International Journal of Biomedical and Pharmaceutical Sciences ©2010 Global Science Books

Modulatory Roles of Antioxidants against the Aqueous Stem Bark Extract of *Alstonia boonei* (Apocynaceae)-induced Nephrotoxicity and Testicular Damage

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ABSTRACT

Alstonia boonei is a commonly used medicinal plant that has been documented to be nephrotoxic and capable of inducing testicular damage. The protective effect of vitamins E and C against *A. boonei*-induced toxicity was examined using albino rats. Oral administration of 1000 mg/kg of aqueous *A. boonei* stem bark extract (AABSBE) alone, AABSBE + a therapeutic dose of vitamin E, AABSBE + a therapeutic dose of vitamin C and AABSBE + a therapeutic dose of vitamins E + C were carried out for 8 weeks using 5 rats per group. The AABSBE-treated group showed a significant ($P \leq 0.05$) increase in the levels of urea and creatinine compared to the control group. Oxidative stress tests of both the kidneys and testes showed a significant increase in the level of malondialdehyde in the AABSBE-treated group while a significant decrease was observed in the levels of Catalase (CAT), Superoxide dismutase (SOD), Reduced glutathione (GSH) and Glutathione-*S*-transferase (GST) activities of the AABSBE- treated group compared with the control. The results further showed the AABSBE + vitamins E and C-treated group demonstrated a significant ($P \leq 0.05$) decrease in the levels of urea, creatinine and MDA compared with the AABSBE-treated group. There was also a significant increase in the levels of CAT, SOD, GSH and GST activities of the AABSBE + vitamins E and C-treated group compared with the AABSBA-treated group. These findings indicate that antioxidants (vitamins E and C) have the potential to protect against *A. boonei*-induced renal and testicular damage. Thus, traditional practitioners should be encouraged to always include either synthetic or natural antioxidants in the AABSBE-containing remedy.

Keywords: oxidative stress, renal damage, testes, vitamins C and E

INTRODUCTION

The use of plants as a source of medicine to treat disease is an ancient practice and generations have long prepared poultices and infusions from indigenous plants, dating back to pre-history (Cowan 1999). Plants produce a diverse range of bioactive molecules making them rich sources of different types of medicines (Nair *et al*. 2005). Thus, in recent times, attention has been re-reverted to plants as sources of therapeutic agents due to their higher properties than other sources.

Alstonia boonei (Apocynaceae) is a large evergreen tree, one of the widely used medicinal plants in Africa. It is distributed throughout the tropics and rain forests of West and Central Africa (Oliver-Bever 1986; Olajide 2000). It is not edible, but possesses medicinal properties contained in the stem bark. The major phytochemicals in the stem bark are saponins, alkaloids, tannins and cardiac glycosides (Fasola and Egunyomi 2005). The stem bark is commonly used by traditional practitioners for treating febrile illness, jaundice, painful micturition, rheumatic conditions, as an antivenom against snake bite and as an antidote against arrow poisoning (Ojewole 1984; Asuzu and Anaga 1991). Moreover, the extract of the stem bark is often used as a febrifuge in treating malaria and is listed in the African Pharmacopoeia as an antimalarial drug (Olajide *et al*. 2000). Terashima (2003), Betti (2004) and Abel and Busia (2005) have shown that the *A. boonei* bark extract could be used in intestinal helminthes, rheumatism and hypertension. Interaction with the traditional practitioners revealed that *A. boonei* is one of the 10 most commonly used medicinal plants in the South-west region of Nigeria (Akintonwa *et al.* 2009).

Despite the numerous traditional uses of *A. boonei* it is known to cause nephrotoxicity, especially at higher doses and after chronic application (Oze *et al*. 2007). It has also been shown to be toxic to the reproductive function in males by inducing testicular damage (Raji *et al.* 2005; Oze *et al*. 2007). Probably due to lack of awareness of these toxicities by traditional practitioners, there is continual use of this medicinal plant for traditional medicine practice. Current understanding of drug toxicity has shown that free radicals are usually generated by the action of some drugs and thus cause damage to cells, tissues and human organs. However, antioxidants have been known to scavenge free radicals thereby preventing adverse destruction of architecture and functions of the body components by these radicals (Awodele *et al.* 2010a, 2010b).

