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Original Research Article

Prophylactic Effects of Ethanolic Extract of *Alstonia boonei* Stem Bark Against DDVP-induced Toxicity in Albino Rats

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ABSTRACT

The prophylactic effect of ethanolic extract of *Alstonia boonei*(AB) stem bark on(2,2-dichlorovinyl dimethyl phosphate)DDVP-induced oxidative damage in male albino rats' liver was investigated. Male Wistar rats were divided into control, DDVP and treatment groups. In the prophylactic experiment, AB, (200 and 400 mg/kg body weight) was administered by oral gavage for 21th days before exposure to DDVP. Lipid peroxidation (LPO), reduced glutathione (GSH) levels, catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were then determined in the liver and heart alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were monitored and histological examination was carried out. Results indicate that DDVP-induced rats had significantly increased relative weight of liver and heart when compared to controls. Treatment with AB at 200 and 400mg/kg caused a significant reduction in relative weight of the organs. In DDVP-induced rats, serum ALT and AST activities and levels of LPO were increased whereas hepatic and cardiac SOD, CAT and GPx were significantly decreased. Furthermore, histological alteration in the liver and aorta were observed in DDVP untreated rats and were ameliorated in DDVP-induced treated rats with AB. In conclusion, the extract possesses antioxidant and hepatoprotective properties that eliminate the deleterious effects of toxic metabolites of DDVP.

KEYWORDS: Antioxidant, DDVP; hepatoprotective; prophylactic; *Alstonia boonei*.

INTRODUCTION

Recent studies have showed that increased formation of free radicals or reactive oxygen species (ROS) contribute to cardiovascular disease (CVD) progression[1]. Generation of large amounts of ROS can overwhelm the intracellular antioxidant defence, causing lipid peroxidation, protein variation and DNA breaks[2]. Studies showed that ROS induced depletion of antioxidants are a key reason in developing CVD[3].The liver is an organ in man and animals responsiblefor detoxifying thousands of chemicals from bodies every single day and if it cannotrole, toxinsbegins

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to build up in the tissues. It aids in digestion and removes waste products, worn-out cells from the blood and in protein synthesis. The liver is our greatest chemical factory, it builds complex molecules from simple substances absorbed from the digestive tract, it makes bile which aids fat digestion and removes toxins through the bowels[4]. But the ability of the liver to perform these rolesis by many substances exposed to daily. These substances include certain medicinal agents which when taken in overdoses and sometimes when introduced within therapeutic ranges injures the organ[5]. It is important to understand that with the present-day human, so many chemical additives and pollutants found in the air, water and food this signals to important care after the health of our liver[6].

Dichlorvos or 2,2-dichlorovinyl dimethyl phosphate (Trade Names: DDVP, Vapona, etc.) is a volatile organophosphate, widely used as an organo-phosphorus insecticide to control household pests, in public health, and protecting stored product from insects. Dichlorvos has high to extreme acute toxicity from oral or dermal exposure, and extreme acute toxicity from inhalation. Taking in large doses may cause nausea and vomiting, restlessness, sweating, and muscle tremors. Large doses may cause coma, inability to breathe, and death[7]. Therefore, these adverse effects, the search for natural products with hepatoprotective potential and slight side effect needed.

Alstonia boonei De Wild is large deciduous evergreen tree, usually up to 45m tall and 1.2m in diameter, belonging to the family Apocynaceae consisting of about 40-60 species. It is a native of tropical and subtropical Africa, Southeast Asia, Central America and Australia. 'Alstonia' named after Dr. C. Alston (1685-1760), a Professor of Botany at Edinburgh University. It's reported for treatment of malaria, intestinal helminthes, rheumatism, muscular pain, insomnia, and hypertension. It contains phytochemicals such as saponin, alkaloids, tannins and steroids[8,9]. In folk medical practice, an infusion of the extract of stem bark serves as anti-snake venom and as antidote to some arrows poisons. Antimalarial of various fractions of the stem bark extract of *Alstonia boonei* reported by Bello IS & Iyiola OA [10,11] while Odugbemi TO et al., Idowu OA and collegues, Gbadamosi IT and collegues[12,13,14] confirmed indigenous medicinal usefulness of *Alstonia boonei* for malaria therapeutic use in Southwestern part of Nigeria. Although, medicinal importance of stem bark extract of *Alstonia boonei* in ameliorating some disease conditions reported by several authors, however, to the best of our knowledge, there is lack of information on the effect of this plant on protective effect. To carry out further scientific scrutiny on this plant, this study designed to evaluate the hepatoprotective effect of *Alstonia boonei* ethanolic stem bark extract on DDVP-induced toxicity in rats.

