World Journal of Biomedicine and Pharmaceutical Sciences
Open Access freely available online
ISSN: 2476-7972
Biosciences Research Support Foundation
http://www.brsfoundation.org/wjbps

Research Article

Assessment of hepatoprotective effect of extracts of *Alstonia boonei* leaf in isoniazid and rifampicin co-treated rats

Judith E. Taiwo[1], Ufuoma B. Shemishere[2]* and Ehimwenma S. Omoregie[1]

[1]*Malaria Research, Molecular Biology and Toxicology unit, Department of Biochemistry, University of Benin, Benin City, Edo State, Nigeria*
[2]*Department of Biochemistry and Molecular Biology,, Federal University, Birnin Kebbi, Kebbi State, Nigeria*

ABSTRACT

Anti-tuberculosis drugs, rifampicin and isoniazid, in their mode of action can generate toxic reactive metabolites upon oxidative bio-activation of the parent compound by the cytochrome P_{450} monooxygenase system leading to liver injury. Currently, there is increased awareness of the benefits in the use of medicinal plants in the management of various oxidative stress-related diseases. This study therefore evaluated the hepatoprotective effect of aqueous and ethanol extracts of *Alstonia boonei* leaf in rats co-treated with isoniazid (INH) and rifampicin (RIF). Sets of albino Wistar rats were placed into eight groups. The animals in each group (except the normal control) were orally co-treated with isoniazid (200mg/Kg) and rifampicin (200mg/Kg).The results revealed that the INH+RIF co-treated rats significantly ($p<0.05$) increased the activities of liver function enzymes (ALT, AST and ALP) when compared with the normal control. There were significantly high levels ($p<0.05$) of malondialdehyde (MDA) and low levels of reduced glutathione (GSH) in the INH + RIF co-treated groups in contrast to the control. However, administration of extracts of *A. boonei* (250mg/kg and 500mg/kg doses) protected the animals against various degrees of oxidative damage, particularly in the aqueous extract treated rats. A similar trend was observed in the silymarin and vitamin E treated rats which showed normal levels of these parameters. The overall results from this study suggest that the aqueous extract of *A. boonei* leaf showed better hepatoprotective ability in contrast to the ethanol extract.

Citation: Taiwo JE, Shemishere UB and Omoregie ES (2018). Assessment of hepatoprotective effect of extracts of *Alstonia boonei* leaf in isoniazid and rifampicin co-treated rats. *World Journal of Biomedicine and Pharmaceutical Sciences* 3: 1-6. http://doi.org/10.5281/zenodo.3243894

Received November 24, 2018; Revised June 3, 2019; **Accepted** June 10, 2019; **Published** June 12, 2019.

Copyright: © 2019 Taiwo *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. WJBPS is a journal publication of BRSF.

Competing Interests: The authors have declared that no competing interests exist.

*** E-mail:** shemishere.ufuoma@fubk.edu.ng

Keywords: hepatotoxicity, isoniazid, rifampicin, reactive metabolites, Alstonia boonei

1. INTRODUCTION

The liver regulates many metabolic, detoxification and secretory functions in the body. It is the major target organ of chemical-induced toxicity [1]. Liver damage, in most cases involves oxidative stress and is characterized by progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma [2]. Although, the precise mechanisms of the pathogenesis of liver cirrhosis are not completely understood, the role of free radicals and lipid peroxides has garnered considerable attention [3].

Isoniazid (INH) and rifampicin (RIF), being the first line drugs used as anti-tubercular chemotherapy, are known to be associated with hepatotoxicity [4]. The incidence of hepatotoxicity is 1-2% with INH alone and increases to 8-10% when it is combined with RIF. The rate of hepatotoxicity has been reported to be much higher in developing countries like India (8-30%) compared to advanced countries (2-3%) with a similar dose schedule [5].

