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Research Article
Effect of *Desmodium velutinum* Stem Bark Methanolic Extract on Some Antioxidant Enzymes and Vitamins in Acetaminophen-intoxicated Rats

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Abstract

Background and Objective: This study evaluated phytoconstituents of the methanol extract of *Desmodium velutinum* (*D. velutinum*) stem bark and the effect of the extract on the antioxidant status and malondialdehyde (MDA) of acetaminophen-intoxicated rats. **Materials and Methods:** Twenty four male Wistar albino rats were used for this study and were divided into six groups of four rats each: Group 1 rats were normal control, group 2 rats were intoxicated with acetaminophen (APAP), group 3 rats were acetaminophen-intoxicated and administered with standard drug silymarin (100 mg kg^{-1} b.wt.), rats in groups 4, 5 and 6 were acetaminophen-intoxicated and pre-treated with methanol extract of *Desmodium velutinum* (MEDV) (100, 200 and 500 mg kg^{-1} b.wt., respectively). Phytochemical analyses, activities of glutathione-S-transferase, catalase (CAT), glutathione peroxidase, glutathione reductase (G-Red), reduced glutathione (GSH) and antioxidant vitamins (vitamins A, C and E) were determined using standard methods. **Results:** Phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, tannins, glycosides, steroids, carotenoids, anthocyanins, terpenoids and phenols. Rats in group 2 showed a significant ($p<0.05$) decrease in levels of superoxide dismutase (SOD), CAT, G-Red and GST, GSH and vitamins A and C compared to those in group 1 (normal control). Groups 4, 5 and 6 rats which were acetaminophen-induced and treated with different doses of MEDV, showed a significant ($p<0.05$) increase in the levels of SOD, catalase, glutathione transferase and glutathione reductase and levels of reduced glutathione when compared to rats in group 2. There was also a significant increase in the vitamins A and C levels of rats in groups 4, 5 and 6 compared to those in group 2. A significantly ($p<0.05$) higher MDA level was observed in group 2 rats compared to those in the treated and normal control groups. **Conclusion:** The result of this study suggests that the methanol extract of *Desmodium velutinum* stem bark contains some bioactive compounds that possess antioxidant properties and reduces the concentration of lipid peroxidation product (MDA) in acetaminophen-intoxicated rats.

Key words: *Desmodium velutinum*, antioxidant enzymes, antioxidant vitamins, acetaminophen, phytochemicals, methanol extracts

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore, contains chemical components that are medically active. These non-nutrients plant chemical compounds or bioactive components are often referred to as phytochemicals. They act as a natural defence system for host plants and provide colour, aroma and flavour. Phytochemicals are protective and disease-preventing particularly for some form of cancer and heart disease[1-4].

The study of natural products, on the other hand, is called phytochemistry. Certain photochemical from fruits such as grapes and apples, vegetables such as broccoli and onion, spices such as turmeric, beverages such as green tea and red wine, as well as many other sources have been isolated and characterized[4,5].

Antioxidants protect cells against free radicals such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynite, the damaging effects of reactive oxygen species which results in oxidative stress leading to cellular damage[6]. Natural antioxidants are significant in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischemia, carcinogenesis, neurodegenerative disorders, pregnancy, rheumatic disorder, DNA damage and ageing[7,8]. Antioxidants exert their activity by scavenging the 'free-oxygen radicals' thereby giving rise to a fairly 'stable radical'. The free radicals are metastable chemical species, which tend to trap electrons from the molecules in the immediate surroundings. There may be damage to crucial bio molecules like lipids, proteins including those present in all membranes, mitochondria and the DNA if radicals are not scavenged effectively in time also causing abnormalities leading to disease conditions[7]. Thus, free radicals are involved in a number of diseases including: Tumour inflammation, haemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal ulcerogenesis, asthma, rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, neurodegenerative diseases (e.g., Parkinsonism, Alzheimer's diseases), AIDS and even early senescence[7,9].