METHODS

CHEMICALS

Dichlorvos or 2,2-dichlorovinyl dimethyl phosphate(Trade Names: DDVP, Vapona, etc.) Bought from a local chemist in Ibadan, Nigeria. Thiobarbituric acids (TBA) bought from Aldrich Chemical Co. (Milwaukee, WI, USA). Glutathione, hydrogen peroxide, 5,5'-dithios-bis-2-nitrobenzoic acid (DNTB) and Epinephrine bought from Sigma Chemical Co., Saint Louis, MOUSA. Trichloroacetic acid (TCA) and Thiobarbituric acid (TBA) bought from British Drug House (BDH) Chemical Ltd., Poole, UK. Other reagents were of analytical grade and the purest quality available.

COLLECTION AND EXTRACTION OF *ALSTONIA BOONEI* STEM BARK

The stem bark of *Alstonia boonei* collected in Ado-Ekiti (EkitiState) and authenticated at the Department of Plant Science, Ekiti State University with herbarium number U.H.A.E 65. The stem bark of *Alstonia boonei* air-dried and crushed into fine powder. The powdered part extracted with ethanol using maceration and the extract concentrated in vacuum at 400C with rotary evaporator and water bath to dryness. The yield of the extraction was 5.01%.

ANIMALS

Inbred male Sprague Dawley rats (*Rattus novergicus*) weighing between 100-190g bought from the animal house of the Department of Chemical Sciences, Biochemistry Unit, Afe Babalola University, Nigeria. Animals kept in aired cages at room temperature (28-30°C) and preserved on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*.

ETHICAL APPROVAL

Rats handling and treatments conform to guidelines of the National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use. The ethical committee of the Afe

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Babalola University approved this study. All animals in this study follow the institutional Animal Ethical Committee according to guidelines given by Committee for Control and Supervision of Experiments on animals (CPCSEA).

INDUCTION Of EXPERIMENTAL ANIMAL

DDVP [O,O-dimethyl O-(2, 2-dichlorovinylphosphate) induced in groups II, III and IV. Briefly, DDVP dissolved in distilled water and then managed by intravenous injection (through tail vein) at a dose of 50 mg/kg body weight.

STUDY DESIGN

Twenty male rats randomly divided into four groups of five rats each. Group I served as the negative control and accessed to normal rat diet and water *ad libitum* only, Group II served as the positive control and given 50mg/kg body weight of DDVP. Group, III and IV given 200 mg/kg and 400 mg/kg body weight of the stem bark extracts after exposure to 50mg/kg body weight of DDVP respectively.

PREPARATION OF TISSUES

Rats fasted overnight and sacrificed 24 hours after the last dose of drugs. Liver and heart quickly removed and washed in ice-cold 1.15% KCl solution, dried and weighed. A section of liver and aorta samples fixed in 10% formalin for histological examination. The remaining parts of liver and heart homogenized in 4 volumes of 50 mM phosphate buffer, pH 7.4 and centrifuged at 10,000g for 15 minutes to get post-mitochondrial supernatant fraction (PMF). All procedures carried out at temperature of 0-4°C.

PREPARATION OF SERUM

Blood collected from the heart of the animals into plain centrifuge tubes and allowed to stand for 1 hour. Serum prepared by centrifugation at 3,000g for 15 minutes in a Beckman bench centrifuge. The clear supernatant used for estimating serum lipid profile and enzymes.

