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Effects of Methanol Leaf Extract of *Vernoniaamygdalina* on Paraquat-Induced Hepatic Injuryin Albino Rats

NWOZOR C.M*[1], UGHACHUKWU P.O[2], OKONKWO P.O[2], ILO C.E[3]
[1]*Department of Physiology, Faculty of Basic Medical Sciences, ChukwuemekaOdumegwuOjukwu University, Uli Campus, Anambra State, Nigeria.*
[2]*Department of Pharmacology and Therapeutics, College of Medicine, ChukwuemekaOdumegwuOjukwu University, Awka Campus, Nigeria.*
[3]*Department of Pharmacology and Therapeutics, College of Health Sciences, NnamdiAzikiwe University, Nnewi Campus, Anambra State, Nigeria.*
**Corresponding author: NWOZOR C.M.*

Abstract
Paraquat is an herbicide that is widely used in agriculture for weed control. Vernoniaamygdalina is a medicinal plant used in traditional medicine for the treatment of a variety of ailments. This research was undertaken to evaluate the effects of methanol leaf extract of V. amygdalina on paraquat-induced hepatic injury in Wistar rats. Twenty-four apparently healthy Wistar (albino) rats were randomly divided into four groups of six rats per group. Group A served as control. Group B received 10 mg/kg paraquati.p weekly for three weeks. Group C and D received paraquat plus 100 mg/kg and 200 mg/kg V. amygdalina. At the end of the experiment blood samples were taken via cardiac puncture for liver enzymes: alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP). Liver tissues were taken for histology. The results showed that paraquat caused liver injury as evidenced by marked increase in liver enzymes. V. amygdalina at 100 mg/kg and 200 mg/kg blocked paraquat and caused a reduction in the levels of these enzymes. Histologically paraquat caused significant hepatic inflammation. V. amygdalina was able to ameliorate this effect. Therefore, methanol leaf extract of V. amygdalina showed significant hepatoprotective effect.

Key Words: *Paraquat, V. amygdalina, AST, ALT, Wistar rats.*

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I. Introduction

Paraquat is an herbicide that is widely used in agriculture for eradication of weeds. It is very toxic. It kills all green parts of plants rapidly. Because of widespread use by farmers accidental poisoning can occur. The common routes of exposure in human and animals are by ingestion or through direct skin contact [1]. In terms of its chemical structure, paraquat resembles the neurotoxin, 1-methyl-4-phenylpyridinium ion (MPP+). This implies that it belongs to the class of redox cycling compounds capable of inducing mitochondrial damage, increase in reactive oxygen specie (ROS) production and oxidative stress [2].

Vernoniaamygdalina is commonly called bitter leaf. It is called onugbu in Igbo, and ewuro (Yoruba). It is an important part of our diet in Nigeria, especially in the South-Eastern region of the country. The macerated leaves of this plant are used to cook soup. Some authors have reported its use in traditional medicine especially as anti-helmith, anti-malarial, appetizer [3,4].

Furthermore, *V. amygdalina* has several pharmacological properties such as hypoglycemic/antidiabetic [5], antioxidant [6,7], anti-cancer [8]. This research was done to find out the effects of the methanol extract of *V. amygdalina* on paraquat-induced liver injury.

II. Materials And Methods

Plant Materials

The leaves of *vernoniaamygdalina* were purchased from afor-egbu market at Uli, Ihiala local government area, Anambra State. The leaves were authenticated by a botanist, DrChukwujekwu G. Ukpaka, Department of Biological Sciences, ChukwuemekaOdumegwuOjukwu University (COOU), Uli campus.

Preparation of Crude Extracts

The leaves were detached from the stems, washed twice with distilled water to remove dust. Subsequently they were shade-dried and powdered with an electric blender.The powdered sample of *V. amygdalina* weighing 50 g were extracted with methanol solvent (500 mL) by using Soxhlet extractor for 72 h.

