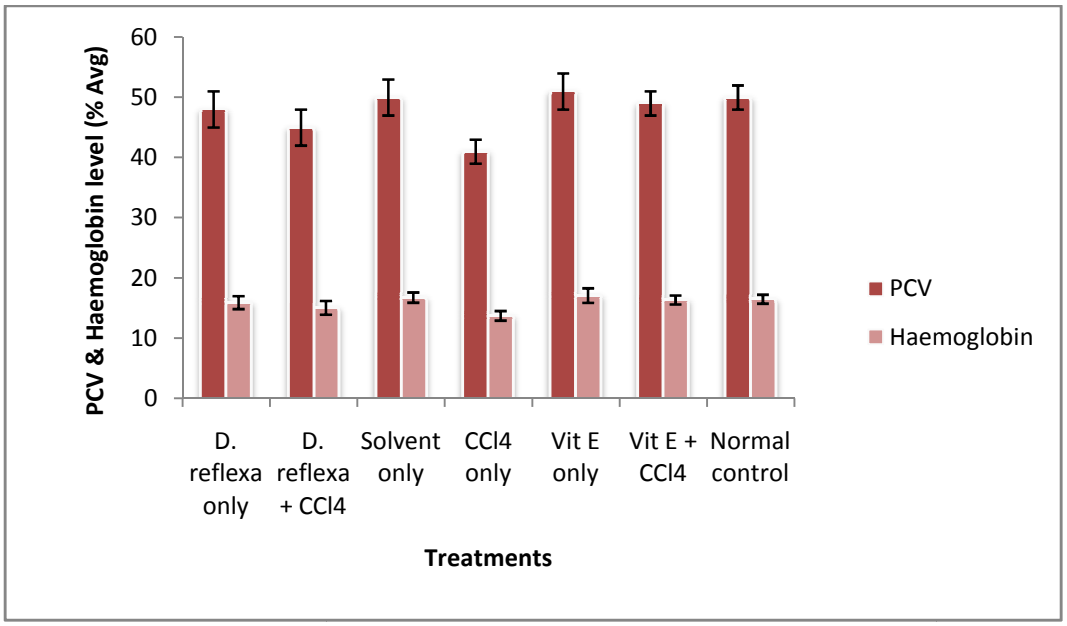

Fig. 3. Mean packed cell volume (PCV) and haemoglobin concentration of rats after 10 days daily administration of *D. reflexa* extract (2.5mg) with 72 hourly carbon tetrachloride intoxication