The human body produces insufficient amount of antioxidants which are essential for preventing oxidative stress. Free radicals generated in the body can be removed by the endogenous antioxidant defences such as glutathione or catalases[10]. Therefore, this deficiency had to be compensated by making use of natural exogenous antioxidants, such as vitamin C, vitamin E, flavones, β-carotene and natural products in plants[11-13]. Plants contain a wide variety of free radicals scavenging molecules including phenols, flavonoids, vitamins, terpenoids have rich antioxidant activities[11,14]. Many plants, citrus fruits and leafy vegetables are the source of ascorbic acid, vitamin E, carotenoids, flavanols and phenolics which possess the ability to scavenge the free radicals in human body. Significant antioxidant properties have been recorded in phytochemicals that are necessary for the reduction in the occurrence of many diseases[15,16]. Many dietary polyphenolic constituents derived from plants might contribute significantly to protective effects *in vivo* thus are more effective antioxidants *in vitro* than vitamins E or C[8,12]. Antioxidants are often added to foods to prevent the radical chain reactions of oxidation and they act by inhibiting the initiation and propagation step leading to the termination of the reaction and delay the oxidation process. Food industries have focused on finding natural antioxidants to replace synthetic compounds due to safety concerns of synthetic compounds. Furthermore, there is growing trend in consumer preferences for natural antioxidants, all of which has given rise to exploration of natural sources of antioxidants[16].

Desmodium velutinum has been reported in traditional medicine to have medicinal properties. Extracts of *D. velutinum* are used traditionally in treatment of some diseases, hence may be used as sources of pharmacological active agent in the treatment of aches and pains[17]. *Desmodium velutinum* is also used in traditional medicine as antipyretic and analgesic agent. Earlier studies demonstrated the antipyretic activity of the plant[18], also intraperitoneal administration of the methanol: Methylene chloride leaf extract was shown to reduce intestinal motility[19]. To authors' knowledge, the effect of the methanolic extract stem bark on acetaminophen-induced oxidative stress has not yet been studied. This study was, therefore, aimed at determining the phytochemical constituents of the methanol extract of *Desmodium velutinum* stem bark and the effect of the extract on some antioxidant enzymes and vitamins in acetaminophen intoxicated rats.

MATERIALS AND METHODS

Materials

Plant material: Plant material used in this study was fresh stem bark of *Desmodium velutinum*.

Collection and authentication of plant material: Fresh stem bark of *Desmodium velutinum* was collected within Nsukka Community and was authenticated at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Reagents and chemicals: All chemicals used in this study were of analytical grade and were obtained from commercial dealers in Nsukka and Onitsha, both in Nigeria. They include products of May and Baker, England; BDH Chemical Limited, Poole, England and Merck, Germany. Reagents used for all the determining concentration were commercial kits and products of Randox Laboratories Limited, United Kingdom and TECO Diagnostics, USA.

Equipment and instruments: Equipment and instruments used for this study were obtained from the laboratory unit of Department of Biochemistry, University of Nigeria, Nsukka and Shalom Research Laboratory, Nsukka. Others were purchased from commercial dealers in Nsukka and Onitsha, both in Nigeria. They include: Spectrophotometer (model SPM721-2000, Biodiagnostic Inc., USA), centrifuge (model 800D), micropipette (volume range 0-100 µL) (Swastic Scientific Instrument Private Ltd., Mumbai, India), test tubes (Pyrex, England) and refrigerator (Haier thermocool, China).

Methods

Method of extraction of plant materials: Stem of *D. velutinum* (1.5 kg) was washed in running water to remove unwanted materials and chopped into small pieces. The pieces were air-dried for 2 weeks and ground into coarse powder. About 1200 g of coarse powdered sample was weighed and soaked in 7.5 L of methanol solution, stirred and allowed to stand for 48 h with occasional stirring. The suspension was filtered using muslin bag followed by Whatman No. 42 filter paper. The filtrate was evaporated under reduced pressure and dried using a rotary evaporator at 55°C. The concentrated extract was stored in a labelled sterile screw capped bottle at 2-8°C. The percentage yield of the extract was as follows.

Preparation of acetaminophen (paracetamol): The stock concentration of acetaminophen was prepared by dissolving 600 mg of the standard drug in 2 mL of distilled water bringing the stock concentration to 60 mg mL^{-1}. Paracetamol was induced intraperitoneally at the dose of 2 g kg^{-1} b.wt.[20].

Management of experimental animals: A total of 24 male Wistar albino rats were purchased from the Faculty of Biological Science Animal House, University of Nigeria, Nsukka, Enugu State, Nigeria and were about 12 weeks old. The animals were kept under standard conditions for 7 days with free access to water and food before starting the experiment. The animals were housed in separate standard cages and provided with palletized feed (Grand cereals and oil mills Nigeria Limited) and water *ad libitum* at room temperature. Albino mice of average weight, 20.50±4.27 g were used in determination of median lethal dose (LD_{50}). They received human care throughout the experimental period in accordance with the ethical rules and recommendations of the University of Nigeria committee on the care and use of laboratory animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23, revised 1985)[21].