BIOCHEMICAL TESTS

Protein contents of the samplestested by the method of Lowry et al.[15] using bovine serum albumin as standard. The alanine and aspartate amino transferases (ALT and AST) tested by the combined methods of Mohun and Cook[16] and, Reitman and Frankel[17]. Lipid peroxidation level tested by the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA), aproduct of lipid peroxides as described by Buege and Aust[18]. The tissue superoxide dismutase (SOD) measured by the nitro blue tetrazolium (NBT) decrease method of McCord and Fridovich[19]. Catalase (CAT) tested spectrophotometrically by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi[20]. Reduced glutathione level measured by the method of Beutler et al.[21], this method is on developing a stable (yellow) color when 5',5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) added to sulfhydryl compounds. The chromophoric product resulting from Ellman's reagent with the reduced glutathione (2-nitro-5-thiobenzoic acid) holds a molar absorption at 412 nm which is proportion to the reduced glutathione in the test sample. The glutathione peroxidase (GPx) assessed by the method of Rotruck et al.[22].Glutathione-S-transferase (GST) determined according to Habig et al.[23], the principle is on all known GST explain a high with 1-chloro-2,4-dinitrobenzene as the second substrate. When this substance mixed with reduced glutathione, its absorption shifts to a longer wavelength 340 nm and increase at this wavelength provides a direct measurement of the enzymatic reaction.

HISTOPATHOLOGY OF TISSUES

Tissues fixed in 10% formalin dehydrated in 95% ethanol and then cleared in xylene before set in paraffin. Sections (about 4 µm) prepared and stained with hematoxylin and eosin (H&E) dye, and examined under a light microscope by a Histopathologist who was ignorant of the treatment groups.

STATISTICAL ANALYSIS

All values expressed as the mean ± S.D. of five animals each group. Data analyzed using one-way ANOVA followed by the post-hoc Duncan multiple range test for analysis of biochemical data using SPSS(16.0).Values considered statistically significant at $p< 0.05$.

RESULTS

Effects of *Alstonia boonei* stem bark on body weight and relative weight of organs of DDVP-induced toxicity rats

In table 1, there were significant increases ($p<0.05$) in the relative weight of liver and heart of DDVP untreated rats when compared with the control, while treatment with ethanolic extract of *Alstonia boonei* stem bark (100 and 200 mg/kg) significantly reduced the relative weight of heart and liver of DDVP rats to values that were

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statistically similar ($p>0.05$) to the control. All these changes induced by DDVP intoxication were significantly ($p < 0.05$) restored to near normal levels on administration of *Alstonia boonei* stem bark.

Table 1. Changes in the body weight and relative weight of organs of DDVP-induced toxicity rats treated with ethanolic extract of *Alstonia boonei.*

Treatment	Body weight (g)		Weight of organs (g)		Relative weight of organs (g)	
	Initial	Final	Liver	Heart	Liver	Heart
Control	100.25±0.21	117.46±5.32	6.35±0.27	0.51±0.02	2.22±0.05	0.22±0.04
DDVP untreated	112.08±1.12	128.10±4.96	5.24±0.60	0.44±0.07	3.35±0.08*	0.36±0.01*
DDVP + 200mg/kg	121.55±2.23	149.36±7.09	5.71±0.74	0.54±0.05	2.10±0.03**	0.28±0.01**
DDVP + 400mg/kg	151.02±3.35	176.22±4.99	6.13±0.57	0.59±0.04	2.25±0.05**	0.24±0.02**

Values are means ± S.E.M. of 5 animals per group, DDVP = at 50mg/kg DDVP Treated = *Alstonia boonei*at 200mg/kg, DDVP treated = *Alstonia boonei* at 400mg/kg, *significantly different from Control (p< 0.05), ** significantly different from DDVP untreated (p< 0.05).

EFFECTS OF *ALSTONIA BOONEI* STEM BARKON ANTIOXIDANT PARAMETERS AND MARKER ENZYMES IN DDVP-INDUCED TOXICITY IN RATS

Administration of DDVP significantly increased (p<0.05) serum, hepatic and cardiac lipid peroxidation (LPO) products measured as thiobarbituric acid reactive substances respectively (Table 2). However, treatment with *Alstonia boonei* extract completely ameliorated DDVP-induced increase in LPO. In DDVP -induced rats, the activities of hepatic and cardiac GSH, SOD and CAT as well as cardiac GPx decreased significantly relative to the control (Table 3). Excellent performance of extract at (400 mg/kg) reversed the adverse effect of DDVP by normalizing this enzymatic antioxidant. *Alstonia boonei* pre-treatment prior to DDVP exposure caused a significant increase in GPx activities as well as a noticeable increase in GSH level. In DDVP-induced rats, serum ALT and AST were significantly increased (Table 4) relative to the control. Treatment with *Alstonia boonei* before DDVP exposure resulted in significant protection of the liver and heart, as indicated by reductions in the elevated levels of ALT and AST; however, there was evidence of amelioration in the treated group.

