

# The Cushioning Effect of Extracts of *Amaranthus Viridis* Linn (Green Amaranth) on Acetaminophen-Induced Hepatotoxicity.

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## ABSTRACT

Vegetables serve as indispensable constituents of the human diet, supplying the body with minerals, vitamins and certain hormone precursors, in addition to protein and energy. Huge quantities of *Amaranthus viridis* (Green amaranth), is consumed all over Nigeria vegetable for various dishes. In the current study, the hepatoprotective properties of aqueous and ethanol extract of *A. viridis* was studied. Thirty (30) male Wister albino rats weighing between 120-140g were used for the study. The animals were divided into five (5) groups of six animals each. These include three (3), control groups; Normal Control (Water and Feeds only), Negative Control (Water, Feed and 2g/kgbw of Acetaminophen) and Positive control (Water, Feed, Sylimarin and 2g/kgbw of Acetaminophen). The other groups were two groups administered with the aqueous and ethanol leaf extracts of the plant, respectively. The results obtained shows that oral administration of a single 2g/kgbw dose of acetaminophen led to a marked increase in the serum activities of the ALT, AST and ALP of the animals in the negative control group compared to the normal control group. The groups pre-treated with 400mg/kgbw of aqueous leaf extracts of the plant showed significantly ( $p < 0.05$ ) decreased activity of ALT and AST but insignificant change in the serum activity of ALP of the animals. It can therefore, be suggested that *Amaranthus viridis* produced effects which may contribute to the preservation of cellular GSH levels in the acetaminophen-intoxicated rats, which further provides cellular defense both as a hydroxyl radical scavenger and also a detoxifying agent against the toxic intermediate of acetaminophen in a GST-catalysed reaction. Therefore, it can be a good source of raw material for the production of drugs used for the prevention and treatment of liver diseases and other pathological conditions associated with oxidative stress. This encourages and supports **its use as edible vegetables and for therapy respectively.**

## INTRODUCTION

Vegetables serve as indispensable constituents of the human diet, supplying the body with minerals, vitamins and certain hormone precursors, in addition to protein and energy, (Aja *et al.*, 2010). Leafy vegetables have been found to boost the concentration of Red Blood cell and significantly increase the serum activity of AST in experimental animals, (Ezekwe *et al.*, 2013). With the increasing focus on plant research all over the world, a large body of evidence has been collected to show immense potential of medicinal plants for treatment purposes or for the production of drugs (Dahanukar *et al.*, 2001; Olamide and Mathew, 2013; Udochukwu, *et al.*, 2015). The use of plants in ethnomedicine for the management of ailments stem from the presence of phytochemicals, (Aja, *et al.*, 2010). It has been suggested that ethanol

extracts of vegetables could be used in treating diseases caused by microorganisms (Ladipo, *et al.*, 2010). Polyphenols and flavonoids in plant extracts had been implicated for their antioxidant potentials by improving the functionality of the antioxidant system of test rats, (Edeoga, *et al.*, 2005; Sudha *et al.*, 2011; Imaga and Bamigbetan 2013; Alamgir *et al.*, 2016; Enemali *et al.*, 2018). Many remedies from plant origin have been evaluated for their potential antioxidant and hepatoprotective effect in experimental animal model (Enemali and Udedi 2018; Enemali, *et al.*, 2019).

Acetaminophen (APAP) is an analgesic and antipyretic substance used in the production of the drug paracetamol. Although safe at therapeutic doses of 1000mg/75kgbw, APAP had been found to cause severe liver injury (Erica and Emily. 2014) at higher doses (Enemali *et al.*, 2018). Mitchell, *et al.* (1973), reported that Acetaminophen (APAP) overdose is the predominant cause of acute liver failure in the United States and that toxicity begins with a reactive metabolite that binds to proteins. These findings indicated that acetaminophen was metabolically activated by cytochrome P<sub>450</sub> enzymes to a reactive metabolite that depleted glutathione (GSH) and covalently bonded to protein. It has also been shown by James, *et al.* (2009) that repletion of glutathione (GSH) prevented the toxicity. Depletion of GSH which is an intrinsic antioxidant is capable of introducing peroxidation of cell membrane lipids, regeneration of reactive oxygen free radicals and hepatocellular fatty regeneration with centriolobular necrosis of the liver.