Toxic reactive metabolites generated during hepatic biotransformation of some of the anti-tubercular drugs are thought to covalently bind with cellular macromolecules to generate free radicals, which in turn bring about cellular injury [6]. Isoniazid metabolites, acetylhydrazine and hydrazine have been implicated as the causative hepatotoxins. Oxidative activation of these metabolites in the liver by cytochrome P450 (Cyt P450) monooxygenase system generates electrophilic intermediates and free

radicals which are capable of causing liver injury in animals [7-8]. Rifampicin, a powerful inducer of mixed function oxidase, contributes to hepatotoxicity of INH by enhancing the production of these toxic metabolites [9]. Thus, oxidative stress has been found to be an important mechanism in hepatotoxicity of anti-tubercular drugs in young rats, indicated by the altered profile of antioxidant enzymes along with increased lipid peroxidation in treatment with combination of INH and RIF [10].

Clinical and experimental examinations have shown that cirrhosis can be reversed. Various pharmaceutical drugs have been used to minimize and reverse the insult, but most of them lead to appreciable side effects during long-term treatment. For this reason, the use of an effective alternative without side effects is crucial to reduce the oxidative stress, which leads to hepatic disorders [11-12].

Currently, there is a great awareness of the health benefits of phenolic and polyphenolic compounds because of their antioxidant property [13]. Dietary plants such as quince, avocado pear, strawberries, mangoes, citrus fruits, broccoli, cabbage, celery, etc, which possess phenolic and polyphenolic compounds have been shown to exert various biological actions. These include scavenging of free radicals, metal chelation, increases in enzyme activity. More recently, they have been shown to influence signal transduction, release of transcription factors, and gene expression. They have received considerable attention in the past decade because of their reputed role in the prevention of several human disorders, especially hepatotoxicity mainly caused by oxidative stress [14]. Medicines from indigenous plants form the basis of primary health care for a majority of people living in urban and rural areas of the third world countries. This is due to the perceived low cost, easy access and ancestral experience as well as the belief that these medicines are devoid of adverse effects [15].

Alstonia boonei, commonly called cheese wood or stool wood belongs to the family Apocynaceae. Its plant parts are employed in the treatment of various ailments. Anti-malarial, analgesic and anti-inflammatory properties of the aqueous and ethanol extracts of *Alstonia boonei* stem bark have been reported [16];[17]. The leaves and latex are reported to be used for the treatment of rheumatic and muscular pain and hypertension [18]. Stem bark of *A. boonei,* which is commonly used in the treatment of malaria is listed in the African pharmacopoeia as an anti-malarial drug [19].

The aim of this study was to investigate the hepatoprotective potential of aqueous and ethanol leaf extracts of *A. boonei* in male wistar rats.

2. MATERIAL AND METHODS

2.1 Experimental Animals

Male Wistar Albino rats (150-250g) were used for this study. The rats were acclimatized for 2 weeks before starting the experiments. Animals were maintained in a 12-hour light/dark cycle at room temperature. The rats were fed with pelleted grower's mash and tap water *ad libitum*.

2.3 Plant Materials and Preparation of the Extracts

Leaves of *Alstonia boonei* were collected from the surroundings of Faculty of Pharmacy, University of Benin, Benin City and authenticated by the plant taxonomist of Pharmacognosy Department, University of Benin, Benin City, Nigeria. The leaves were shade-dried at room temperature, and the dried leaves were pulverized.

***Alstonia boonei* Aqueous Extract (ABAE)**: The coarse powder of the leaves was soaked in deionized water for 24 hours and then filtered using muslin cloth. The extract was concentrated by evaporation in a boiling water bath and dried in an oven at 40^0C. The dried aqueous extract was dissolved in 0.9% physiological saline as the vehicle.

***Alstonia boonei* Ethanolic Extract (ABEE):** The coarse powder of the leaves was soaked in 2 litres of ethanol (90%) for 72 hours and then filtered using a muslin cloth. The extract was concentrated with a rotary evaporator, and dried in the oven at 40^0C. The dried ethanol extract was dissolved in 0.9% physiological saline.