Table 2. Changes in the levels of lipid peroxidation in DDVP-induced toxicity rats treated with ethanolic extract of *Alstonia boonei.*

Treatments	LIVER (µmol MDA/mg protein)	HEART (µmol MDA/mg protein)	SERUM (µmol MDA/mg protein)
Control	1.52±0.01	2.05±0.02	**2.82±0.08**
DDVP untreated	3.98±0.02*	4.15±0.03*	**3.98±0.06***
DDVP + 200mg/kg	1.22±0.03**	1.45±0.02**	**1.78 ±0.04****
DDVP + 400mg/kg	**1.42±0.01****	**1.85±0.04****	**2.56±0.07****

Values are means ± S.E.M. of 5 animals per group, DDVP = at 50mg/kg DDVP Treated = *Alstonia boonei*at 200mg/kg, DDVP treated = *Alstonia boonei* at 400mg/kg, *significantly different from control (p< 0.05), ** significantly different from DDVP untreated (p< 0.05).

Table 3. Changes in the levels of hepatic and cardiac antioxidant parameters in DDVP-induced rats treated with ethanolic extract of *Alstonia boonei.*

Treatment	Liver				Heart			
	GSH (mg /g tissue)	GPx	SOD (U/mg protein)	CAT	GSH (mg /g tissue)	GPx	SOD (U/mg protein)	CAT
Control	25.85±0.15	35.55±0.83	37.36±1.01	35.57±1.08	36.06±1.11	45.74±5.74	57.03±0.01	9.37±0.81
DDVP untreated	11.23±0.21*	13.65±0.41*	16.23±0.68*	19.22±0.58*	20.21±1.41*	22.26±1.10*	20.26±0.71*	4.57±0.31*
DDVP + 200mg/kg	21.38±0.24**	34.58±0.45**	35.88±0.03**	32.59±0.41**	34.23±2.14**	38.56±1.44**	54.16±0.37**	7.12±0.36**
DDVP + 400mg/kg	24.21±0.27**	35.48±0.21**	36.34±1.00**	34.78±0.81**	35.76±1.32**	42.54±1.22**	56.85±0.21**	8.98±0.47**

Values are means ± S.E.M. of 5 animals per group, DDVP = at 50mg/kg DDVP Treated = *Alstonia boonei*at 200mg/kg, DDVP treated = *Alstonia boonei* at 400mg/kg, *significantly different from control (p< 0.05), ** significantly different from DDVP untreated (p< 0.05).