In view of the above, this present study investigates the possible roles of vitamins E and C in protecting *A. boonei*-induced nephrotoxicity and male reproductive organ damage. The outcome of this investigation might form the basis of intervention and counseling of traditional practitioners on the rational use of *A. boonei* so as to prevent unintended adverse effects.

Received: 1 December, 2009. Accepted: 17 November, 2010.

Original Research Paper

MATERIALS AND METHODS

Plant collection and identification

The stem bark of an approximately 12-months-old *A. boonei* was collected from a secondary forest in Ikire Osun state, Nigeria. It was authenticated by T. K. Odewo, a senior superintendent of the Forestry Research Institute of Nigeria (FRIN) where voucher specimen FHL108275 was deposited for reference, and confirmed by A. Adeleke of the Pharmacognosy Department, University of Lagos, Nigeria.

Extraction of stem bark of *A. boonei*

The stem bark was air dried and grounded to a fine powder, which was then dissolved in distilled water (10 g/L). The liquid was decanted 24 hrs later. The filtrate was evaporated to dryness in a 40°C oven. Yield ranged between 12 and 13.5% on a dry weight basis. The dried extract was stored in a refrigerator and dissolved in distilled water just before use. The pH of the extract was 7.8.

Animal collection

Adult male Albino rats (150-250 g) and Swiss albino mice (18-20 g) were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria.

Drugs

Vitamin C tablets (100 mg/tablet) and Vitamin E tablets (100 mg/tablet) were obtained from the outpatient Pharmacy Department of the Lagos University Teaching Hospital, Lagos, Nigeria.

Experimental procedure

1. Acute toxicity test

An oral acute toxicity study was performed using 30 male Swiss mice (18-20 g). The animals were acclimatized in a temperature controlled environment with a 12-h light-dark cycle, fed mice with pellet (Pfizer pellet) and water *ad libitum* and fasted for a period of 24 hrs prior to administration of the extract. The animals were divided into 6 groups and single oral doses of 500, 1000, 2000, 4000, 6000 and 8000 mg/kg of aqueous *A. boonei* stem bark extract (AABSBE) were administered. The control group was administered 0.1 ml of distilled water. The animals were examined for 24 hrs for any behavioral signs and death. The lethal dose (LD_{50}) was calculated using a probit analysis as described by Miller and Tainter (1944).

2. Sub-chronic toxicity test

25 male albino rats (150-250 g) were used for the chronic toxicity experiment. The dose of the extract used was not greater than ⅓ of the LD_{50} obtained. The animals were divided into 5 treatment groups, all of which were administered orally for 2 months: Group 1 (control group): 0.25 ml of distilled water; Group 2 (positive control): 1000 mg/kg AABSBE; Group 3: 5 mg/kg Vitamin E + 1000 mg/kg AABSBE; Group 4: 8 mg/kg Vitamin C + 1000 mg/kg AABSBE; Group 5: 5 mg/kg Vitamin E + 8 mg/kg Vitamin C + 1000 mg/kg AABSBE.

The administration of vitamins E and C started a month before the incorporation of AABSBE into the treatment. After 60 days' administration, rats were sacrificed by cervical dislocation for internal macroscopic analysis; weight of organs was measured and the kidneys and testes were examined histologically. Urea and creatinine were estimated using a fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany). Oxidative stress parameters {Malondialdehyde (MDA), Catalase (CAT), Superoxide dismutase (SOD), Reduced glutathione (GSH) and Glutathione-*S*-transferase (GST)} were determined using the methods of Beers and Seizer (1952) and Beuge and Aust (1978).