2.4 Acute Toxicity Study

Acute toxicity testing was performed according to the OECD up and down procedure. 14 rats were used, which were fasted overnight before the administration of 0, 250, 500, 1000, 2000, 3000 and 5000mg/Kg bw for both aqueous and ethanol extracts orally using an intubator. 0 dose (control) was administered 1ml of vehicle (physiological saline).
After administration, rats were observed periodically for symptoms of toxicity and death within 24 hours and then daily for 14 days.

2.5 Experimental Procedure

A total of 40 rats were divided into 8 groups of 5 rats each and were exposed to the following treatment for 16 days.
Group 1 (control): physiological saline (po)
Group 2: 200mg/kg INH (po) + 200mg/kg RIF (po)
Group 3: 200mg/kg INH (po) + 200mg/kg RIF (po) + 100mg/kg vitamin E (ip)
Group 4: 200mg/kg INH (po) + 200mg/kg RIF (po) + 100mg/kg silymarin (po)
Group 5: 200mg/kg INH (po) + 200mg/kg RIF (po) + 250mg/kg ABAE (po)

Group 6: 200mg/kg INH (po) + 200mg/kg RIF (po) + 500mg/kg ABAE
Group 7: 200mg/kg INH (po) + 200mg/kg RIF (po) + 250mg/kg ABEE (po)
Group 8: 200mg/kg INH (po) + 200mg/kg RIF (po) + 500mg/kg ABEE (po)

Physiological saline was used as vehicle for all plant extracts and drugs except Vitamin E, which was dissolved in olive oil. 1ml of physiological saline, silymarin and the various doses of plant extracts each were administered to respective groups via oral gavage (po) using an intubator, while 1ml of vitamin E (dl-α-tocopheryl acetate) was administered intraperitoneally (ip). After 1 hour, 1ml of the given doses of isoniazid and rifampicin each were administered via oral gavage with an intubator to the respective groups. The experiment lasted for 14 days.

The animals were then sacrificed after an overnight fast, under chloroform anaesthesia. Blood was collected from each rat by cardiac puncture and from abdominal aorta. The liver were harvested, washed in phosphate buffer saline (PBS) and weighed. Liver tissue weighing 1g was homogenized in 10mls of PBS, centrifuged at 4000rpm for 20 minutes and the supernatant was collected for antioxidant assay. The blood serum were obtained after centrifugation at 3000rpm for 10 minutes and stored at -20^{o}C until use for liver function tests.

2.6 Biochemical Analyses

Antioxidant Assay (Oxidative Stress Assessment)
Malondialdehyde (MDA) and reduced glutathione (GSH) were estimated by [20] and Ellman's methods respectively.

Liver Function Tests
Plasma ALP, ALT, AST, bilirubin, total protein and albumin levels were estimated by commercially available kits (Randox of UK).

Histopathology
Histopathological analysis was carried out on the liver tissue preserved in 10% formalin to detect morphological changes.

Statistical Analysis
The Statistical Package for the Social Sciences (SPSS) version 20 was used to analyse the data obtained. Results are expressed as mean ± SEM (standard error of mean). Comparison of data within respective groups was done using One Way Analysis of Variance (ANOVA). The differences were considered significant when $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Acute Toxicity Study

There was no mortality among the graded dose groups of animals and the aqueous extract groups did not show any toxicity or behavioural changes at a dose level of 5000mg/kg body weight, but the ethanol extract groups were observed to be sluggish. This finding suggests that aqueous leaf extract of *A. boonei* is safer than the ethanol leaf extract. Hence, lower doses of 250mg/kg and 500mg/kg were selected for this study.

3.2 Biochemical Assays

Results for all the biochemical assays are shown below.