At the end of the extraction process, the methanol solvent was evaporated by using rotary evaporator (Yamato Rotary Evaporator, model-RE801) under reduced pressure to obtain methanol crude extract. The formula for calculating the yield of the crude extract is as follows:

$$\text{Percentage yield} = \frac{\text{Final weight of extract}}{\text{Weight of dry leaves}} \times 100$$

This crude extract of methanol was suspended in water (60 mL). Whatman No. 41 was used to filter the crude extracts. This helped to remove particles. This was subsequently evaporatedby using rotary evaporator under reduced pressure. The residue left in the separator funnel was re-extracted twice by following the same procedure and filtered. The combined extracts were concentrated and dried by using rotary evaporator under reduced pressure [9].

Determination of Acute Toxicity (LD50) of *V. Amygdalina*

Acute toxicity of *V. amygdalina* was done using the method of Lorke [10]. Three groups of three rats each were orally administered with *V. amygdalina* at doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg body weight. The animals were observed for 24 hours. If there was no death in phase I the next phase was phase II. In phase II three groups of one rat each were given the following doses of *V. amygdalina* orally: 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg body weight. They were observed for 24 hours and the number of death was recorded. LD50 value was determined using the following formula: $LD50 = \sqrt{D0 \times D100}$
Where D0 = Maximum dose that did not cause death.
D100 = Minimum dose that caused death.

Experimental Animals

Apparently healthy rats (about 24) from Francis farms Ltd, Nnewi were used. Average weight of each rat was between 140 g to 200 g.The rats were randomly distributed into four groups of 6 rats per group:
Group A (control), Group B (paraquat only), Group C (paraquat + *V. amygdalina* 100 mg/kg), Group D (paraquat + *V.amygdalina* 200 mg/kg). Animals were housed at 22 ± 1^0C (12-hour light-dark cycle).They were acclimatized for one week before commencement of experiment. Feed and water were made available to the rats *ad libitum.* All experiments were carried out in accordance with standard procedure.

STUDY DESIGN

Experimental Studies

Group A (control) received water and feed *ad libitum*. Groups B, C, D animals received intraperitoneal injection of paraquat (10mg/kg) weekly for three weeks according to previously published guidelines [11].Groups C and D received 100 mg/kg and 200 mg/kg respectively of methanol fraction of leaf extract of *vernoniaamygdalina*by naso-gastric tube daily for 20 days starting 1 hr. after first paraquat injection. On the 6^{th} day after the last dose of paraquat the animals were sacrificed and their blood sampled. The serum samples were used to check for the following liver enzymes: alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT).

Determination of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) Activities (Teco Diagnostics, USA)

AST and ALT were determined using the spectrophotometric method of Bergermeyer*et al.*, [12].

Principle

This assay is based on the principle that AST and ALT catalyse the transfer of amino group from L-aspartate/L-alanine to a-ketoglutarate to yield oxaloacetate/pyruvate respectively. Oxaloacetate/pyruvate can oxidize NADH to NAD+ in the presence of malate dehydrogenase/lactate dehydrogenase. The decrease in absorbance at 340nm in a spectrophotometer due to the oxidation of NADH is monitored kinetically and is proportional to AST/ALT activity.

Procedure:

One (1.0) ml of ALT or AST reagent was added into a test tube and allowed stand for 3 minutes to equilibrate to 37°C. 0.10 ml of specimen was added to the ALT or AST reagent and mixed gently and the

solution was still maintained at 37°C. Then, the absorbance was read three times at 60 seconds interval at 340 nm.

Calculation:
The mean change in absorbance readings were calculated thus (ΔA/min). Therefore, ALT or

$$\text{AST activity (IU/L)} = \frac{\Delta A/\text{min.} \times TV \times 1000}{Є \times SV \times LP}$$

Where: ΔA/min = Average absorbance change per minute
TV =Total reaction volume (ml)
1000 = Conversion of IU/mL to IU/L
Є = Millimolar absorptivity of NADH (6.22)
LP = Light path (cm)
Note: samples with values above 500 IU/L were diluted 1:1 with normal saline, and re-assayed and the results mulplied by two.

