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Effects of *Vernonia Amygdalina* Leaf Extract on Total Antioxidant Capacity and Malondialdehyde Levels in Acetaminophen-Induced Toxicity in Mice

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ABSTRACT

Background*:** Many plants have been shown to possess antioxidant properties. ***Aim: To determine the effects of *V. amygdalina* on Total antioxidant status and malondialdehyde levels in mice treated with acetaminophen overdose**.** ***Methodology:*** Mice were separated into 5 groups of 6 mice each. 300mg/kg of acetaminophen was administered to all groups except group 1. Then *V. amygdalina* extract was administered to groups 1 and 3 at 50mg/kg and group 4 at 100mg/kg, and group 5 received Vitamin C at 500mg/kg, all treatments were given orally. 8 hours later blood samples were collected by cardiac puncture, livers were excised, homogenised and used for biochemical analysis. ***Results:*** Acetaminophen produced a significant reduction in TAOC and a marked increase in MDA levels of mice in groups 2, 3, 4 and 5. These effects were significantly attenuated by *V. amygdalina* administration, in a dose dependent manner. These effects were comparable with those of Vitamin C. ***Conclusion:*** Results suggests that *Vernonia amygdalina* possesses significant antioxidant effects.

KEYWORDS: *Vernonia amygdalina*, Oxidative stress, Lipid Peroxidation.

INTRODUCTION

Oxidative stress and lipid peroxidation reactions are frequent accompaniments of many pathological processes. In chronic kidney diseases, cardiovascular disorders, diabetes mellitus, degenerative disorders, inflammatory and autoimmune disorders, lipid peroxidation reactions and depletion of antioxidant defence systems have been increasingly reported in literature [1-3]. The results of these might be biological damage to cellular membranes and vital micro-molecular components such as proteins and nucleic acids, with or without concomitant activation of nuclear transcription mechanisms that may yield pro-inflammatory gene products [4-6].

The body has intrinsic mechanisms to attenuate the noxious effects of reactive oxygen and nitrogen species, including glutathione, superoxide dismutase, and peroxidases. However, in many pathological conditions these defence systems are overwhelmed, resulting in oxidative stress. Conventional therapies utilise antioxidants such as vitamins and minerals to support this intrinsic system, and literature shows impressive outcomes with these therapies. However, a significant body of evidence is available supporting the utility of natural products as antioxidants. *Vernonia amygdalina* a plant that grows freely in many parts of Zambia is reported to be rich in polyphenolics, flavonoids, saponins, tannins, and alkaloids [7-9]. It is commonly used in herbal medicine, and as a spice or condiment in some African recipes. In view of the phytochemical evidence, this study evaluated the effectiveness of leaf extract of *Vernonia amygdalina* as an antioxidant in a mice model of oxidative stress. The specific objectives were to evaluate the effects of methanolic leaf extract of the plant on blood and liver levels of malondialdehyde and total antioxidant capacity (TAOC) in mice treated with toxic levels of acetaminophen. This information will contribute to the literature on the pharmacology of this plant, and

provide justification for its use in folk medicine for the treatment of diseases characterised by depletion of antioxidant levels.

MATERIALS AND METHODS

To achieve the above objectives, a quantitative experimental study was designed involving acetaminophen induced mice model of oxidative stress [10]. The effects of intervention with the leaf extract were compared to the effects of a standard drug (vitamin C) and a positive control which received no plant extract as detailed below. The study was approved by ERES Converge, Lusaka, an independent ethical review board. The ethical review reference number was 2015-May-013.

Preparation of plant extract:
Vernonia amygdalina was obtained locally from Lusaka and authenticated at the Botany Section of Department of Biological Sciences, University of Zambia. A voucher specimen was deposited at the Departmental herbarium. Leaves of the plant were shade-dried and size-reduced to powder using a mortar and pestle. A portion of the powder weighing 100 g was then extracted with 80 % methanol using a Soxhlet apparatus at 60 ^{o} C. The extract was concentrated and dried with the aid of a rotary evaporator at 40 ^{o} C. The resulting powdery extract was stored refrigerated at +4 ^{o} C until required for the study. For use, a 500 mg/L stock solution of the extract was prepared in distilled water.