Reactive oxygen species (ROS) are produced in the cells by cellular metabolism and other exogenous environmental agents. They are generated by a process known as redox cycling and are catalysed by transition metals such as Fe<sup>2+</sup> and Cu<sup>2+</sup> (Halliwell and Gutteridge, 1999). Over production of ROS can damage cellular biomolecules like nucleic acids, proteins, lipids, carbohydrates and enzymes resulting in several diseases. Living systems have specific pathways to overcome the adverse effects of various damages. However, sometimes these repair mechanism fail to keep pace with such deleterious effects (Nilsson *et al.*, 2004). In chronic liver diseases caused by oxidative stress (alcoholic and non-alcoholic fatty liver diseases, drug- and chemical-induced hepatic toxicity), conventional therapeutics options available for managing liver hepatotoxicity include a commonly used drug called silybon 140, whose active ingredient is silymarin. (Feher and Lengyey, 2017). Silymarin is the active ingredient in the branded drug, Silybon 140 (a known hepatoprotective drug), manufactured by Micro Laboratory Ltd, India. Silymarin has cytoprotective activity mediated by its antioxidative and radical-scavenging properties, (Křena and Walterovab, 2005). As an antioxidant, silymarin scavenges for free radicals that can damage cells exposed to toxins. Silymarin has been said to be at least ten times more potent in antioxidant activity than vitamin E.

In a poor resource country like Nigeria, access to quality drugs continue to be a big challenge to rural dwellers and Those in extreme poverty. In such countries, leafy vegetables contribute greatly to the nutritional and medicinal needs of local populations where they are used as staples, flavours, condiments, spices, drinks and beverages in most developing countries (Mbang *et al.*, 2008). It is generally known that the consumption of a variety of local herbs and vegetables by man contributes significantly to the improvement of human health, in terms of prevention and cure of diseases because plants have long served as a useful and natural source of therapeutic agents (Chevellier, 1996). Living systems have specific pathways to overcome the adverse effects of various damages, but sometimes, these repair mechanism fail to keep pace with such deleterious effects (Stegmark and Akesson, 2004; Nilsson *et al.*, 2004). Therefore, there is need to seek an alternative to augment the effort of the internal protection and repair mechanisms.

Townsend (1985) typically described the plant, *Amaranthus viridis* as an annual herbaceous plant, of the family Amaranthaceae, genus *Amaranthus* and specie *Amaranthus viridis* L (USDA, 2008). *A. viridis* grows in heavy organic to very sandy soil, including muck soil after the water has gone down for the season. *Amaranthus viridis* is grown and utilized in many areas of the world as both a wild and cultivated pot herb (Uphof, 1968). The plant is rich in calcium and iron and it is a good source of vitamin B and C (Morton, 1981). *Amaranthus viridis*' common names include: green amaranth, slender amaranth, African spinach,

wild amaranth, (English), inine (Igbo), tete (Yoruba).

Huge quantities of *Amarranthus viridis* (Green amaranth), are consumed all over Nigeria for flavouring and spicing of various types of food. Available information on the medicinal property is scanty, therefore, it is expedient to establish the hepatoprotective potentials of aqueous and the ethanol leaf extracts of this plant

## **MATERIALS AND METHODS**

### **Experimental Animals**

Thirty (30) male Wister albino rats weighing between 120-140g were used for the study. These rats were purchased from the animal house of the National Veterinary Research Institute (NVRI), Vom, in Plateau state. They were housed in clean, well ventilated metal cages in the animal house of the Department of Biological Sciences (Zoology unit), Nasarawa State University Keffi. The animals were kept under 24 hours light/dark cycling. They were allowed access to unlimited food and water supply and allowed to acclimatize for two (2) weeks before the commencement of the study.

### **Plant Material**

The leaf of the plant under study were harvested from a farm at Angwan Lambu in Keffi, Nasarawa State (North Central Region of Nigeria). They were taken to the Department of Plant Science and Biotechnology for identification and assignment of voucher number (NSUKPSB112V), before some samples were deposited in the Herbarium.