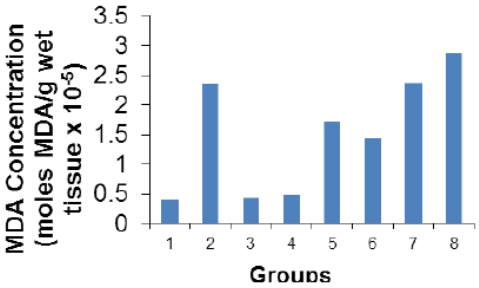


Figure 1. Malondialdehyde estimation after 14 days treatment.

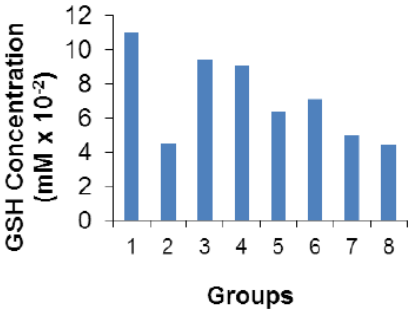


Figure 2. Reduced glutathione estimation after 14 days treatment.

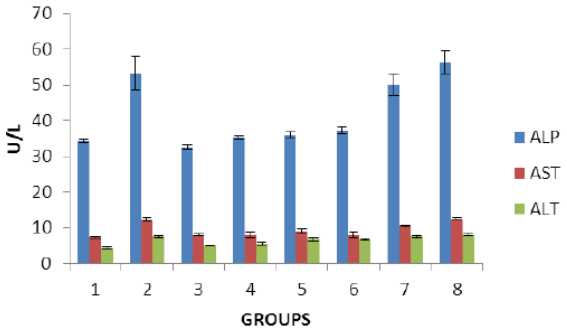


Figure 3. Liver function enzymes activity after 14 days treatment.

Total Bilirubin

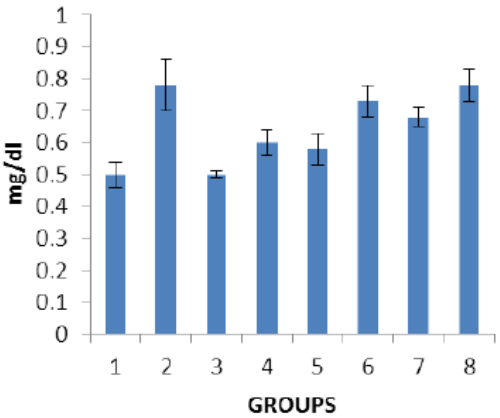


Figure 4. Total bilirubin concentration after 14 days treatment.

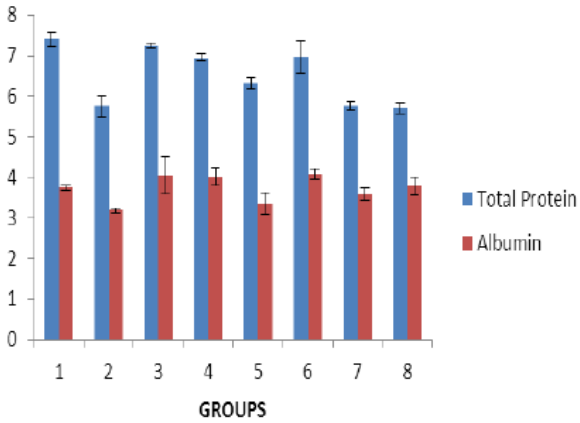


Figure 5. Total protein and Albumin concentration after 14 days treatment.

The results from this study indicated that there was incidence of hepatotoxicity in the isoniazid and rifampicin treated rats as suggested by previous studies [4,9-10]. High MDA and low glutathione values (figure 1 and 2) confirm the incidence of hepatotoxicity due to generation of free radicals during hepatic biotransformation of the anti-tubercular drugs [6].The administration of INH+RIF resulted in a significant increase ($p < 0.05$) in the plasma AST, ALT, ALP (Figure 3) and total bilirubin (figure 4)\ levels. This is attributed to the damaged structural integrity of the liver, hence they are released into systemic circulation. Moreover, decrease in albumin and total protein levels (figure 5) also showed that administration of INH+RIF has caused impairment of liver function; specifically its capacity to synthesize albumin. This is because the liver is the major organ that synthesizes proteins and albumin is only produced in the liver. Thus, the incidence of toxicity reduces the liver's ability to carry out this function.