Experimental animals:
Thirty albino mice weighing 20 – 29 g were obtained from the animal house of the Department of Physiological Sciences of the School of Medicine. These were habituated to laboratory conditions for two weeks before commencement of the experiment at the Pharmacology lab of the Department. The environmental conditions were room temperature of 22 – 26 ^{o} C, 12 hours light-dark cycles, metal cages with free access to feed and water, ad libitum. The mice had access to mice shells obtained from Livestock Services Cooperation at the Showgrounds in Lusaka.

Study design:
Based on an LD50 value of 560+/- 1.21mg/kg previously reported for *V. amygdalina* leaf extract prepared under similar conditions [11], the protocol below was developed from literature [12, 13] and used for the study. The mice were randomly divided into 5 groups of six mice each. The mice were fasted for 12 hours before administration of the treatments, and after another 4 hours of fasting, they were returned to their routine chows.
Group 1 received oral dose of 50 mg/Kg extract but no acetaminophen (negative control). Group 2 (positive control) were administered only 300 mg /Kg acetaminophen orally (P.O.). The acetaminophen was dissolved in warm saline. Group 3 received 300 mg/Kg P.O. dose of acetaminophen followed by 50 mg/Kg oral dose of the extract. Group 4 received 300 mg/Kg P.O dose of acetaminophen and 100 mg/Kg oral dose of extract. Group 5 received 300 mg/Kg P.O dose of acetaminophen and 500 mg/Kg oral dose of vitamin C.

Sample collection and processing:
At 8 hours post-treatment, blood samples were collected by cardiac puncture into sterile EDTA bottles and mixed. After centrifugation, the plasma were separated into sterile vials and stored refrigerated until required for biochemical analysis. The mice were immediately sacrificed and their livers were excised and washed with ice-cold phosphate buffered saline, followed by 0.15 M Tris buffer at a pH of 7.4, blotted and weighed [10]. The liver samples were homogenised in 0.15 M Tris buffer to give a concentration of 100mg/ml.

Biochemical analysis:
Total antioxidant capacity: This was estimated by the ferric reducing ability of plasma (FRAP) assay method of Benzie and Strain [14] which depends on the ability of the sample to reduce ferric to ferrous iron at low pH. This in turn causes the formation of blue coloured ferrous tripyridyl-s-triazine (TPTZ) which can be measured spectrophotometrically at a wavelength of 593 nm.

Malondialdehyde assay: Malondialdehyde levels in plasma and liver homogenate was determined by the thiobarbituric acid reaction method described by Ohkawa et al [15]. Thiobarbituric acid forms a coloured complex with malondialdehyde and this is measured spectrophotometrically at 532 and 600 nm.

Data analysis: Data were analysed with SPSS version 23, expressed as mean +/- standard error of the mean, and compared by one way analysis of variance (ANOVA). A p value < 0.05 was considered significant.

RESULTS

The percentage yield for 100g of *V. amygdalina* leaves which were pounded was 50%. The administration of 300 mg/Kg acetaminophen induced significant decreases in total antioxidant capacity (TAOC) in the liver and blood (see Figure 1). Therefore, liver and plasma levels of TAOC in group 1 were significantly higher than values seen in group 2, $p < 0.05$ (see Tables 7 and 8). Concomitant administration of methanolic leaf extract of *V. amygdalina* produced a dose-

dependent attenuation of the oxidative stress, manifesting as significantly higher levels of TAOC in groups 3 and 4 when compared to group 1 p <0.05 (see table 1).