Determination of Alkaline Phosphatase (ALP) Activity (Teco Diagnostics, USA)
ALP was assayed using the spectrophotometric method of Schlebusch*et al.*, [13].
Principle
At alkaline pH, ALP catalyzes the hydrolysis of p-nitrophenyl phosphate to yellow coloured p-nitro phenolate and phosphate; the change in absorbance measured at 405nm is directly proportional to the enzyme activity.
p-Nitrophenyl phosphate + H_2O $\xrightarrow{\text{Alkaline Phosphatase}}$ p-Nitrophenol + H_3PO_4
Procedure:
One (1.0) ml of ALP reagent was added into a test tube and allowed to equilibrate to 37°C. The spectrophotometer was blanked with water at 405 nm. 0.025 ml of specimen was added to the reagent and mixed gently while the solution was still maintained at 37°C. Then, the absorbance was read three times at 60 seconds interval at 405 nm.
Calculation:
The mean change in absorbance readings were calculated thus (ΔA/min). Therefore, ALP activity (IU/L) = $\frac{\Delta A/\text{min.} \times TV \times 1000}{Є \times SV \times LP}$
Where: ΔA/min = Average absorbance change per minute
TV =Total reaction volume (ml)
1000 = Conversion of IU/mL to IU/L
Є = Millimolar absorptivity of NADH (18.75)
LP = Light path (1cm)
Note: samples with values above 800 IU/L were diluted 1:1 with normal saline, and re-assayed and the results multiplied by two.

Statistical Analysis
Data obtained were expressed as Mean ± SEM. Data were analyzed statistically using SPSS version 21. One-way ANOVA was done followed by post-hoc Bonferroni. A p-value < 0.05 was considered significant.

III. Results

LD50 of Methanol leaf extract of *V. amygdalina*

Phase I	
10 mg/kg	0/3
100 mg/kg	0/3
1000 mg/kg	0/3
Phase II	
1600 mg/kg	0/1
2900 mg/kg	0/1
5000 mg/kg	0/1

No death was recorded with the maximum dose of 5000 mg/kg.
LD50, therefore, was > 5000 mg/kg

Effect of *V. Amygdalina* on Alanine Transaminase (ALT) Levels

The results of the effect of *V. amygdalina* on ALT are shown in table I below. Control rats had 15.57±1.01 IU/L. This was significantly increased by paraquat (63.60±7.55 IU/L). *V. amygdalina* (both 100 mg/kg and 200 mg/kg) significantly blocked paraquat and caused a marked reduction in ALT levels. Therefore *V. amygdalina* demonstrated significant hepatoprotective effect.

Effect of *V. Amygdalina* on Aspartate Transaminase (AST)

The results of the effect of *V. amygdalina* on AST are shown in table II below. AST concentration in control rats was 16.43±0.64. The value in paraquat-injected rat was 66.14±5.73 IU/L. *V. amygdalina* (both 100 mg/kg and 200 mg/kg) blocked paraquat. The values were 38.35±3.41 and 40.62±1.67 IU/L respectively. *V. amygdalina* demonstrated significant hepatoprotective effect.

Effect of *V. Amygdalina* on Alkaline Phosphatase (ALP)

The results of the effect of *V. amygdalina* on ALP are shown in table III below. ALT concentration in control rats was 45.22±8.73 IU/L. The level in paraquat-injected rats was 126.14±11.09 IU/L. This represented a 2.8-fold increase.Thusparaquat significantly increased ALP level. *V. amygdalina*blocked paraquat and caused a decrease in ALT levels. The values for 100 mg/kg and 200 mg/kg were 86.48±6.82 and 77.92±6.15 IU/L respectively.*V. amygdalina* demonstrated significant hepatoprotective effect.

Histopathological Analysis of the Hepatoprotective Effects of *V.Amygdalina*.

