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EFFECTS OF VERNONIA AMYGDALINA (BITTER LEAF) ON THE BIO-MARKER OF OXIDATIVE STRESS IN ACCETAMINOPHEN INDUCED LIVER DAMAGE OF ALBINO RATS

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ABSTRACT

The effect of daily oral administration of Bitter leaf (Vernonia amygdalina) extract (2ml/kg body weight) for 14days on the alkaline phosphatase, aspartate and alanineaminotransferase activities on rat liver, kidney, Serum and Biomarker Enzymes in acetaminophen-induced-hepatotoxicity in albino rats respectively were investigated. A noticeable change in the biomarker enzyme activities such as (Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) of the albino rats treated with acetaminophen were observed. Induction of acetaminophen caused significant (P<0.05) increase in the activities of AST, ALT and ALP when compared to the control. Oral administration of the extract of Bitter leaf (Vernonia amygdalina) reduced the activities of AST, ALT and ALP in rats (Table 1, 2 and 3) when compared to the untreated group (positive control). This is an indication that the extract might prevent liver damage by maintaining the integrity of the plasma membrane and suppressing the leakage of the enzymes through the membrane. The significant (P<0.05) reduction in the activity of AST, ALT and ALP by Silymarin, and Bitter leaf (Vernonia amygdalina) extract treatment may be correlated to their hepatoprotective effects.

***KEYWORDS**: Rat, Liver, Oxidative Stress, Acetaminophen, Vernonia Amygdalina*

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INTRODUCTION

The laboratory rat (*Rattusnorvegicus*) has long been favoured as an experimental model for nutritional research because of its moderate size, profligate reproduction, adaptability to diverse diets, and tractable nature. It is now the species of choice for many experimental objectives because of the large body of available data and the development of strains with specific characteristics that facilitate the study of disease and other processes.

Biomarker is a measurable indicator of the severity or presence of some disease state. More generally a biomarker is anything that can be used as an indicator of a particular disease state or some other physiological state of an organism. A biomarker can be a substance that is introduced into an organism as a means to examine organ function or other aspects of health. For example, rubidium chloride is used in isotopic labeling to evaluate perfusion of heart muscle. It can also be a substance whose detection indicates a particular disease state, for example, the presence of an antibody may indicate an infection.

More specifically, a biomarker indicates a change in expression or state of a protein that correlates with the risk or progression of a disease, or with the susceptibility of the disease to a given treatment. Biomarkers can be characteristic biological properties or molecules that can be detected and measured in parts of the body like the blood or tissue. They may indicate either normal or diseased processes in the body. Biomarkers can be specific cells, molecules, or genes, gene products, enzymes, or hormones. Complex organ functions or general characteristic changes in biological structures can also serve as biomarkers.

Although the term biomarker is relatively new, biomarkers have been used in pre-clinical research and clinical diagnosis for a considerable time. For example, body temperature is a well-known biomarker for fever. Blood pressure is used to determine the risk of stroke. It is also widely known that cholesterol values are a biomarker and risk indicator for coronary and vascular disease, and that C-reactive protein (CRP) is a marker for inflammation.

Biomarkers are useful in a number of ways, including measuring the progress of disease, evaluating the most effective therapeutic regimes for a particular cancer type, and establishing long-term susceptibility to cancer or its recurrence. The parameter can be chemical, physical or biological. In molecular terms biomarker is "the subset of markers that might be discovered using genomics, proteomics technologies or imaging technologies. Biomarker has played major roles in medicinal biology. Biomarkers help in early diagnosis, disease prevention, drug target identification, drug response etc. Several biomarkers have been identified for many diseases such as serum LDL for cholesterol.

Oxidative stress is also a predisposing factor and direct cause of various disorders in both man and animals, including renal ones. This fact encouraged pharmacologists to utilize antioxidants, especially those of natural source, for prophylaxis and treatment of such disorders and improving the health status of normal subjects as well. In the last few decades, several models have been suggested to define the interconnection and the biological pathways of aging. The widely accepted theory is the “oxidative stress hypothesis” (Ghezziet al., 2017) that advanced and modified the free radical theory of aging. Based on the oxidative stress hypothesis, oxidative damage is not solely triggered by the unrestricted ROS production, but it also caused by other oxidants, such as reactive lipid species and reactive nitrogen species (RNS).

The hypothesis of oxidative stress highlights the crucial role of antioxidant defenses as an important component of the overall redox balance of the organism. However, several studies demonstrated that avoiding oxidative stress damage does not increase longevity (Buffensteinet al., 2008).Oxidative stress is considered as an imbalance between pro and antioxidant species, which results in molecular and cellular damage (Conti et al., 2016). Mitochondria are major organelles that are accountable for generation of energy through oxidative phosphorylation to generate adenosine triphosphate (ATP), a molecule which is crucial for cellular actions (Weinberg et al., 2015).