Accompanying the oxidative stress was significant increases in lipid peroxidation product (malondialdehyde) levels in the plasma and liver (see figure 2). Thus, MDA levels in group 2 were significantly higher than values in group 1, P<0.05 (see tables 9 and 10). *V. amygdalina* extract also reduced the increase in MDA levels in a dose dependent manner (see table 2). These effects of *V. amygdalina* extract are similar to those produced by vitamin C

DISCUSSION

The main objective of our study was to determine the effects of *Vernonia amygdalina* on the Total antioxidants and malondialdehyde levels in an acetaminophen induced heapatotoxicity. In our study, acetaminophen administration produced a significant reduction in the total antioxidant capacity of the mice as was evident in groups 2, 3, 4 and 5, and this was consistent with published data.[13] Administration of *V. amygdalina* methanolic leaf extract to the mice, produced a significant attenuation of oxidative stress, as there was an increase in the total antioxidant status in a dose dependent manner, this was also shown by other researchers.[13, 16] The increase in the total antioxidant status of the mice by the extract was comparable with that produced by Vitamin C.
The reduction in the total antioxidant status following the administration of the acetaminophen overdose, could be due to the increase in the amount of the antioxidants being used in the mopping up of the ROS brought about by the process of oxidative stress.[17]

Similarly, acetaminophen administration was associated with a marked increase in MDA levels in both blood and liver (Figure 2). These levels were significantly($P \leq 0.05$) attenuated in a dose dependent manner by administration of the *V. amygdalina* leaf extract. These results were consistent with previously done research. [13, 18, 19] The attenuation of MDA levels was also comparable with that produced by Vitamin C, consistent with other research data available. [20, 21]
Since lipid peroxidation occurs due to the oxidation of polyunsaturated fatty acids in the cells membranes, another mechanism by which plant extract may have caused a reduction in MDA levels could be due to the presence of antioxidant Vitamins namely A, C, E, and probably other substances which protect the membrane structure of the cells.[22, 23] However, according to the study done by Swee et al[24] the Vitamin C content of the plant was considered not to be an effective contributor to the antioxidant effects of the plant.

The plant *V. amygdalina* is believed to carry out its effects of antioxidant activity through various mechanisms that include, free radical scavenging, or possibly antioxidant status modulation, regeneration of damaged tissue, or by protein metabolism maintenance. [13]

There are other possible mechanisms by which the plant may carry out its effects. It is believed that the plant may restore the oxidant-antioxidant balance by either contributing to the endogenous antioxidants or by using its own antioxidant to scavenge the free radicals.[25] Another possible mechanism could be through the phenolic content which may be neutralising free radicals which are responsible for initiating oxidative processes or through the radical chain reaction termination.[26, 27] The other mechanism could be the ability of the plant to restore the in vivo antioxidant levels of the liver which increases the levels of the endogenous antioxidant [16] or by possibly increasing antioxidant molecules synthesis [19]. The plant also contains high amounts of phenolics and flavonoids which are free radicals and may be responsible for the free radical scavenging properties of the plant.[16]

Glutathione an important non-protein thiol, that has a central role of coordinating the antioxidant defence system in the body, has been shown to be significantly increased through the administration of *V. amygdalina* extract.[28] Reduced Thiols are believed to be essential for recycling of antioxidant vitamins like Vitamin C and E .[28] Therefore, the extract could also work by a mechanism that probably allows the free radicals to react with the components of the plant, such that this could lead to an increase in the levels of glutathione or other antioxidants. The plant has also been found to possess some micronutrients like selenium, phosphorus, iron and zinc, chromium and copper. [29, 30]The enzymes involved in free radical scavenging are metalloenzymes, therefore the presence of these micronutrients in the plant extract may have been essential in boosting their production thus increasing their activity. [31]

CONCLUSION

The *V. amygdalina* methanolic leaf extract was shown to possesses the antioxidant effects as it was able to attenuate the effects of acetaminophen induced toxicity.

RECOMMENDATIONS

We are recommending that more studies should be done to determine the active components of *V.*

amygdalina responsible for the antioxidant effects shown.

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Table 1: Plasma and liver total antioxidant activities in the 5 groups of mice.