The results of the histopathological analysis of the hepatoprotective effect of *V. amygdalina* are shown in fig 1 below. The control rats showed normal liver tissue with portal tract, hepatocytes, and sinusoid (Group A). In the paraquat-injected rats there was inflammation as evidenced by the presence of inflammatory cells. This is presented in Group B. The rats treated with 100 mg/kg of *V. amygdalina* showed liver tissue with focal inflammation (Group C).The rat treated with 200 mg/kg of *V. amygdalina* showed Photomicrograph of Normal Liver Tissue With Portal Tract, Hepatocytes and Sinusoid.(H&E)X100 (Group D).

TableI: Shows serum Alanine Transaminase (ALT) concentrations.

Groups	ALT (IU/L)	P-value
A	15.57±1.01	
B	63.60±7.55***	0.000
C	32.50±3.53	0.064
D	37.84±2.28**	0.007

Table II: Shows serum Aspartate Transaminase (AST) concentrations.

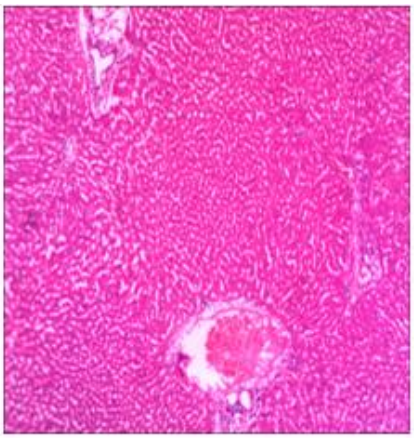
Groups	AST (IU/L)	P-value
A	16.43±0.64	
B	66.14±5.73***	0.000
C	38.35±3.41**	0.001
D	40.62±1.67***	0.000

Table III: Shows serum Alkaline Phosphatase concentrations.

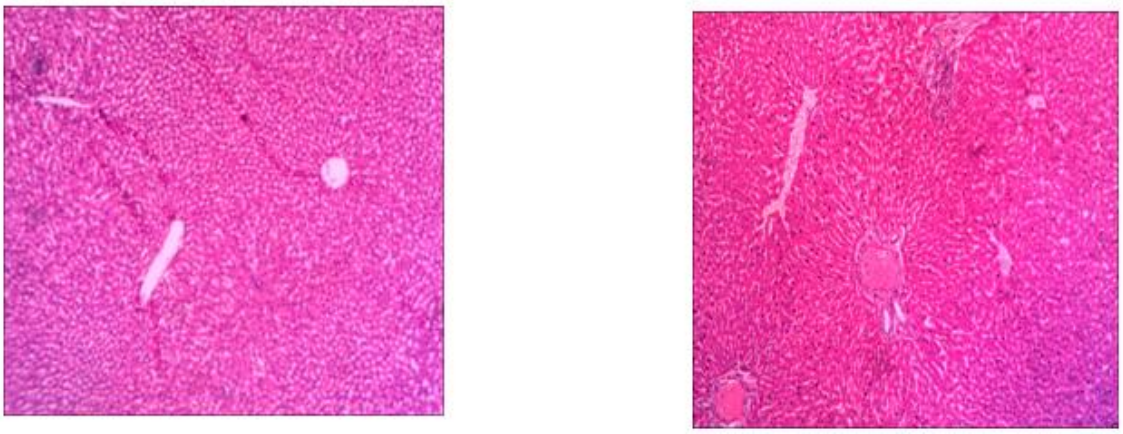
Groups	ALP (IU/L)	P-value
A	45.22±8.73	
B	126.14±11.09***	0.000
C	86.48±6.82*	0.019
D	77.92±6.15	0.101

***P< 0.001; **P< 0.01; *P< 0.05

Group A



Group B



Group C **Group D**

Fig 1: Shows histology of the liver

KEY. Slide a (control rats): Showing Photomicrograph Of Normal Liver Tissue With Portal Tract, Hepatocytes And Sinusoid.(H&E)X100. Group B (paraquat injected rats): Showing Photomicrograph of Liver Tissue with Some Inflammatory Cells. (H&E)X100. Group C (Rats treated with 100 mg/kg of *V. amygdalina*) Showing Photomicrograph of Liver Tissue With Focal Inflammation.(H&E)X100.Group D (Rats treated with 200 mg/kg of *V. amygdalina*):Showing Photomicrograph of Normal Liver Tissue With Portal Tract, Hepatocytes and Sinusoid.(H&E)X100.