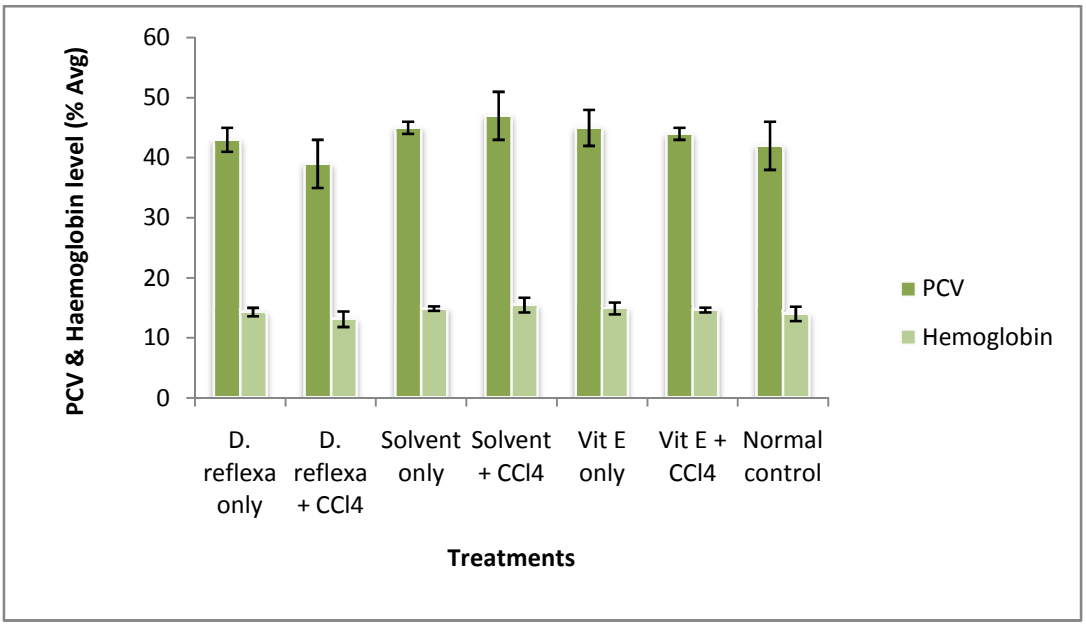

Fig. 4. Mean packed cell volume (PCV) of rats in serum of rats on intraperitoneal injection of CCl_4 following 2 days pre-treatment with methanolic extract of *D. reflexa* 5mg/kg

3.2 Effect of *D. reflexa* Extract on Levels of Urea and Creatinine

To evaluate the effect of *D. reflexa* extract pre-treatment on kidney function in rats chronically and acutely intoxicated with CCl_4, the serum levels of urea and creatinine were measured. In both models, extract pre-treatment greatly prevented the CCl_4 induced elevation in the level of both urea (Tables 1 and 2) and creatinine (Tables 1 and 2) while no such significant difference occurred between the vitamin E control, *D. reflexa* extract only and untreated control groups (Table 1).

Table 1. Mean creatinine and urea level in serum of rats after 10 days daily administration of *D. reflexa* methanolic extract (2.5mg) with 72 hourly carbon tetrachloride intoxication

Urea and creatinine concentration mg/dl			
Group	**Treatment**	**Urea**	**Creatinine**
1	*D. reflexa* only	67 ± 5^{a}	1.0 ± 0.0^{a}
2	*D. reflexa* + CCl_4	69 ± 5^{a}	1.2 ± 0.2^{a}
3	Solvent only	87 ± 6^{b}	1.2 ± 0.1^{a}
4	CCl_4 only	130 ± 6^{c}	2.4 ± 0.2^{b}
5	Vit E only	70 ± 6^{a}	1.2 ± 0.1^{a}
6	Vit E + CCl_4	82 ± 6^{b}	1.2 ± 0.2^{a}
7	Normal control	85 ± 5^{b}	1.0 ± 0.0^{a}

Values are Mean±SD; Values having different letters across the column are significantly different (p<0.05)

Table 2. Mean creatinine and urea concentration in serum of rats on intraperitoneal injection of CCl_4 following 2 days pre-treatment with methanolic extract of *D. reflexa* (5mg/kg)

Urea and creatinine levels mg/dL			
Group	**Treatment**	**Urea**	**Creatinine**
1	*D. reflexa* only	46 ± 4^{ab}	1.0 ± 0.0^{a}
2	*D. reflexa* + CCl_4	55 ± 6^{b}	1.8 ± 0.2^{b}
3	Solvent only	32 ± 3^{a}	1.2 ± 0.1^{a}
4	CCl_4 only	92 ± 7^{c}	2.2 ± 0.2^{b}
5	Vit E only	46 ± 5^{ab}	1.2 ± 0.1^{a}
6	Vit E + CCl_4	54 ± 4^{b}	1.2 ± 0.2^{a}
7	Normal control	37 ± 3^{a}	1.0 ± 0.0^{a}

Values are Mean ± SD; Values having different letters across the column are significantly different (p<0.05)

4. DISCUSSION

The levels of packed cell volume, hemoglobin (Figs. 3 and 4) were significantly lower ($P<0.05$) in the CCl_4 control group than in the extract or vitamin E-treated groups. Also the CCl_4 – induced significant elevation in the levels of bilirubin (Figs. 1 and 2). Elevated levels of urea and creatinine (Tables 1 and 2) were prevented by treatment with the methanolic extract of *D. reflexa* seed or vitamin E.