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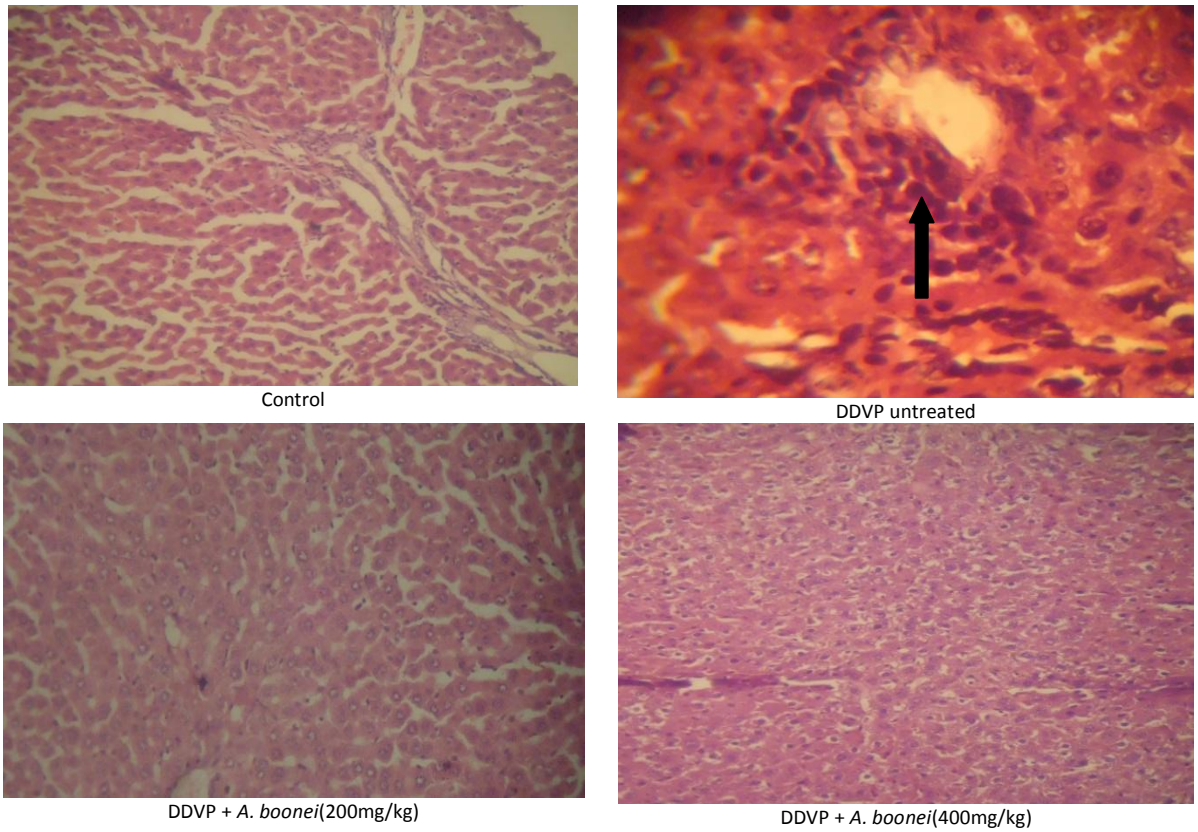
Table 4. Changes in the activities of serum, hepatic and cardiac alanine and aspartate aminotransferases in DDVP-induced rats treated with ethanolic extract of *Alstonia boonei.*

Treatments	LIVER (U/L)		HEART (U/L)		SERUM (U/L)	
	AST	ALT	AST	ALT	AST	ALT
Control	57.80±3.42	48.45±2.29	48.45±0.02	55.56±2.24	4.54±1.77	6.37±1.46
DDVP untreated	28.04±3.62*	26.78±2.56*	26.22±3.38*	23.24±3.34*	8.39±0.56*	9.89±2.24*
DDVP + 200mg/kg	41.56±2.88**	41.12±1.22**	38.02±2.25**	45.42±2.24**	4.13±1.50**	5.23±1.48**
DDVP + 400mg/kg	55.32±1.34**	46.11±1.05**	45.02±1.45**	52.12±1.12**	4.35±1.42**	6.01±1.58**

Values are means ± S.E.M. of 5 animals per group, DDVP = at 50mg/kg DDVP Treated = *Alstonia boonei*at 200mg/kg, DDVP treated = *Alstonia boonei* at 400mg/kg, *significantly different from control (p< 0.05), ** significantly different from DDVP untreated (p< 0.05).

EFFECTS OF *ALSTONIA BOONEISTEM* BARK ON THE HISTOLOGY OF AORTA AND LIVER

The histology of liver slide of DDVP untreated rats showed marked portal congestion, severe periportal cellular infiltration by mononuclear cells and mild diffuse vacuolar degeneration of hepatocytes (Fig. 1). The aorta from DDVP-induced toxicity rats revealed large focal area of myofibril necrosis with severe hemorrhages and fibrous connective tissue laid down (Fig. 2). Treatment with *Alstonia boonei* stem bark extract (200 and 400mg/kg) reversed the adverse effect of DDVP-induced toxicity on the histological architecture of the aorta and liver of the rats, which is similar to their control. The histological results further corroborated the biochemical findings suggesting the useful effects of *Alstonia boonei* in DDVP induced-toxicity in rats.