IV. Discussion

Administration of paraquat (10 mg/kg i.p weekly for three weeks) caused marked elevation of ALT, AST, and ALP (P=0.000).Thus significant hepatic injury was well established. In the control rats the ALT value was 15.57±1.01 IU/L. In the paraquat-injected rats it was 63.60±7.55 IU/L. This represented a four-fold increase. For the AST the concentration in the control rats was 16.43±0.64 IU/L.In the paraquat-injected it was 66.14±5.73 IU/L. Again this represented a four-fold increase.Paraquat caused 2.8-fold increase in ALP levels.

V. amygdalina at 100 mg/kg caused ALT decrease to 32.50±3.53 IU/L. It was not statistically significant (P=0.064).However at 200 mg/kg it was 37.84±2.28 IU/L. This decrease was highly significant (P=0.007). *V. amygdalina* at 100 mg/kg caused AST decrease to 38.35±3.41 IU/L. This was highly significant (P=0.001). At 200 mg/kg it was 40.62±1.67 IU/L. It was highly significant (P=0.000).

ALT and AST are two of the most important and established markers of hepatic injury. The concentrations can be elevated in a variety of hepatic insults caused by either toxins or drugs. Of the two ALT is thought to be more specific for hepatic injury because it is present mainly in the cytosol of the liver and in low concentrations elsewhere. AST has cytosolic and mitochondrial forms and is present in tissues of the liver, heart, skeletal muscle, kidneys, brain, pancreas, lungs, and blood cells (both white and red blood cells [14].

The mechanism of paraquat toxicity in the brain is similar to its toxicity in the liver. Upon entering the liver tissue, paraquat induces oxidative stress causing injury to the hepatocytes. This leads to the release of transaminases into the blood. Thus elevated blood levels of these transaminases indicate hepatic injury.*V. amygdalina* showed significant hepatoprotective effect. This is in agreement with the findings reported by Iwalokun et al., [7] and Momoh Johnson et al., [15].

Quantitative and qualitative phytochemical constituents of the extract of *V. amygdalina* showed significant amounts of alkaloids, tannins, saponins, flavonoids, phenolics, and glycosides [16]. Flavonoids are known to be good antioxidants. Luteolin (a flavonoid formed in *V. amygdalina*) has been reported to be a strong antioxidant [17,3]. Therefore, the mechanism by which *V. amygdalina* exerted its hepatoprotective effect may be attributed to its antioxidant properties.

Histopathological analysis equally confirmed the hepatoprotective effects of

V. amygdalina (fig.1). In the paraquat-injected rats there was inflammation as evidenced by the presence of inflammatory cells, suggesting that paraquat had hepatotoxic effect. The control rats showed normal liver tissue with portal tract, hepatocytes, and sinusoid. In the rats treated with 100 mg/kg of *V. amygdalina* there was focal inflammation, suggesting that inflammation was resolving and was being contained by the medicinal plant.In the rats treated with 200 mg/kg of *V. amygdalina* the liver architecture appeared normal.

V. Conclusion

Paraquat, an herbicide used widely in agriculture for weed control was used to induce hepatic injury. Indeed there was hepatotoxicity as evidenced by elevation in liver enzymes namely: aspartate transaminase (AST), alanine transaminase (ALT), and alkaline Phosphatase (ALP). Methanol leaf extract of *V. amygdalina* at both 100 mg/kg and 200 mg/kg was able to ameliorate this injury. The mechanism by which this medicinal plant exerted this effect may be attributed to the abundant antioxidants it contains.

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