Experimental design for acute toxicity study: Acute toxicity (LD_{50}) of the methanol extract was carried out using the modified method of Lorke[22]. Eighteen albino mice (both sexes) were used in this study. The acute toxicity test consisted of two phases: Phase I and phase II. In the phase I, nine mice were randomly assigned into three groups of three mice each. Groups 1, 2 and 3 were administered orally, methanol extract at the doses of 10, 100, 1000 mg kg^{-1} b.wt. Based on the observation of the phase I, the procedure was repeated using another set of nine mice which were randomly assigned into three groups of three mice each. Groups 4, 5 and 6 of phase two were administered the methanol extract at doses of 1600, 2900 and 5000 mg kg^{-1} b.wt., respectively. The mice in phase I and II were observed for general behavioural, neurological and autonomic profile for 24 h.

Experimental design: The twenty-four rats used in this study were divided into six groups of four rats each and treated as shown in Table 1.

Treatment with vehicle, extract and standard drug started on day 1, (i.e., the day after 14 days acclimatization). On day 12, rats in groups 2-6 were intoxicated (induction of hepatotoxicity) orally with acetaminophen and treatment was stopped on day 14.

Table 1: Experimental groups

Groups	Description	Treatment
1	Normal control	Administered with 5 mL kg^{-1} b.wt., vehicle, no intoxication
2	Experimental control	Intoxicated with acetaminophen and untreated
3	Standard control	Pre-treated with 100 mg kg^{-1} b.wt., of silymarin and intoxicated with acetaminophen
4	Treatment group 1	Pre-treated with 100 mg kg^{-1} b.wt., of extract and intoxicated with acetaminophen
5	Treatment group 2	Pre-treated with 200 mg kg^{-1} b.wt., of extract and intoxicated with acetaminophen
6	Treatment group 3	Pre-treated with 500 mg kg^{-1} b.wt., of extract and intoxicated with acetaminophen

Extract: Methanol extract of *D. velutinum* stem, number of rats per group (n) = 5

Blood sample collection and processing: On day 15, after an overnight fasting, blood samples were collected from all the rats by ocular puncture into plain tubes and were allowed to clot for 15 min. This was then centrifuged at 4000 rpm for 10 min. The serum was used for biochemical analyses.

Qualitative phytochemical analysis of the extract: The methods described by Harborne[23] and Trease and Evans[24] were used.

Quantitative phytochemical analyses of the extract: This was determined according to the method of Harborne[23].

Quantification of glycoside content: The glycoside content of the extract was determined according to the method of Singleton *et al.*[25].

Assay of catalase activity: Catalase activity was assayed by the method described by Aebi[26].

Assay of superoxide dismutase activity: Superoxide dismutase (SOD) activity was assayed using the method described by Fridovich[27] as contained in the commercial kit.

Determination of glutathione concentration: The concentration of glutathione was determined according to the method of Habig *et al.*[28].

Determination of vitamin A concentration: Vitamin A concentration was determined using the method described by AOAC[29].

Determination of vitamin C concentration: Vitamin C concentration was determined using the method described by Goodhart and Shils[30].

Determination of vitamin E concentration: The concentration of vitamin E was determined using the method described by Desai[31].

Determination of malondialdehyde concentration: The malondialdehyde (MDA) concentration was determined by the method of Wallin *et al.*[32].

Statistical analysis: Data obtained from the laboratory were analyzed using IBM Statistical Product and Service Solutions (SPSS), version 18. The results were expressed as mean±standard deviation (SD) and presented in tables. One-way analysis of variance (ANOVA) was used to compare means across the groups. Mean values with $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Result of the phytochemical analysis of *Desmodium velutinum* methanol extract as seen in Table 2 shows that alkaloids, carotenoids, saponins, steroids and flavonoids were detected in moderate concentrations and anthocyanin, phenols, tannins and glycosides were discovered in low amount. Saponins, flavonoids, tannins, steroids and alkaloids are very useful in the scavenging of free radical while steroids show some analgesic properties[33]. The presence of these phytochemicals suggest that methanol stem extract of *Desmodium velutinum* (MEDV) could have various medicinal values such as antidiarrhoeic, analgesic, antioxidative, hypolipidemic and antidiabetic activities since they contain saponins, flavonoids, tannins, alkaloids and steroids[33].