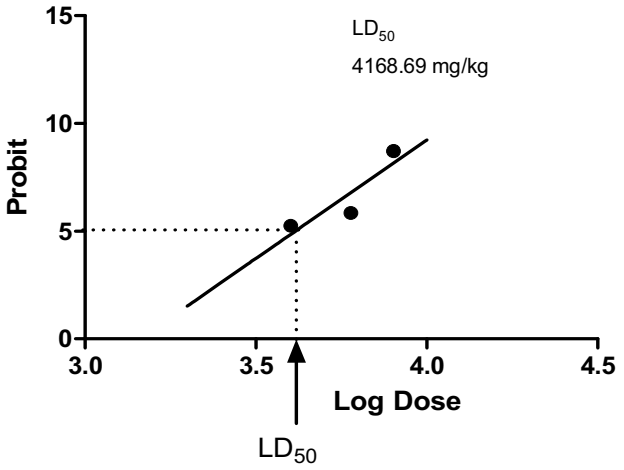

Fig. 1 Probit analysis for the determination of LD_{50} of *A. boonei* in the treated mice.

Statistical analysis

Results are presented as mean ± S.E.M. Statistical significance between the control groups and the test groups were analyzed by a student's *t*-test at $P < 0.05$.

RESULTS

Mortality results show that AABSBE at different graded doses (4000, 6000 and 8000 mg/kg) induced 60, 80 and 100% mortality, respectively (**Table 1**). The LD_{50} was 4168.69 mg/kg using a probit analysis (**Fig. 1**).

There were no statistically significant differences in the weight of the kidneys, testes and epididymis of the rats treated with AABSBE alone, AABSBE + vitamin E, AABSBE + vitamin C and AABSBE + vitamins E and C (**Table 2**).

There was a statistically significant difference in the level of creatinine and urea between the AABSBE-treated group and the control (**Table 3**). There was a significant in-

Table 1 Mortality induced in mice (n = 5) 24 hrs after acute oral treatment with *Alstonia boonei*.

Dose (mg/kg)	Log dose	% Mortality	Probit
800	2.602	0	-
1000	3.000	0	-
2000	3.301	0	-
4000	3.602	60	5.25
6000	3.778	80	5.84
8000	3.903	100	8.72

Table 2 Organ weight of the rats (n =5) after 60 days of treatment with *Alstonia boonei* and antioxidants.

Group	Mean organ weight/100 g body weight (means ± SEM)		
	Kidney	Epididymis	Testes
Control	0.396 ± 0.03	0.720 ± 0.09	0.239 ± 0.06
Alstonia boonei	0.410 ± 0.02	0.751 ± 0.12	0.241 ± 0.02
A. boonei + Vit E	0.379 ± 0.02	0.750 ± 0.13	0.222 ± 0.02
A. boonei + Vit C	0.441 ± 0.06	0.795 ± 0.07	0.221 ± 0.09
A. boonei + Vit E+C	0.444 ± 0.05	0.785 ± 0.13	0.225 ± 0.08

Table 3 Renal function parameters in rats (n =5) after 60 days of treatment with *Alstonia boonei* and antioxidants.

Group	Creatinine	Urea
Control	73.05 ± 2.88	9.75 ± 0.95
Alstonia boonei	95.42 ± 6.37 a	17.15 ± 4.40 a
A. boonei + Vit E	74.40 ± 4.42 b	7.08 ± 0.58 b
A. boonei + Vit C	61.37 ± 3.37 b	6.75 ± 0.14 b
A. boonei + Vit E+C	71.59 ± 1.75 b	9.45 ± 1.26 b

a or b letter within a column indicates significant differences at $P \leq 0.05$ compared with the control or *A. boonei* respectively using the student's t-test

Table 4 Kidney oxidative stress parameters in rats (n = 5) after 60 days of treatment with *Alstonia boonei* and antioxidants.