Group	**Treatment**	**Plasma** (U/mg of protein)	**Liver** (U/mg of protein)
Grp 1	*V. amygdalina* 50mg/Kg only (Negative control)	0.81±0.01	0.86±0.01
Grp 2	*Acetaminophen 300*mg/Kg (Positive control)	0.54±0.01	0.49±0.02
Grp 3	Acetaminophen 300mg/Kg + *V. amygdalina* 50mg/Kg	0.64±0.01	0.68±0.01
Grp 4	Acetaminophen 300mg/Kg + *V. amygdalina* 100mg/Kg	0.70±0.00	0.77±0.00
Grp 5	Acetaminophen 300mg/Kg + Vitamin C 500mg/Kg	0.69±0.01	0.75±0.01

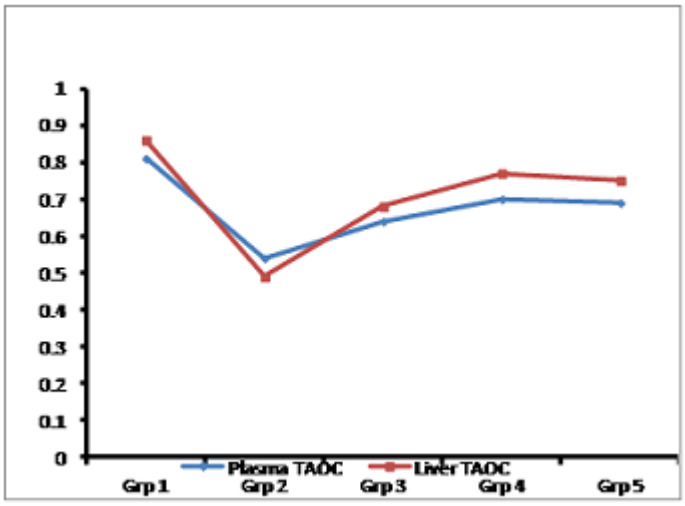

Figure 1. TAOC values in the blood and liver of the 5 groups

Table 2. Malondialdehyde levels in the 5 groups of mice.

Group	**Treatment**	**Plasma (nmol/ml)**	**Liver (nmol/m)**
Grp 1	*V. amygdalina* extract 50mg/Kg (Negative control)	0.23±0.05	0.20±0.03
Grp 2	Acetaminophen 300mg/Kg only (Positive control)	4.07±0.23	5.37±0.25
Grp 3	Acetaminophen 300mg/Kg + *V. amygdalina* 50mg/Kg	1.91±0.04	1.65±0.02
Grp 4	Acetaminophen 300mg/Kg + *V. amygdalina* 100mg/Kg	0.56±0.02	0.83±0.01
Grp 5	Acetaminophen 300mg/Kg + Vitamin C 500mg/Kg	0.61±0.04	0.59±0.02

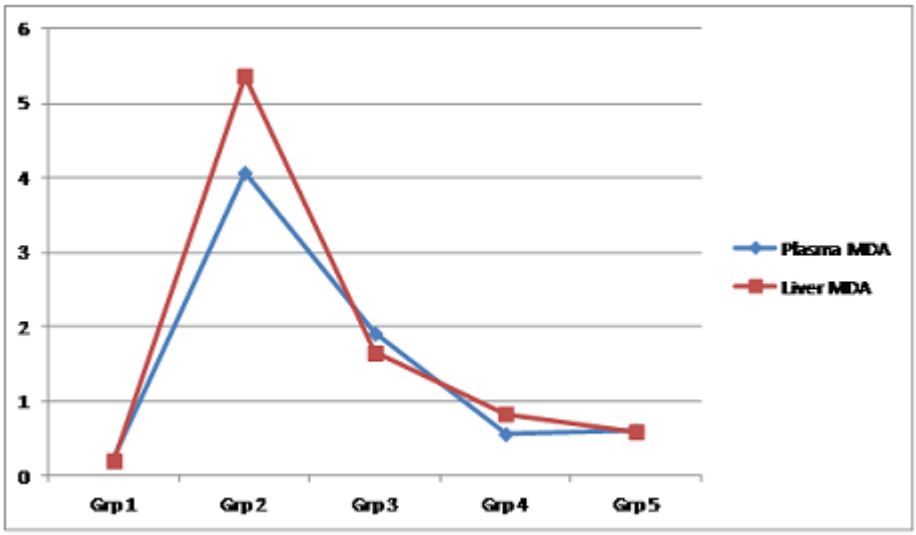

Figure 2. MDA levels in the blood and liver of the 5 groups

Table 3. Showing the ANOVA readings of TAS for blood sample

ANOVA

Levels of TAS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.239	4	.060	258.837	.000
Within Groups	.006	25	.000		
Total	.245	29			