Control

DDVP untreated

DDVP + *A. boonei*(200mg/kg)

DDVP + *A. boonei*(400mg/kg)

Figure 1.Changes in histology of liver samples of DDVP-induced toxicity in rats treated with *Alstonia boonei* ethanolic stem bark extract.

Black arrows show portal congestion, periportal cellular infiltration and vacuolar degeneration of hepatocytes.

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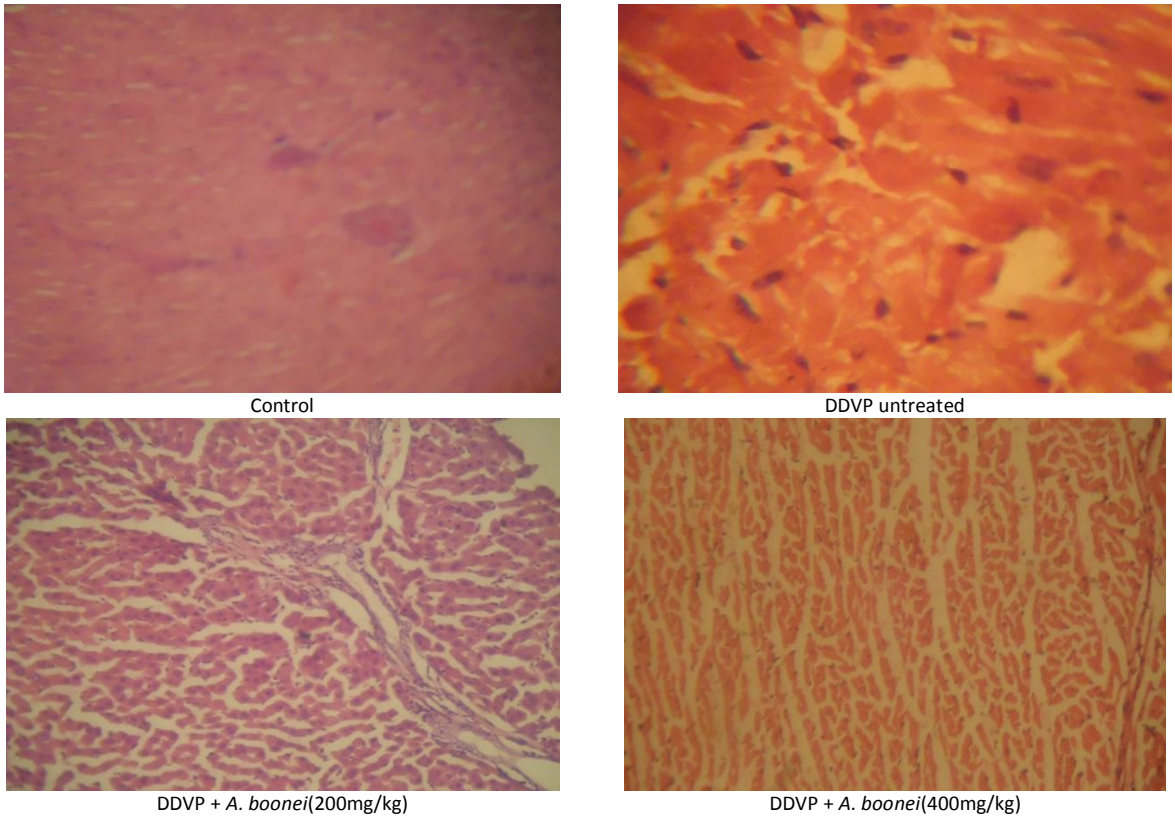

Figure 2.Changes in histology of aorta from DDVP-induced toxicity in rats treated with Alstonia boonei ethanolic stem bark extract.