Group	MDA	CAT activity	GSH	GST	SOD
Control	2.78 ± 0.04	149.25 ± 0.19	0.08 ± 0.01	0.88 ± 0.01	0.81 ± 0.01
Alstonia boonei	7.76 ± 0.04 a	54.00 ± 0.11 a	0.06 ± 0.01 a	0.54 ± 0.01 a	0.53 ± 0.02 a
A. boonei + Vit E	3.46 ± 0.07 b	86.60 ± 0.33 b	0.06 ± 0.01 b	0.75 ± 0.06 b	0.62 ± 0.01 b
A. boonei + Vit C	3.19 ± 0.07 b	145.63 ± 0.29 b	0.07 ± 0.01 b	0.81 ± 0.01 b	0.70 ± 0.01 b
A. boonei + Vit E+C	4.58 ± 0.07 b	147.86 ± 0.22 b	0.07 ± 0.01 b	0.83 ± 0.01 b	0.96 ± 0.01 b

a or b letter within a column indicates significant differences at $P \leq 0.05$ compared with the control or *A. boonei* respectively using student's t-test; MDA, malondialdehyde (U/g proteins); GSH, reduced glutathione (U/g proteins); GST, Glutathione-*S*-transferase (U/g proteins); SOD, Superoxide dismutase (U/g proteins)

Table 5 Testes oxidative stress parameters in rats (n = 5) after 60 days of treatment with *Alstonia boonei* and antioxidants.

Group	MDA	CAT activity	GSH	GST	SOD
Control	7.81 ± 0.07	74.25 ± 0.19	0.09 ± 0.03	0.87 ± 0.01	0.71 ± 0.01
Alstonia boonei	11.71 ± 0.07 a	44.50 ± 2.93 a	0.06 ± 0.01 a	0.42 ± 0.01 a	0.35 ± 0.01 a
A. boonei + Vit E	8.82 ± 0.04 b	77.88 ± 0.22 b	0.08 ± 0.01 b	0.67 ± 0.01 b	0.68 ± 0.01 b
A. boonei + Vit C	8.50 ± 0.02 b	88.50 ± 0.12 b	0.07 ± 0.01	0.73 ± 0.02 b	0.54 ± 0.02 b
A. boonei + Vit E+C	7.87 ± 014 b	79.25 ± 0.19 b	0.07 ± 0.01	0.74 ± 0.01 b	0.68 ± 0.01 b

a or b letter within a column indicates significant differences at $P \leq 0.05$ compared with the control or *A. boonei* respectively using student's t-test; MDA, malondialdehyde (U/g proteins); GSH, reduced glutathione (U/g proteins); GST, Glutathione-*S*-transferase (U/g proteins); SOD, Superoxide dismutase (U/g proteins)

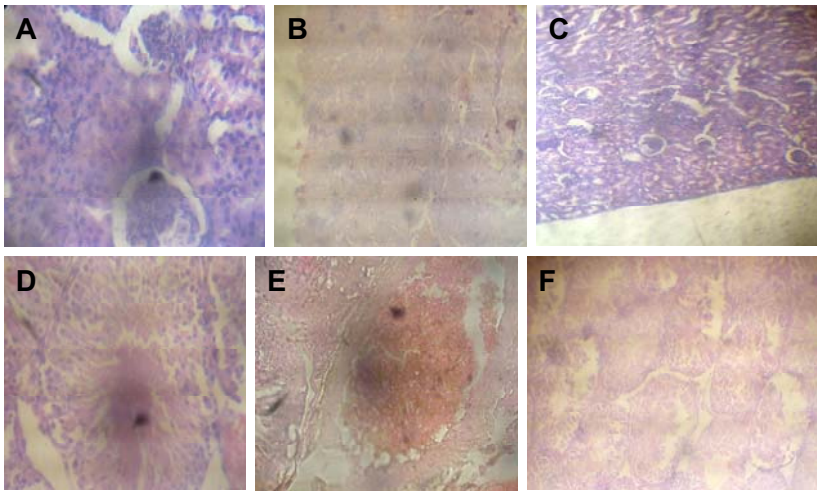

Fig. 2 Histological sections. (**A**) Kidney of control rat. (**B**) Renal tissue of rat treated with *A. boonei*. (**C**) Kidney tissue of rat treated with *A. boonei* + Vit C and E. (**D**) Testicular tissue of control rat. (**E**) Testicular tissue of rat treated with *A. boonei*. (**F**) Testicular tissue of rat treated with *A. boonei* + Vit C and E. **A-F**: Magnification ×40.