Table 4. Showing the ANOVA readings of TAS for liver sample

ANOVA

TAS Levels

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.468	4	.117	122.147	.000
Within Groups	.024	25	.001		
Total	.492	29			

Table 5. Showing the ANOVA readings of MDA for blood sample

ANOVA

MDA Levels

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	60.327	4	15.082	221.051	.000
Within Groups	1.706	25	.068		
Total	62.033	29			

Table 6. Showing the ANOVA readings of MDA for liver sample

ANOVA

MDA Levels

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	106.326	4	26.582	338.068	.000
Within Groups	1.966	25	.079		
Total	108.292	29			

Table 7. Showing the Post Hoc Tests of TAS status for blood sample

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Levels of TAS
LSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Negative Control	Positive Control	.27500*	.00877	.000	.2569	.2931
	Low Extract	.17333*	.00877	.000	.1553	.1914
	High Extract	.11500*	.00877	.000	.0969	.1331
	Vitamin C	.12333*	.00877	.000	.1053	.1414
Positive Control	Negative Control	-.27500*	.00877	.000	-.2931	-.2569
	Low Extract	-.10167*	.00877	.000	-.1197	-.0836
	High Extract	-.16000*	.00877	.000	-.1781	-.1419
	Vitamin C	-.15167*	.00877	.000	-.1697	-.1336
Low Extract	Negative Control	-.17333*	.00877	.000	-.1914	-.1553
	Positive Control	.10167*	.00877	.000	.0836	.1197
	High Extract	-.05833*	.00877	.000	-.0764	-.0403
	Vitamin C	-.05000*	.00877	.000	-.0681	-.0319
High Extract	Negative Control	-.11500*	.00877	.000	-.1331	-.0969
	Positive Control	.16000*	.00877	.000	.1419	.1781
	Low Extract	.05833*	.00877	.000	.0403	.0764
	Vitamin C	.00833	.00877	.351	-.0097	.0264
Vitamin C	Negative Control	-.12333*	.00877	.000	-.1414	-.1053
	Positive Control	.15167*	.00877	.000	.1336	.1697
	Low Extract	.05000*	.00877	.000	.0319	.0681
	High Extract	-.00833	.00877	.351	-.0264	.0097

*. The mean difference is significant at the 0.05 level.

Table 8. Showing the Post Hoc Tests of TAS status for liver sample

Post Hoc Tests

Multiple Comparisons

Dependent Variable: TAS Levels
LSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Negative control	Positive control	.37333*	.01787	.000	.3365	.4101
	Low Extract	.18000*	.01787	.000	.1432	.2168
	High Extract	.09167*	.01787	.000	.0549	.1285
	Vitamin C	.11333*	.01787	.000	.0765	.1501
Positive control	Negative control	-.37333*	.01787	.000	-.4101	-.3365
	Low Extract	-.19333*	.01787	.000	-.2301	-.1565
	High Extract	-.28167*	.01787	.000	-.3185	-.2449
	Vitamin C	-.26000*	.01787	.000	-.2968	-.2232
Low Extract	Negative control	-.18000*	.01787	.000	-.2168	-.1432
	Positive control	.19333*	.01787	.000	.1565	.2301
	High Extract	-.08833*	.01787	.000	-.1251	-.0515
	Vitamin C	-.06667*	.01787	.001	-.1035	-.0299
High Extract	Negative control	-.09167*	.01787	.000	-.1285	-.0549
	Positive control	.28167*	.01787	.000	.2449	.3185
	Low Extract	.08833*	.01787	.000	.0515	.1251
	Vitamin C	.02167	.01787	.237	-.0151	.0585
Vitamin C	Negative control	-.11333*	.01787	.000	-.1501	-.0765
	Positive control	.26000*	.01787	.000	.2232	.2968
	Low Extract	.06667*	.01787	.001	.0299	.1035
	High Extract	-.02167	.01787	.237	-.0585	.0151

*. The mean difference is significant at the 0.05 level.