### **Methods**

#### **Plant Sample preparation**

They were rinsed in water to remove dust and sand particles, and then dried under room temperature for fourteen (14) days. The dried leaves were then ground into powder using electric blender. Part of the ground leaf materials were used to determine the proximate composition while the other was used for preparation of aqueous and ethanol extracts.

#### **Proximate Analysis of the Plant Material**

Standard methods of the Association of Analytical Chemist (AOAC), 2006 was used to determine the moisture content, crude protein, crude fat, total ash, crude fiber and carbohydrate content of the materials.

#### **Preparation of Leaf Extracts**

Ground plant material were divided into two; one was extracted with ethanol by soaking 200g of the ground samples in 1000ml of 98% ethanol (i.e. ratio 1:5; weight to volume) for 48hrs. The extracts were filtered using muslin cloth and then concentrated by heating in a water bath and stored in airtight containers.

The other part was extracted by soaking 200g of the powdered samples in 1000ml of distilled water (i.e. ratio 1:5; weight to volume) for 24hrs. The extracts were filtered using muslin cloth and then concentrated by heating to evaporate the water and stored in airtight containers.

#### **Phytochemical Screening of the Leaf Extracts:**

Portions of the concentrated extracts were used for phytochemical screening using standard procedures of the Association of Analytical Chemist to identify the constituents as described by Odebiyi and

Sofowora(1990), Fadeyi (1983), Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

### **Animal Handling**

The animals were divided into five (5) groups of six animals each. These include three (3), control groups; Normal Control (Water and Feeds only), Negative Control (Water, Feed and 2g/kgbw of Acetaminophen) and Positive Control (Water, Feed, Sylimarin and 2g/kgbw of Acetaminophen). The other groups were two groups administered with the aqueous and ethanol leaf extracts of the plant, respectively prior to administration of acetaminophen (2g/kgBW) through oral gavage.

### **Preparation of Biological Samples**

#### **Collection of Blood Samples**

At the end of the experimental period, (after 9 hours of intoxication), the animals were made unconscious by exposure to chloroform in an enclosed container, according to the method described by Ekor, *et al.* (2006) Incisions were quickly made into the animals' cervical region with the aid of sterile blades. Blood samples were collected by cervical decapitation into plain tubes. Serum was collected by centrifuging the clotted blood in a HSC (1000-4000rpm) bench centrifuge at 3000 rpm for 10 minutes.

#### **Preparation of Liver Homogenate:**

The liver homogenate was prepared according to the method described by Enemali *et al.* (2019). The sample was stored at the temperature of -4°C and later used for the assay of the activities of the antioxidant enzymes (Super Oxide Dismutase, Catalase and Glutathione Peroxidase).

#### **Determination of the Liver Function**

The prepared serum was analyzed for various biochemical parameters – Aspartate Aminotransferase (AST) (King, 1965a), Alanine aminotransferase (ALT) (Lowry *et al.*, 1951) and Alkaline phosphatase (ALP) (King, 1965b); Total and Direct Bilirubin were estimated by the method of Malloy-Evelyn, (1937). Albumin concentration was determined using Randox kits using the method outlined in the manual for albumin determination.

#### **Histopathological Study on the Liver Tissues**

Histopathological study on the liver tissues was carried out at Federal College of Veterinary and Medical Laboratory Technology, Vom, Plateau State, using the method described by Bancroft *et al.*, (2013).

#### **Statistical Analysis**

Statistical analysis was conducted, for the enzyme assay and lipid profile values using the one-way ANOVA and data were expressed as mean  $\pm$  standard deviation (SD) of six values. The differences between the mean values of the test groups were analysed for statistical significance using one-way ANOVA. The differences between groups were considered to be significant if  $p < 0.05$ .