DISCUSSION

The present decade has witnessed a great and intense resurgence in the interest and use of the plant[24]. The healing power of herbs identified and botanical medicine has been one of the oldest practiced professions by humanity[25]. In fact, the use of synthetic pharmaceutical products and hepatotoxic agent reported not only to connect or some serious adverse effects but these drugs are costly and not within the reach of all. However, traditional use of herbs to promote healing is not an alien in any continent. The present examine the hepatotoxic effects of the stem bark extract of *A. boonei*.[26] reported the therapeutic importance of the extract of the stem bark of *A. boonei* in folk medicine. Apart from the lack of information about the adverse or toxicity of this plant extract despite its wide spread use in folk medicinal or traditional practice, there is lack of information underlying biochemical mechanism responsible for some of the observable and reported properties of this plant. The results of the present study suggest that mean body weight of DDVP untreated group decreased with the increase in relative liver weight, agrees with the findings[27]. However, treatment with *A. boonei* (100 and 200 mg/kg) significantly decrease the relative weight of liver and heart of the DDVP induced rats.

Oxidative stress, defined as a disruption of the balance between oxidative and antioxidative[28]. Studies in animal models and human clinical trials have proved a between lipid peroxidation and hepatic damage[29]. Our results show increased levels of MDA in the serum and tissues of DDVP induced rats when compared to controls. On the other hand, treatment with *A. boonei* caused a significant decrease in the levels of MDA in these organs. This protective effect is on the antioxidant of *A. boonei*, which reduced the oxidative damage by blocking free radicals produce, and thus inhibited lipid peroxidation. The first metabolite of DDVP; dichloroacetaldehyde free radical, is believed to initiate the biochemical processes leading to oxidative stress, which is the direct cause of many pathological conditions such as diabetes mellitus, cancer, hypertension, kidney damage, liver damage and death[30]. These activated radicals bind to micromolecules and reduce lipids peroxidative degradation of polyunsaturated fatty acids. This leads to the formation of lipid peroxides which in turn gives products like malonylaldehyde that cause damage to membranes [31]. This lipid peroxidative

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degradation of biomembranes is one of the main causes of hepatotoxicity by DDVP. This is usually evidenced by a rise in theserum marker enzymes of the liver and heart namely AST and ALT. SOD and CAT are considered primary antioxidant enzymes, since they are involved in direct elimination of ROMs[32]. Effect of DDVP in LPO, CAT and SOD activities was found to be tissue dependent. In spite of decreased liver and cardiac CAT and SOD activities in DDVP rats, increase in LPO was observed. Higher LPO and low SOD and CAT activity indicates an oxidative stress condition. The effect on LPO, CAT, and SOD was reversed by *A. boonei* extract treatments. The observation suggests that in order to overcome the oxidative damage in liver and heart, some other compensatory mechanisms exist in addition to antioxidant enzymes. Reversal of decreased enzymes and inhibition of LPO appears to be due to free radical scavenger activity of ethanol extract of *A. boonei* stem bark in liver and heart. The experimental results indicated that LPO played a role in tissue injury in DDVP-induced toxicity in rats. Ethanol extract of *A. boonei* stem reduced the LPO in liver and, thus effectively protected cell functions and structure. The results indicate that ethanolic extract of *A. boonei* stem bark showed significant protection against the oxidative damage induced by DDVP in liver and heart of rats. *A. boonei* stem bark may act as cardio-protective and free radical scavenger agent.

Glutathione is the strong nucleophilic molecules found in most cells with its function as an antioxidant. Free radicals are usually destroyed by GSH. DDVP may be determined by increasing the release of cellular transaminases, increasing the production of MDA and decreasing cellular level ofGSH[33-35]. Histopthological studies showed normal integrity of the liver and aorta in the control group. The liver and heart of rats given DDVP, showed severe congestion, necrosis, calcification of hepatocytes, mononuclear cell infiltration, with areas of vacoulation and interstitial hemorrhage; while rats treated with 200 and 400 mg/kg body weight of the ethanol extract of *Alstonia boonei* plus DDVP shows minimal blood congestion, reduction in steatosis, fatty degeneration and peripheral hyalinazation of the hepatocytes of the liver tissue. This adds credence that physiologic recovery preceded obvious histological changes and the results may suggest that diets supplemented with *Alstonia boonei* will improve hepatic-protection against oxidative liver damage.

CONCLUSION

In conclusion, the results of the present study indicate that *Alstonia boonei* possess antioxidant and hepatoprotective properties, eliminating the deleterious effects of toxic metabolites from DDVP when administered orally. Further studies are required to identify the active component(s) and mechanism(s) underlying the beneficial effects of this plant.

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