Oxidative stress was induced with acetaminophen (APAP), which is an analgesic for treating pain and fever[34]. At therapeutic doses, it causes no discomfort. However, an overdose of the drug can result to severe hepatic damage[35]. From Table 3, a significant increase ($p<0.05$) was observed in the SOD activities of rats in groups 4, 5 and 6 (APAP-intoxicated and treated with MEDV) compared to group 2 (induced and untreated). This increase in SOD activity scavenges the superoxide anion to form hydrogen peroxide and thus, diminishing the toxic effect caused by this radical. It is said to be the first line of defense against free radicals[36]. There seemed to also be damage to the liver; a target of most toxicant and the organ responsible for the modulation of lipid levels in the body. Damaged liver cannot effectively synthesize antioxidants leading to free radical accumulation in the blood. A significant ($p<0.05$) increase was observed in the SOD concentration in groups 4, 5 and 6 (induced and treated with

Table 2: Phytochemical constituents of methanol extract of *Desmodium velutinum* stem

Phytochemicals	Bioavailability	Amount (%)
Saponins	++	6.00
Tannins	++	0.11
Alkaloids	++	8.00
Flavonoids	++	4.00
Glycosides	+	0.02
Terpenoids	+++	20.0
Phenols	+	0.33
Steroids	++	4.00
Carotenoids	++	7.00
Anthocyanins	+	0.50

+++: High content, ++: Moderate content, +: Low content. Values are mean of a single run

Table 3: Enzymatic antioxidant status of experimental rats

	Antioxidant status				
Groups	SOD (IU L^{-1})	CAT (IU L^{-1})	GST (IU L^{-1})	G-Red (IU L^{-1})	GSH (mg dL^{-1})
1	3.87±0.33^{e}	5.01±0.31^{c}	27.05±1.67^{d}	24.45±2.61^{cd}	4.76±0.14^{c}
2	1.14±0.03^{a}	2.30±0.27^{a}	14.59±1.91^{a}	16.44±1.10^{a}	1.97±0.13^{a}
3	1.61±0.23^{b}	3.02±0.21^{b}	3.36±0.43^{b}	19.46±1.13^{b}	16.44±1.10^{a}
4	2.48±0.30^{c}	3.62±0.14^{c}	20.58±0.52^{b}	23.94±0.83^{c}	3.51±0.28^{b}
5	3.15±0.04^{d}	4.11±0.12^{d}	23.46±0.86^{c}	26.71±2.14^{de}	4.42±0.30^{c}
6	3.73±0.17^{e}	5.37±0.13^{f}	26.68±1.32^{d}	28.48±1.78^{e}	5.45±0.23^{c}

Data are Mean±SD (n = 4). Values with different superscripts are significant at $p<0.05$

different doses of MEDV) compared to group 3 (induced and treated with silymarin). The lowest dose (100 mg kg^{-1} b.wt.) of MEDV showed a significant ($p<0.05$) increase in SOD concentration compared to those treated with silymarin (200 mg kg^{-1} b.wt.) suggesting that the synthetic drug is not as effective as MEDV.

Treatment of acetaminophen-induced rats in groups 4, 5 and 6 with different doses of the MEDV also showed a significant ($p<0.05$) increase in the catalase concentration as observed in Table 3 compared to those in group 2 (induced and untreated) in a dose dependent manner. This finding that MEDV can increase catalase concentration implies that it may have antioxidant and hepatoprotective effects against acetaminophen-induced oxidative stress. Earlier study has shown that *Carica papaya* fruit extract produced a significant increase in catalase concentration of acrylamide-intoxicated rats and this was attributed to the presence of alkaloids and flavonoids which scavenge free radicals[37].