## **RESULTS**

### **The Proximate Compositions of the Leaves of *Amaranthus viridis*:**

The leaves of *Amaranthus viridis* were found to be rich in protein, having percentage compositions of 25.09  $\pm$  0.07%. The leaf also has high ash content of above 11% and protein content of above 20% as shown in

Table 1

**Table 1: The proximate Compositions of *Amaranthus viridis* the leaf**

Proximate Parameters	Composition
Crude protein (%)	25.09 ± 0.07
Crude Fibre (%)	9.54 ± 0.10
Ash (%)	17.77 ± 0.07
Total Lipids (%)	11.97 ± 0.05
Moisture (%)	9.80 ± 0.06
Total Carbohydrate (%)	4.88 ± 0.10

Values are mean ± SD of triplicate (X3) results

**The Qualitative Phytochemical Compositions of Aqueous and Ethanol Leaf Extracts of the Plants**

The results from the phytochemical analysis of the aqueous and ethanol leaf extracts of the plant showed the presence of Phenols, Tannins, Terpenoids and Cardiac glycosides both aqueous and ethanol extracts. While other phytochemicals were differently distributed in the aqueous and ethanol leaf extracts of the plant. The water extracted higher quantities of tannin and saponin from all the plant and ethanol leaf extract contained more Phenol and alkaloids, see Table 2

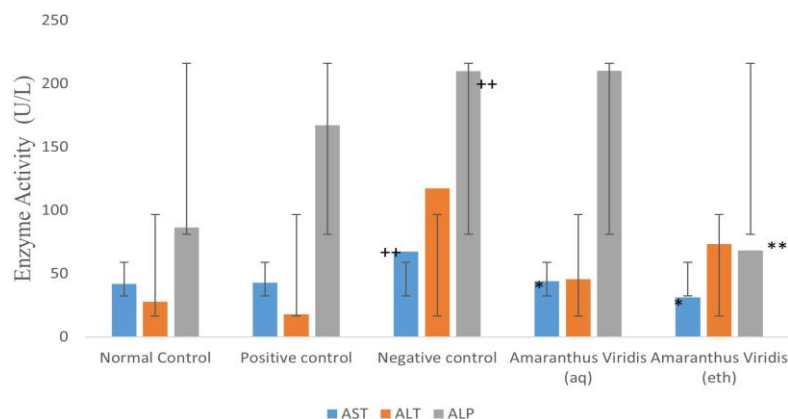
**Table 2: The qualitative phytochemical compositions of aqueous and ethanol leaf extracts of *Amaranthus viridis***

Phytochemical	Aqueous Extract	Ethanol Extract
Alkaloid	–	+
Tannin	+	+
Total Phenol	+	+
Cardiac Glycoside	+	+
Triterpenoid	–	+
Sterol	–	+
Saponin	+	–
Balsam	+	+
Cardenolides	+	–
Resin	+	–
Glycoside	+	–
+ indicates presence; – indicates absence		

**The Effect of Pre-Treatment with 400gm/Kgbw Of Leaf Extracts of *Amaranthus Viridis* on the Serum Activities of AST, ALT And ALP of Rats Intoxicated with 2g/Kgbw Single Dose of Acetaminophen.**

The oral administration of a single 2g/kgbw dose of acetaminophen led to a marked increase in the serum activities of the ALT, AST and ALP of the animals in the negative control group compared to the normal control group .The groups pre-treated with 400mg/kgbw of aqueous leaf extracts of the plant showed significantly (p<0.05) decreased activity of ALT and AST but insignificant change in the serum activity of

ALP of the animals (Table 3).

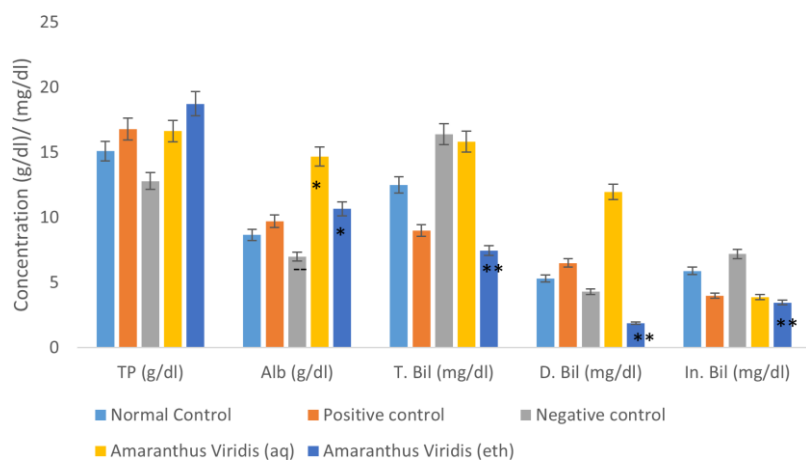


**\*\*Fig 1: The effect of pre-treatment with 400gm/kgbw leaf extracts of *Amaranthus viridis* on the serum activities of AST, ALT and ALP of rats intoxicated with 2g/kgbw single dose of acetaminophen.**