Pre-treatment with the methanolic extract of *D. reflexa* significantly ($P<0.05$) prevented the CCl_4 mediated elevation in the levels of bilirubin ($P<0.05$). That serum bilirubin (total, direct

and indirect) levels which were also significantly (P<0.05) elevated by CCl_4 treatment, was remarkably reduced by pre-treatment or administration of the extract and vitamin E (Fig. 1), strongly suggest that the seed methanolic extract of *Dioclea reflexa* protected the rat liver against hepatotoxicity as much as vitamin E, since high level of bilirubin which is excreted by the liver is an important index of liver dysfunction. Thus, the consumption of this popular condiment may have wide ranging implication in preventing and ameliorating oxidative stress related illnesses in the population where it is popularly consumed.

The decrease in the packed cell volume (Fig. 3) and hemoglobin concentration (Fig. 3) of the CCl_4 control group compared with the extract or vitamin E-treated groups is consistent with the bilirubin result (Fig. 1), since haem degrade following red blood cell destruction consequent on CCl_4 intoxication [17]. Hence, while the lowered level of PCV and hemoglobin suggest oxidative damage and red blood cell membrane destruction in the CCl_4 group, the higher levels of PCV and hemoglobin in the extract treated group is a demonstration of the capacity of *D. reflexa* to protect red blood cell membrane, and possibly boost the haemopeitic system [18].

From Tables 1 to 2, it can be observed that there were high levels of urea and creatinine in the CCl_4 control group which was significantly different (P<0.05) from the groups treated with extract, vitamin E and normal control group. This is due to the fact that direct or indirect exposure of kidney to nephrotoxic agents may result in ultra-structural damage to any of the principal component of the nephrons [19]. This results in elevated level of blood urea which is derived from normal metabolism of protein and is usually excreted in the urine and hence elevated blood urea usually indicates glomerular damage. Similarly, creatinine, a metabolite of creatine, is also excreted completely in the urine via glomerular filtration. Thus, an elevation of its level in the blood is an indication of impaired kidney function [20]. That the extract treated groups showed significantly (P<0.05) lowered concentrations of creatinine and urea, confirms the potency of *Dioclea reflexa* seed extract in protecting and ameliorating the kidney from oxidative stress-related damages. The ability of the *D. reflexa* seed methanol extract to protect and ameliorate the organs of the rat against oxidative stress-related damage should be as a result of its polyphenols and flavonoids content, since polyphenols, especially flavonoids have been reported to exert positive effects on human health through their antioxidant properties [2, 3, 5, 6, 21]. However, studies are underway to establish the specific polyphenols in *Dioclea reflexa* seeds that are responsible for these desirable health effects.

5. CONCLUSION

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Atawodi SE. Antioxidant potential of African medicinal plants. Afri J Biotechnol. 2005;4(2):128-33.