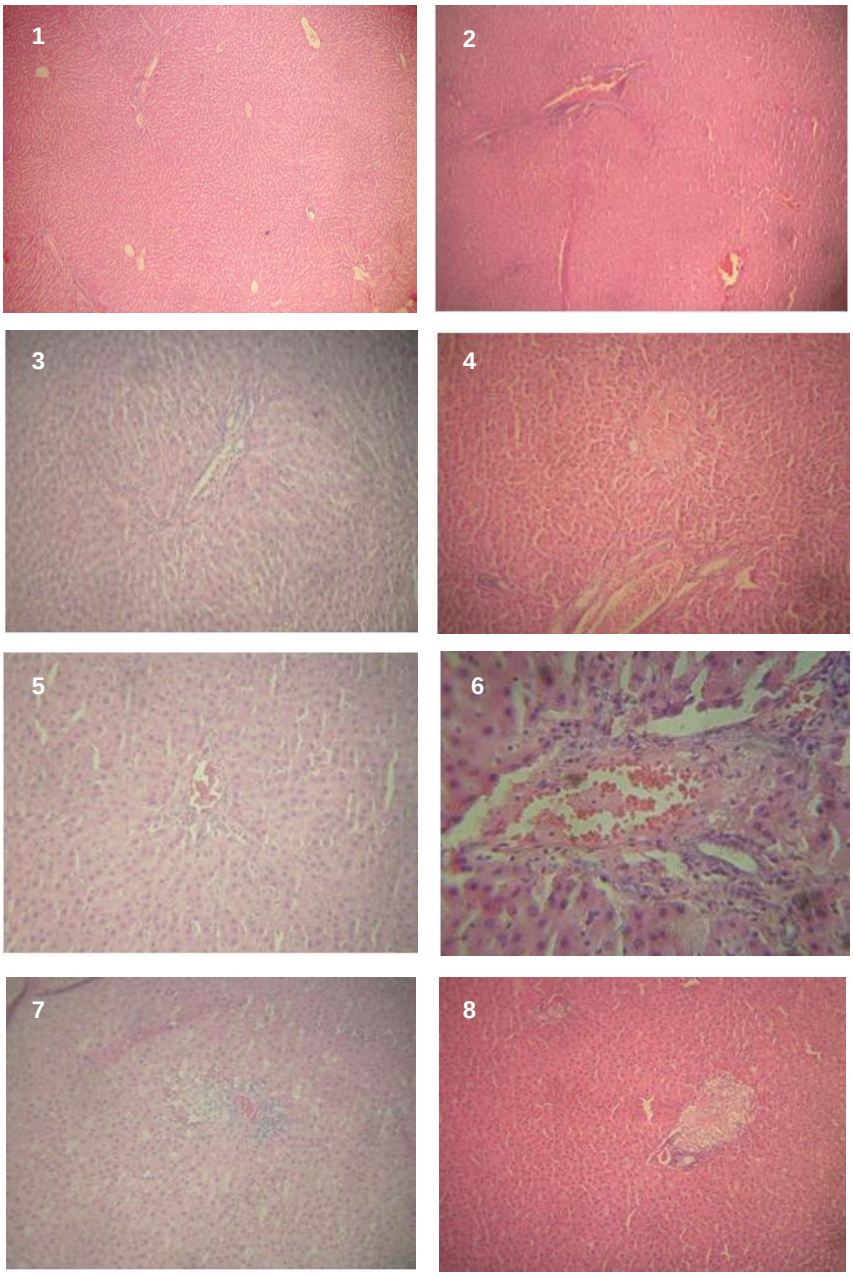
This study revealed that vitamin E, a known antioxidant almost completely restored the liver back to normal [21] as observed by the near normal values of liver function parameters, and this was confirmed by the histopathology result. Vitamin E and silymarin, another known hepatoprotective agent, were seen to protect the liver from oxidative injury [21-22]), as seen in the significantly low values of MDA and high values of reduced GSH in the vitamin E and silymarin treated groups. However, histopathology showed that vitamin E was a better protective agent than silymarin. It was also observed that there was significant increase in the concentrations of total protein and albumin in the vitamin E and silymarin treated groups compared to the INH+ RIF treated rats, this indicates restored liver function.