Values are mean  $\pm$  SD of six (6) results, \* and \*\* show values with significant increase and decrease respectively, compared to the Negative control while ++ and - indicate values with significant increase and decreases respectively compared to the Normal control. AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase

**The Effect of Pre-Treatment with 400gm/Kgbw Aqueous and Ethanol Leaf Extracts of *Amaranthus Viridis* on the Serum Concentrations of Total Protein, Albumin, and Bilirubin of Rats Intoxicated with 2g/Kgbw Single Dose of Acetaminophen.**

The oral administration of a single 2g/kgbw dose of acetaminophen to the rats elicited a significant ( $p < 0.05$ ) decrease on the serum albumin concentration of the rats. However this intoxication caused insignificant ( $p > 0.05$ ), change on the serum concentrations of total protein, total bilirubin, direct bilirubin and indirect bilirubin (fig 2). The 7day pre-treatment of the animals with both aqueous and ethanol leaf extracts of the plant elicited significant ( $p < 0.05$ ) increase on the serum concentrations of the albumin of some of the animals, see Table 4.



**Fig 2: The effect of pre-treatment with 400gm/kgbw leaf extracts of *Amaranthus viridis* on the serum concentrations of total protein, albumin, and bilirubin of rats intoxicated with 2g/kgbw single dose of acetaminophen.**

Values are mean  $\pm$  SD of six (6) results, \* and \*\* show values with significant increase and decrease

respectively, compared to the Negative control while  $^{++}$  and  $^{-}$  indicate values with significant increase and decreases respectively compared to the Normal control.