crease in creatinine and urea between the AABSBE-treated group and all other groups.

The oxidative stress parameters of the kidneys (**Table 4**) showed a statistically significant increase in the level of MDA between the control group and the AABSBE-treated group. There were also a statistically significant decrease in the levels of CAT, GSH, GST and SOD between the control group and the AABSBE-treated group. The results further showed a statistically significant increase in the levels of CAT, GSH, GST and SOD between AABSBE-treated group and the other four groups.

There was a statistically significant increase in the testes MDA level between the control and *A. boonei*-treated group (**Table 5**). The results further showed statistically ($P \leq 0.05$) significant decrease in the levels of CAT, GSH, GST and SOD parameters between the control and *A. boonei* treated group. The levels of CAT, GST and SOD parameters were significantly increased when *A. boonei is* co-administered with vitamins E and/or C. The MDA significantly decreased between *A. boonei* group and *A. boonei* co-administered vitamins E and/or C.

Histopathological results showed AABSBE to cause extensive damage of the renal tissue but the co-administration of AABSBE with vitamins E and C demonstrated protection (**Figs. 2A-C**). There was also testicular damage of the germ cells caused by the administration of AABSBE while protection was observed in the testes when AABSBE was co-administered with vitamins C and E (**Figs. 2D-F**).

DISCUSSION

Alstonia boonei De Wild belongs to the family Apocyanaceae (Majekodunmi *et al*. 2008). The tree is 39 m high and 3 m in girth, with straight trunk, deeply fluted at the base and the branches are whorled (severally radiating from the trunk at the same level especially in the young trees). The leaves are simple whorled and are confined towards the end of the twigs to give a rather small crown. The bark is grayish, rough and has small scattered lenticels. Its slash is thick, granular, mottled yellow and exudes copious white latex. Traditionally the infusion of the stem bark is drunk as a remedy for snake-bite and also for arrow poison. It is also used for treating fever and the infusion of root, stem bark and leaves is drunk as remedy against asthma (Adomi and Umukoro 2010).

The aqueous stem bark extract of *A. boonei* was found not to produce any mortality in treated mice when orally administered with doses up to 2000 mg/kg. Although a dose-dependent mortality was latter recorded between 4000 and 8000 mg/kg of the oral administration. The LD_{50} of the extract was found to be 4168.69 mg/kg which is much higher than the 2 g/kg dose reported to be the ceiling point for medicinal plants toxicity when administered orally in acute toxicity study (Lu and Lavalle 1965). However, this safety assertion may not be applicable to medicinal plants taken for a long period.

The kidney is the primary organ for clearance and excretion of xenobiotics including drugs and drugs product from the body. Study of the effect of the drug extract on the kidney is essential because of the cardinal role the organ

plays in plasma clearance, some detoxification, homeostasis and excretion of xenobiotics. The report of Panda (1999) has shown that determination of some waste metabolic products excreted exclusively via the kidneys provide useful information about the health status of the kidneys such metabolites are urea and creatinine.

The *A. boonei* extract did not produce any significant difference ($P \geq 0.05$) on the mean values of the organs (kidneys, testes and epididymis) weight/100 g body weight of rat in all the treatment groups as compared with the control group. This may be as a result of the length of extract administration which may not be long enough to produce remarkable difference in the organ weight.