The aqueous leaf extracts of *Alstonia boonei* (250 and 500mg/ kg bw) slightly abated the effects of the hepatotoxic drugs . It was observed that they slightly reduced the plasma activities of the liver enzymes, ALP, AST, and ALT that were previously raised in INH + RIF groups. Other biochemical parameters also had slight changes. Whereas the ethanol leaf extracts of *A. boonei (*250mg/kg and 500mg/ kg bw) gave values close to those of INH+ RIF treated group.

4. CONCLUSION

The results obtained in this study indicate that the aqueous and ethanol leaf extracts of *Alstonia boonei* only slightly protected the animals from hepatotoxicity induced by the anti-tubercular drugs, isoniazid and rifampicin during the study periodWe therefore recommend that more studies should be done on the

hepatoprotective activity of the aqueous leaf extract of *A. boonei* for a longer period of time and also the use of alcoholic infusions and decoctions of this plant be discouraged, aqueous infusions and decoctions should rather be used.



Histopathology Results. Plates: (1) Untreated liver (Group 1) showing normal hepatocytes radiating from central vein and portal triad [H&E, X10]; (2) Isoniazid (200mg/kg) and Rifampicin (200mg/kg) treated liver (Group 2) showing generalized central and portal venous congestion and periportal inflammation [H&E, X10]; (3) Isoniazid (200mg/kg), Rifampicin (200mg/kg) and Vitamin E treated (Group 3) liver showing abated effect of the drugs on the liver, with normal hepatocytes and focal portal venous congestion [H&E, X10]; (4) Isoniazid (100mg/kg), Rifampicin (100mg/kg) and Silymarin (Group 4) treated liver showing slightly abated effect of the drugs on the liver, with normal hepatocytes and generalized portal venous congestion; (5) Isoniazid (200mg/kg), Rifampicin (200mg/kg) and aqueous extract of *Alstonia boonei* (250mg/kg)(Group 5) treated liver showing slightly abated effect of the drugs on the liver, with normal hepatocytes, and focal portal venous congestion [H&E, X10]; (6) Isoniazid (200mg/kg), Rifampicin (200mg/kg) and aqueous extract of *Alstonia boonei* (500mg/kg) (Group 6) treated liver showing slightly abated effect of the drugs on the liver, with normal hepatocytes, focal portal venous congestion and mild inflammation [H&E, X40]; (7) Isoniazid (200mg/kg), Rifampicin (200mg/kg) and ethanolic extract of *Alstonia boonei* (250mg/kg) (Group 7) treated liver showing focal periportal hepatocytes necrosis, portal venous congestion and marked inflammation [H&E, X10]; (8) Isoniazid (200mg/kg), Rifampicin (200mg/kg) and ethanolic extract of *Alstonia boonei* (500mg/kg) (Group 8) treated liver Isoniazid showing extensive focal periportal hepatocytes necrosis, portal venous congestion [H&E, X10].