Table 9. Showing the Post Hoc Tests of MDA levels for blood sample

Post Hoc Tests

Multiple Comparisons

Dependent Variable: MDA Levels
LSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Negative Control	Positive Control	-3.84000*	.15081	.000	-4.1506	-3.5294
	Low Extract	-1.67833*	.15081	.000	-1.9889	-1.3677
	High Extract	-.32833*	.15081	.039	-.6389	-.0177
	Vitamin C	-.38333*	.15081	.018	-.6939	-.0727
Positive Control	Negative Control	3.84000*	.15081	.000	3.5294	4.1506
	Low Extract	2.16167*	.15081	.000	1.8511	2.4723
	High Extract	3.51167*	.15081	.000	3.2011	3.8223
	Vitamin C	3.45667*	.15081	.000	3.1461	3.7673
Low Extract	Negative Control	1.67833*	.15081	.000	1.3677	1.9889
	Positive Control	-2.16167*	.15081	.000	-2.4723	-1.8511
	High Extract	1.35000*	.15081	.000	1.0394	1.6606
	Vitamin C	1.29500*	.15081	.000	.9844	1.6056
High Extract	Negative Control	.32833*	.15081	.039	.0177	.6389
	Positive Control	-3.51167*	.15081	.000	-3.8223	-3.2011
	Low Extract	-1.35000*	.15081	.000	-1.6606	-1.0394
	Vitamin C	-.05500	.15081	.718	-.3656	.2556
Vitamin C	Negative Control	.38333*	.15081	.018	.0727	.6939
	Positive Control	-3.45667*	.15081	.000	-3.7673	-3.1461
	Low Extract	-1.29500*	.15081	.000	-1.6056	-.9844
	High Extract	.05500	.15081	.718	-.2556	.3656

*. The mean difference is significant at the 0.05 level.

Table 10. Showing the Post Hoc Tests of MDA levels for liver sample

Post Hoc Tests

Multiple Comparisons

Dependent Variable: MDA Levels
LSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Negative Control	Positive Control	-5.17167*	.16189	.000	-5.5051	-4.8382
	Low Extract	-1.44500*	.16189	.000	-1.7784	-1.1116
	High Extract	-.63333*	.16189	.001	-.9668	-.2999
	Vitamin C	-.38667*	.16189	.025	-.7201	-.0532
Positive Control	Negative Control	5.17167*	.16189	.000	4.8382	5.5051
	Low Extract	3.72667*	.16189	.000	3.3932	4.0601
	High Extract	4.53833*	.16189	.000	4.2049	4.8718
	Vitamin C	4.78500*	.16189	.000	4.4516	5.1184
Low Extract	Negative Control	1.44500*	.16189	.000	1.1116	1.7784
	Positive Control	-3.72667*	.16189	.000	-4.0601	-3.3932
	High Extract	.81167*	.16189	.000	.4782	1.1451
	Vitamin C	1.05833*	.16189	.000	.7249	1.3918
High Extract	Negative Control	.63333*	.16189	.001	.2999	.9668
	Positive Control	-4.53833*	.16189	.000	-4.8718	-4.2049
	Low Extract	-.81167*	.16189	.000	-1.1451	-.4782
	Vitamin C	.24667	.16189	.140	-.0868	.5801
Vitamin C	Negative Control	.38667*	.16189	.025	.0532	.7201
	Positive Control	-4.78500*	.16189	.000	-5.1184	-4.4516
	Low Extract	-1.05833*	.16189	.000	-1.3918	-.7249
	High Extract	-.24667	.16189	.140	-.5801	.0868

*. The mean difference is significant at the 0.05 level.

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