This study showed significant decrease ($P \leq 0.05$) in the values of urea and creatinine of the control group as compared with *A. boonei*-treated group. This increase in the renal function markers (Cheesebrough 1998) may indicate renal damage in the *A. boonei*-treated rats. This present findings corroborate the study of Cheesebrough (1998) and Oze *et al.* (2007) that showed *A. boonei* to be nephrotoxic. The mechanism of this nephrotoxicity may be due to excessive free radical generation in the kidneys of the animals as shown by a significant ($P \leq 0.05$) increase in the value of MDA and a significant ($P \leq 0.05$) decrease in the values of CAT activity, GSH, GST and SOD oxidative stress parameters in the *A. boonei*-treated group as compared with the control group. The decrease in GST observed in this present study may be due to the inhibition of the enzyme (GST) by the extract of *A. boonei* as earlier shown by the work of Fakae *et al*. (2000).

However, the co-administration of antioxidants (vitamins E and C) with *A. boonei* showed a significant ($P \leq 0.05$) decrease in the values of urea and creatinine as compared with the *A. boonei* alone treated group. This observation may demonstrate the protective capability of vitamins E and C against the *A. boonei* induced renal damage. Furthermore, the results of the kidneys oxidative stress obtained in this study showed that co-administration of antioxidants with *A. boonei* significantly ($P \leq 0.05$) increase the level of catalase activity, GSH, GST and SOD and significantly ($P \leq 0.05$) decrease the level of MDA as compared with the *A. boonei* alone treated group. The study of Ebuehi *et al.* (2009) had earlier shown that presence of free radicals may be one of the plausible explanations for the elevated MDA levels and the catalytic actions of anti-oxidants enzymes are important for the effective removal of oxygen radicals.

The histological section of renal tissues of animals that received only *A. boonei* showed extensive tissue necrosis. There is destruction of the tubulo-interstitial as well as involvement of the glomeruli to a large extent. This irreversible tissue damage was not seen in the sections of tissues from control animals and those that were given vitamin C, vitamin E as well as the animal that received the combined vitamins simultaneously in addition to *A. boonei*. The latter tissues showed reversible cellular changes in form of interstitial infiltrate of mononuclear inflammatory cells mainly lymphocytes and plasma cells. They showed vascular congestion and mild cellular oedema. There was no evidence of glomerular lesions, tubular necrosis, or interstitial fibrosis. The inflammatory response is mildest in the kidneys of animals that received both vitamins in addition to the extract.

The histological section of the testicular tissues from animals that received only *A. boonei* showed marked destruction of the testicular tissue. The seminiferous tubules with their contents were destroyed. The studies of Baldessarini (1980) and Oze *et al*. (2008) have shown that *A. boonei* extract is able to permeate blood testis barrier thus exerting toxic effect on the testis. The testicular damage caused by the extract is also expressed by the significant ($P \leq 0.05$) increase in the MDA level of the *A. boonei*-treated group. However, co-administration of *A. boonei* with antioxidants showed a significant ($P \leq 0.05$) decrease in the MDA level and increase in the catalase activity, GSH, GST and SOD levels as compared with the *A. boonei* alone-treated group.

The extent of destruction is minimal in the testicular tissues of rats that received vitamin E, vitamin C and both vitamins in addition with *A. boonei*. Though, the histological sections of these tissues showed disorganization and marked reduction in germ cells especially the mature sperm cells, there is preservation of the seminiferous tubules and insterstitium. Sections from rats treated with both vitamins showed lesser damage. The basement membrane is not thickened and there was no evidence of interstitial fibrosis.

It may be concluded that co-administration of *A. boonei* with Vitamins E and C have a protective effect against the *A. boonei* alone induced kidney and testicular damage. The probable mechanism of protection may be by free radical mopping of the radical generated by *A. boonei*. Thus, traditional practitioners should be encouraged to always incorporate antioxidants, either synthetic or natural, into the *A. boonei*-containing remedy.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support of University of Lagos, Lagos-Nigeria.

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