2. Rizwan K, Zubair M, Rasool N, Riaz M, Zia-Ul-Haq M, De Feo V. Chemical and biological study of *Agave attenuata.* Int. J. Mol. Sci. 2012;13:6440-6451.
3. Vauzour D, Rodriguez-Mateos A, Corona G, Oruna-Concha MJ, Spencer JPE. Polyphenols and human health: Prevention of disease and mechanisms of action. Nutr. 2010;2:1106-1131.
4. Lako J, Trenerry VC, Rochfort S. Routine analytical methods for use in South Pacific regional laboratories for determining naturally occurring antioxidants in food. Internl Food Res J. 2008;15(3):1-11.
5. Atawodi SE. Antimutagenic polyphenols in African foods: Sixth Annual Conference on Environmental Mutagens in Human Population. Q Sci Proc. 2012;3:96.
6. Nisar M, Qayum M, Ćavar S, Shah MR, Zia-Ul-Haq M, Khan I, Ahmad KW, Qayum ZA. Chemical constituents and antioxidant activity of n-hexane extract of *Impatiens bicolor* Royle. Chem. Nat. Comp. 2012;48:143-146.
7. Atawodi SE, Atawodi JC, Idakwo P, Pfundstein B, Haubner R, Wurtele G, Spiegelhalder B, Bartsch HRW, Owen RW. Evaluation of the polyphenol composition and antioxidant activity of African variety of *Dacryodes edulis* (G. Don) H. J Lam Fruit. J. Med. Food. 2009a;12:1321-1325.
8. Atawodi SE, Atawodi JC, Idakwo GA, Pfundstein B, Haubner R, Wurtele G, Spiegelhalder B, Bartsch HRW, Owen RW. Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem and root barks of *Moringa oleifera*, Lam. J. Med. Food. 2010a;13:710–716.
9. Atawodi SE, Atawodi JC, Pfundstein B, Spiegelhalder B, Bartsch H, Owen R. Assessment of the polyphenol components and *in vitro* antioxidant properties of *Syzygium aromaticum* (L.) Merr. & Perry. Electron. J. Env. Agric. Food Chem. 2011a;10:1970-1978.
10. Iliemene UD, Atawodi SE. *In vivo* antioxidant and hepatoprotective effects of methanolic extract of dioclea reflexa seed in rats following acute or chronic liver injury. Bangladesh J Pharmacol. 2014;9:112-117.
11. Oladosu IA, Echeme JO, Zubair MF. Anticholinesterase and Antibacterial Activities of Dioclimidazole from *Dioclea reflexa* seeds. *Fitoterapia.* 2006;77:571-5.
12. Nwokocha LM, Williams PA. Isolation and Rheological Characterization of *Mucuna flagellipes* Seed Gum. Materials Science Research Centre. Centre for Water Soluble Polymers; 2009. Available: http://epubs.glyndwr.ac.uk/cwsp/2.
13. Agba OA, Asiegbu JE, Omalik CPE. Effect of length of soakingin water at roomtemerature and hot water treatment on the germination of mucuna flagellipes (vogel Ex hook) seeds. Glob J Agric Sc. 2005;4:(1):15-18.
14. Lasisi AA, Yusuf AA. Compositional Analysis of Horse Eye *(Dioclea Reflexa*) Seed Flour and Its Cake. Agric J. 2006;10:28-31.
15. Sherine MR, Safinaz SI. Attenuation of N-nitrosodiethylamine-induced liver carcinogenesis in rats by naturally occurring diallyl sulfide. Afr. J. Biochem. Res. 2008;2(10):197-205.
16. Stephen OA, Abdulkadir AS, Oladepo WD, Thajasvarie H. Effect of Melantonin on carbontetrachloride induced kidney injury in wistar rats. Afri. J. Biomed. Res. 2007;10:153-164.
17. Colleen S, MD Allan, Micheal L. Marks Basic Medical Biochemistry: A Clinical Approach. Second Edition. 2003;453.
18. Al-Hashem F. Camel's Milk Protects Against Aluminum Chloride-Induced Normocytic Normocromic Anemia, Lipid Peroxidation and Oxidative Stress in Erythrocytes of White Albino Rats. Am. J. Biochem. Biotechnol. 2009;5(3):126-36.

19. Barile FA. Clinical Toxicology: Principles and Mechanisms. CRC PRESS Boca Raton London New York Washinton, D. C. 2004;233-243.
20. Frank CL. Lu's Basic Toxicology. Fourth Edition. Taylor & Francis, 11 New Fetter lane, London EC4P 4EE. 2002;38-97.
21. Norhaiza M, Maziah M, Hakiman M. Antioxidative properties of leaf extracts of a popular Malaysian herb, *Labisia pumila.* J Med Plants Res. 2009;3(4):217-23.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=800&id=11&aid=6726