REFERENCES

1. Lee WM (2003). Acute liver failure in the United States. *Semin. Liver Dis* 23:217–226.
2. Alcado R, Arthur MJ and Iredale JP (1997). Pathogenesis of liver fibrosis. *Clin Sci* 19: 103-112.
3. Gebhardt R (2002). Inhibition of Cholesterol biosynthesis in HEPG 2 cells by artichoke extracts reinforced by glucosidase pretreatment. *Phytother. Res* 16: 368-372.
4. Tasduq SA, Peerzada K., Koul S., Bhat R and Johri RK (2005). Biochemical manifestations of anti-tuberculosis drugs induced hepatotoxicity and the effect of silymarin. *Journal of Hepatology Research* 31: 132-135.
5. Sharma SK (2004). Antitubercular drugs and hepatotoxicity. *Infect Genet* 4: 167-70.
6. Mahashur AA and Prabhudesai PP (1991). Hepatitis and antitubercular therapy. *J Assoc Physicians India* 39(8): 595–596.
7. Adhvaryu MR, Reddy NM and Vakharia BC (2008). Prevention of hepatotoxicity due to anti-tuberculosis treatment: A novel integrative approach. *World J Gastroenterol* 14(30): 4753-4762.
8. Timbrell JA, Mitchell JR, Snodgrass WR and Nelson SD (1980) Isoniazid hepatoxicity: the relationship between covalent binding and metabolism *in vivo*. *J Pharmacol Exp Ther.* ;213:364–369.
9. Steele MA, Burk RF and DesPrez RM (1991). Toxic hepatitis with isoniazid and rifampicin. A meta-analysis. *Chest* 99:465-71.
10. Vuilleumier N, Rossier MF and Chiappe A (2006). CYP2E1 genotype and isoniazid-induced hepatotoxicity in patient treated for latent tuberculosis. *Eur J Clin Pharmacol* 62:423-429.
11. Castilla A, Prieto J and Fausto N (1991). Transforming Growth factors B1 and alpha in chronic liver disease effects of interferon alpha therapy. *National England J Med* 324: 933-940.
12. Toklu H, Akbay T, Velioglu-Ogunc A, Ercan F, Gedik N, Keyer-Uysal M.and Sener G (2008). Silymarin, the antioxidant component of Silybum marianum, prevents Sepsis-induced acute lung and brain injury. *J Surgical Res* 145,214–222.
13. Chatterjee TK (2000). Medicinal plants with hepatoprotective properties. In: Herbal Options. 3rd ed. Calcutta: Books and Allied (P) Ltd. p.135.
14. Nardini M and Ghiselli A (2004). Determination of free and bound phenolic acid in beer. *Food Chemistry* 84(1): 137-143.
15. Ernst E (2005). The efficacy of herbal medicine an overview. *Fundam. Lin Pharmacol* 19: 405-409.
16. Iyiola OA, Tijani AY and Lateef KM (2011). Antimalarial activity of ethanolic stem bark extract of *Alstonia boonei* in mice. *Asian J Biol Sci* 4 235–243.
17. Adomi PO (2008). Screening of the leaves of three Nigerian Medicinal Plants for antibacterial activity. *Afr. J. Biotechnol,* 7(15): 2540-2542.
18. Iwu MM, (1993). Handbook of African Medicinal Plants. CRC Press, London, pp: 183-184.
19. Akinmoladun EO, Ibukun EA, Akinrinola BL, Onibon TR, Akinboboye AO, Obuotor EM and Farombi EO (2007). Chemical Constituents and antioxidant activity of *Alstonia boonei.African Journal of Biotechnology* 6(10): 1197-1201.
20. Buege JA and Aust SD (1978). Microsomal Lipid Peroxidation. *Methods in Enzymology* 52: 302-310.
21. Traber MG, Atkinson J (2007). Vitamin E, antioxidant and nothing more. *Free Radic Biol Med* 43: 4-15.
22. Valenzuela M and Garrido A (1994). Biochemical basis of the pharmacological action of the flavonoid silymarin and its structural isomer silibinin. *Biol Res* 27(2):105-12.