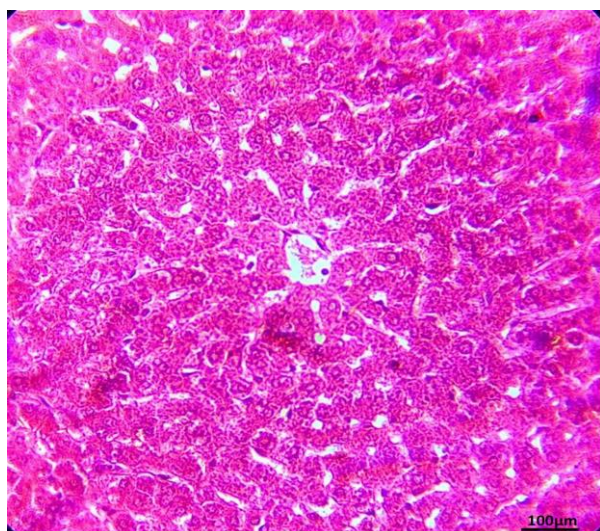
TP: Total protein

Alb: Albumin

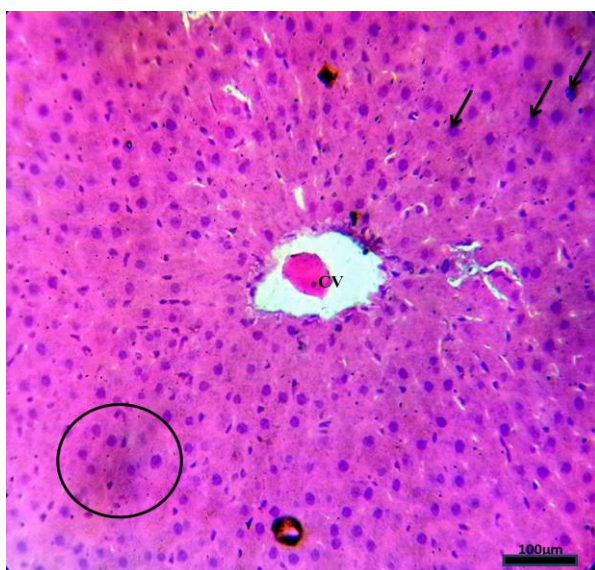
T.Bil: Total Bilirubin

D.Bil: Direct Bilirubin

In.Bil: Indirect Bilirubin

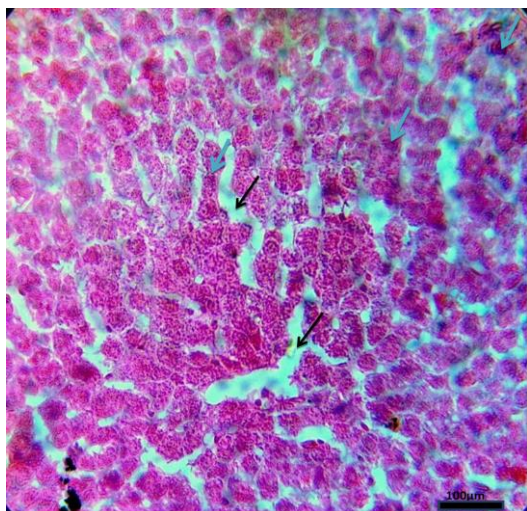


**PLATE 1:** The histological architecture of livers of rats used as Normal Control](X100) – *Haematoxylin and Eosin stain*

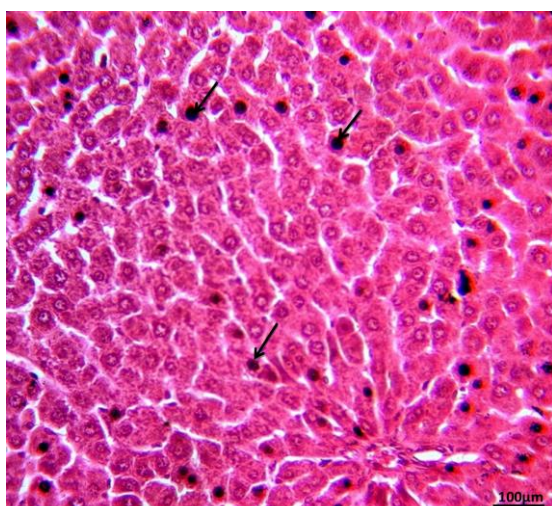


**Plate 2:** The effect of pre-treatment with Silymarin on the histological architecture of livers of rats administered with a single over-dose of acetaminophen, (X100) – *Haematoxylin and Eosin stain*. CV =

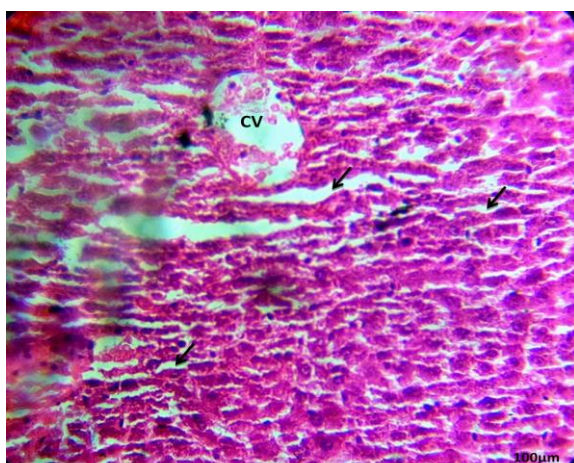
central vein



**PLATE 3:** The effect of a single over-dose of acetaminophen the histological architecture of livers of rats (Negative Control), (X100) – *Haematoxylin and Eosin stain*



**PLATE 4:** The effect of pre-treatment with aqueous extract of *amaranthusviridis*, (Green Amaranth), leaf on the histological architecture of livers of rats administered with a single over-dose of acetaminophen, (X100) – *Haematoxylin and Eosin stain*



**PLATE 5:** The effect of pre-treatment with ethanol extract of *Amaranthusviridis*, (Green Amaranth), leaf



on the histological architecture of livers of rats administered with a single over-dose of acetaminophen (X100) – *Haematoxylin and Eosin stain*. C.V = central vein.