A significant ($p<0.05$) higher glutathione reduced (GSH) concentration was observed in groups 4, 5 and 6 (acetaminophen-induced and treated with different doses of the MEDV) compared to group 2 (induced and untreated). Reduced glutathione acts as a substrate for glutathione peroxidase which makes it possible for conversion of hydrogen peroxide, to oxygen and water. This marked increase in the concentration of GSH reduces the concentration of hydrogen peroxide, thereby, reducing oxidative stress in the experimental rats. This observation implies that MEDV possess antioxidant effect which could be linked to the synergistic effects of the free radical scavenging phytochemicals such as alkaloid, tannin, flavonoids and phenol. The administration of flavonoids-rich extract of MEDV to acetaminophen induced rats improved antioxidant activities and decreased oxidative stress. Earlier reports revealed that *Cynbopogon citratus* is a rich source of phenolic compounds including flavonoids and tannin which are potent antioxidant and is known to reduce oxidative stress[38]. A significant increase was observed in GSH concentration of groups 5 and 6 (induced and treated with different doses of MEDV) compared to that in group 3 (induced and treated with silymarin). The increase in GSH implies that MEDV has more GSH concentration than silymarin.

The main function of GST is to catalyze the conjugation of glutathione with xenobiotic substance making the xenobiotic more water soluble and easily eliminated from the body. There was a significant ($p<0.05$) increase in the GST activities in groups 4, 5 and 6 (induced and treated with different doses of MEDV) compared to group 2 (induced and untreated). This may be due to the presence of some phytochemicals in MEDV which contributes in scavenging the free radicals. Intracellular accumulation of xenobiotics reduces the concentration of GST in group 2 (induced and untreated) because xenobiotics has overwhelmed GST of rats in group 2. The rats in groups 4, 5 and 6 (induced and treated with different doses of MEDV) showed a significant increase in GST activity because the MEDV has high activity of GST which is enough to conjugate with the xenobiotics present and hence reduces the concentration of xenobiotics in the rats. A significant increase was observed in the GST concentration of groups 5 and 6 (induced and treated with different doses of MEDV) compared to those in group 3 (induced and treated with MEDV). Treatment of acetaminophen-induced rats with different doses of the MEDV also showed a significant ($p<0.05$) increase in the glutathione reductase (GS-Red) activities of rats in groups 4, 5 and 6 compared to those in group 2 (induced and untreated). This suggests that MEDV increases GS-Red activity and reduces oxidation. Reduced glutathione acts as a cofactor for the enzyme glutathione peroxidase that converts hydrogen peroxide to oxygen and water which is used up by the body. This shows that administration of plant extract increases the concentration of GS-Red suggesting that the plant stem bark has a high concentration of antioxidant which can be used to manage diseased conditions. This effect could be attributed to the phytoconstituents of the herbal drug. A significant ($p<0.05$) increase was observed in GS-Red activity of groups 4, 5 and 6 compared to group 3(induced and treated with silymarin) which implies that MEDV is more effective in mopping up free radical because GS-Red is present in high concentration.

Table 4: Antioxidant vitamins status of experimental rats

	Antioxidant vitamins (mg dL^{-1})		
Groups	Vitamin A	Vitamin C	Vitamin E
1	4.61 ± 0.12^{e}	4.58 ± 0.82^{d}	0.55 ± 0.01^{a}
2	4.81 ± 0.10^{a}	2.56 ± 0.40^{a}	0.53 ± 0.04^{a}
3	3.14 ± 0.17^{b}	3.17 ± 0.21^{ab}	0.55 ± 0.01^{a}
4	3.32 ± 0.13^{b}	3.35 ± 0.31^{b}	0.52 ± 0.05^{a}
5	3.80 ± 0.18^{c}	3.59 ± 0.25^{bc}	0.53 ± 0.03^{a}
6	4.17 ± 0.04^{d}	4.14 ± 0.38^{cd}	0.52 ± 0.01^{a}

Data are Mean±SD (n = 4). Values with different superscripts are significant at $p<0.05$

Table 5: MDA concentration of normal and acetaminophen-induced rats treated with *D. velutinum* methanolic stem extract

Groups	Malondialdehyde (MDA) concentration (mg dL^{-1})
1	0.67 ± 0.25^{a}
2	3.86 ± 0.22^{b}
3	3.12 ± 0.21^{a}
4	3.12 ± 0.21^{a}
5	1.46 ± 0.35^{b}
6	1.22 ± 0.11^{b}

Results expressed in Mean±SD (n = 4). Values with different letter(s) as superscript across the column are considered significant ($p<0.05$)

Vitamin C (ascorbic acid), a constituent of the MEDV is a strong antioxidant, it destroys toxic free radicals and other reactive oxygen species[34]. Studies have shown that ascorbic acid reacts easily with superoxide radical, hydrogen peroxide (H_2O_2) and the 'singlet' oxygen neutralizing them[39]. In this study, there was a significant ($p< 0.05$) increase in vitamin C in groups 4, 5 and 6 compared to those in group 2 (induced and untreated) as observed in Table 4. This implies that MEDV has a good concentration of vitamin C, an antioxidant vitamin which neutralizes free radicals produced by acetaminophen breakdown. This is line with the findings by Omoregie and Osagie[40] that the vitamin C concentration in rats treated with Jatropha leaf is significantly ($p<0.05$) higher than those in the intoxicated with acetaminophen and untreated. This also supports the view that the vitamin C content in animal's body is directly proportional to the dietary intake of vitamin C.