## DISCUSSION

The proximate composition of the leaf of *Amaranthus viridis* reveal that the vegetable is rich in protein, fibre and ash. The Phytochemical screening of the leaf extracts of *Amaranthus viridis* showed the presence of Phenols, Tannins, Terpenoids and Cardiac glycosides both aqueous and ethanol extracts. Polyphenols and triterpenoids has been shown to possess antioxidant capacity which can ameliorate the effect of oxidative stress on liver cells (Asogwa *et al.*, 2020). The aqueous extract contain higher quantities of tannin and saponin while ethanol leaf extract contained more Phenol and alkaloids. This may be attributed to the different solubility of the phytochemicals in the different solvents used. The oral administration of 2g/kgbw single dose of acetaminophen to the rats (negative control), that were not pre-treated with either the aqueous or ethanol leaf extracts of *Amaranthus viridis*, induced some level of liver injury. This was evidenced by the significant ( $p < 0.05$ ) increase of the serum activities of the Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) of the rats in the negative control group (fig 1). Al-Mamary (2002) reported that the aminotransferase are abundant in the liver and are released into the bloodstream following hepatocellular damage, making them sensitive markers of liver damage. Therefore, a marked increase in the serum ALT and AST activities is indicative of liver damage. Serum levels of aminotransferases are used as an indicator of damage to the liver structural integrity because these enzymes are cytoplasmic in location and are released into the circulating blood only after structural damage (Okediran *et al.*, 2014). A marked rise in the serum activity of ALT, reduction in total serum protein and abnormal increase in serum bilirubin had been reported in hepatotoxicity, (Olamide and Mattew, 2013; Olorunnisola *et al.* 2011; Martin and Friedman, 1992). A decrease in total protein and albumin shows that the ability of the liver to synthesize/metabolize protein (example albumin), may have been impaired, hence indicative of liver damage. Histological profile of the livers of the rats in the negative control group showed poorly preserved hepatic lobular architecture, sharply demarcated hepatocyte, necrosis and some exhibiting peri-portal sinusoidal congestion, marked sinusoidal dilatation which is indicative of liver injury.

The pre-treatment of the animals (rats), with 400mg/kgbw of either aqueous or ethanol leaf extracts of *Amaranthus viridis*, gave protection against toxicity and oxidative stress arising from a 2g/kgbw single dose of the drug over nine hours (9hrs). The protection may be due to the antioxidant properties of these plants, which stem from their phytochemical components. Researchers have found that phytochemicals have the potential to reduce oxidative damage to cells. Polyphenols and flavonoids compounds have been said to play a major role in various antioxidant effects shown by plant extracts (Asogwa *et al.*, 2020). Triterpenoids is one of the phytochemicals present in *Amaranthus* and it has been shown to possess hepato-protective activity according to the report by (Xu *et al.*, 2018). However, it is not known whether the health benefits are the result of individual phytochemicals, the interaction of various phytochemicals, the fibre content of plant foods, or the interaction of phytochemicals and the vitamins and minerals found in the same foods. It was found in this study that *Amaranthus viridis* is a rich source of phytochemicals.

Generally, the administration of aqueous and ethanol leaf extracts of *Amaranthus viridis* led to decrease of the serum activities of ALT and ALP of the animals pre-treated with the extracts, but markedly increased the serum activity of AST. However, the decreases in the activities of ALT and ALP in the serum of the rats pre-treated with these extracts were not as much as that caused by Silymarin. The extracts also markedly increased the serum albumin and total protein of the pre-treated rats with the preservation of the hepatic lobular architecture.

It can therefore, be suggested that *Amaranthus viridis* produced effects which may be attributed to its earlier reported antioxidant activities which may have ameliorated the oxidative damage in the acetaminophen-

intoxicated rats (Ekor, *et al.*, 2006). From the foregoing, it could be suggested that the plant under study possess great deal of hepato-protective potentials which can account for the low activities of ALT and ALP in the serum of the pre-treated rats and the preservation of the livers' lobular architecture, compared to the control group.

### Conclusion:

The present study provides evidence that *Amaranthus viridis* offered protection to the hepatic cells. Therefore, it can be a good source of raw material for the production of drugs used for the prevention and treatment of liver diseases.

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