Vitamin E is a fat-soluble antioxidant whose function is to stop the production of ROS formed due to fat oxidation. There was no significant ($p<0.05$) increase in the vitamin E concentration of rats across the groups compared with the control. This shows that MEDV has the same vitamin E activity with the normal control and also that acetaminophen-induced liver damage didn't affect the activity of vitamin E. Treatment of acetaminophen-induced rats with different doses of the MEDV also showed a significant ($p<0.05$) increase in the vitamin A concentration of rats in groups 4, 5 and 6. This demonstrates that the MEDV has antioxidant vitamin (vitamin A). The observed increase in SOD, CAT, GSH, G-Red, GST, vitamins A and C, concentration in groups 4, 5 and 6 treated with MEDV could be as a result of the synergistic action of antioxidants and phytochemical contents of the extract being able to mop up the free radicals generated as a result of overdose of acetaminophen. This result is in line with findings of Fakurazi *et al.*[41], who observed a restoration of the named parameters to their normal values following treatment with methanol leaf extract of *Hibiscus sabdariffa* after cyclophosphamide-induction. In general, MEDV possess antioxidative effect and may be recommended in managing oxidative-stress induced disorders.

It is well established that excessive formation of N-acetyl-p-benzoquinoneimine (NAPQI), a toxic intermediate of acetaminophen formation after acetaminophen overdose depletes cellular reduced glutathione (GSH) and binds to sulfhydryl groups of cellular proteins[42,43]. These results related to an overproduction of reactive oxygen species which has been shown to inflict damage on lipids through lipid peroxidation (generation of malondialdehyde) and subsequent liver damage. Administration of acetaminophen resulted in significant elevation of the hepatic tissue malondialdehyde (MDA) (a biomarker of lipid peroxidation) concentration to approximately 3-fold of the results observed in normal control. This may be attributed to the metabolite, NAPQI, which induces lipid peroxidation and free radical generation. The NAPQI is a toxic intermediate of Cyt-P450-induced acetaminophen metabolism which can rupture the mitochondrial cell membrane resulting to mitochondrial dysfunction. High level of MDA is an indication of mitochondrial damage leading to decreased ability to produce cellular energy. From Table 5, treatment of acetaminophen-induced rats with different doses of the *D. velutinum* showed a significant ($p<0.05$) decrease in MDA concentration in groups 4, 5 and 6 when compared to group 2. This could be as a result of the presence of flavonoids in moderate concentration which possess antioxidants potentials and has the ability to scavenge free radical hence preventing oxygen consuming lipid peroxidation.

CONCLUSION

Based on the result of this study, it can be suggested that the MEDV can be evaluated for management and prevention of oxidative stress-related diseases such as atherosclerosis, Alzheimer disease, cancer and other cardiovascular diseases. Notwithstanding, further investigation should be carried out to scrutinize the long term effects associated with its use. Conclusively, the result of this study suggests that the methanol extract of *Desmodium velutinum* stem bark contains some bioactive compounds that possess antioxidant properties and reduces the level of lipid peroxidation product (MDA) in acetaminophen induced oxidative stressed rats.

SIGNIFICANCE STATEMENT

This study is the first attempt in evaluating the effect of methanolic extract of *Desmodium velutinum* stem bark on antioxidant enzymes and vitamins in acetaminophen induced liver damage in an *in vivo* animal model. The findings highlight that methanolic extract of *Desmodium velutinum* stem bark possibly possesses antioxidant properties and bioactive compounds capable of reducing levels of lipid peroxidation product (MDA). This study will help the researchers in determining the detail mechanism of medicinal plant against intoxication effect on certain liver biomarkers and vitamins. This research finding is able to further support the growing interest in the employment of natural antioxidants as a protective strategy against oxidative stress in under developed countries and among the rural dwellers.

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