The electron transport chain consumes up to 90% of total oxygen (O2) taken up by the cells. During this process, (ROS) Reactive oxygen species are generated as by-products for the partial four-electron reduction of O2 to produce water molecule, which is the last electron acceptor in the ATP generation process. Nearly 0.1–0.5% of inhaled O2 is converted to superoxide (O−2) during the normal physiological states (Servaiset al., 2009).

In the normal healthy state, the generation and oxidation of ROS occur in a controlled manner. By contrast, the ROS production is increased under high-stress conditions or under disease states. The ROS generated from aerobic respiration caused a cumulative oxidative damage in macromolecules, including lipids, DNA, and proteins, which subsequently lead to cells death (Scheibye-Knudsen et al., 2015), and affect the health span of numerous principal organ systems.

An alteration of the redox status and the dysregulation of the immune system during aging may lead to the elevation of systemic inflammatory status. Both of these processes caused the activation of inflammatory mediators via oxidative stress-induced redox imbalance. The age-related redox imbalance is more likely triggered by the net effect of low anti-oxidative defense systems and incessantly produce of reactive species, including superoxide (O−2), hydroxyl radical (OH), peroxynitrite (ONOO−), hydrogen peroxide (H2O2), reactive lipid aldehydes, and reactive nitric oxide (NO) (Lennickeet al., 2015).

Unresolved chronic inflammation during aging may serve as a pathophysiologic association which converts normal functional changes to the age-related degenerative diseases (Viola and Soehnlein, 2015). Oxidative stress is reinforced by several reactive species, including H2O2, singlet oxygen, other radicals, and non-radicals, which are consistently produced in the body due to the aerobic metabolism, and thereby potentially altering basic structural components such as proteins, lipids, and nucleic acids (Weidinger and Kozlov, 2015).

Template biosynthesis of polypeptide chains on ribosomes usually does not produce a functional protein. The newly developed polypeptide chain must undergo certain chemical modifications outside the ribosome. Thus, these modifications are most often accompanied by enzymes and take place after all the information supplied by the template RNA (mRNA) has been read, that is after mRNA translation. These additional processes are known as posttranslational modifications. There are four primary groups of protein functions which require posttranslational modification of amino acid residue side chains. The functional activity of several proteins requires the presence of certain prosthetic groups covalently bound to the polypeptide chain.

These are usually involving complex organic molecules which take part in the protein activity for instance, the transformation of inactive Apo proteins into enzymes. Another important group of modifications is protein tags, which provide intracellular localization of proteins such as marking the proteins for transport to the proteasome, where they will be proteolyzed and hydrolyzed. Additionally, some of the posttranslational modifications regulate biochemical processes by varying enzymatic activity (Weidinger and Kozlov, 2015).

Naturally, the organism has several antioxidant defenses to protect against hostile oxidative environments, including classical antioxidant enzymes for example catalase, glutathione peroxidase, and superoxide dismutase as well as non-enzymatic ROS scavengers, such as β-carotene, vitamin C, vitamin E, and uric acid (Espinosa-Diezet al., 2015; Harris et al., 2015). Among all the antioxidant enzymes, glutathione peroxidase is the most powerful biological anti-oxidative reductant.

Collectively, maintaining a healthy redox balance status is crucial for the physiological acid-base buffer system in the body for the optimal homeostatic cellular activities. Changing in redox balance would have a great impact on the transcriptional activities and cellular signaling pathways because most of the activation and reactions is dependent on the reduction/oxidation processes.

Acetaminophen (APAP, Paracetamol) is one of the most popular drugs in the world, which was first introduced and used in 1893 by Von Mering to treat pain and fever. The effect of the drug starts about 12 minutes after its oral administration. Due to high drug administration, poisoning with this medicine is one of the most common cases ofpoisoning in the world starting with symptoms such as malaise, vomiting, diarrhea, and sometimes shock.Also, in excessive consumption it can lead to liver necrosis and jaundice in animals and humans. In some cases, it has been observed to cause simultaneous myocardial and renal damages (kumar, 2005).

Although acetaminophen is a safe drug, andthe distance between a therapeutic dose (0.5 g) and its toxic dose (15-25 g) is relatively high and is associated with little risk for its consumers, severe liver toxicity from acetaminophen poisoning often lead to acute liver failure (ALF). Also, studies have found that liver tissue necrosis would occur following the consumption of excessive amounts of acetaminophen as an analgesic and antipyretic drug (Lee, 2012). In individuals receiving toxic doses of acetaminophen, extensive necrosis of liver cells is observed after 24 hours and reaches its maximum in the following 3-4 days (khorsandi, 2010).

It was found through experimental studies in animals that cytochrome P450 inhibitors can inhibit hepatotoxicity caused by acetaminophen, and cytochrome P450 inducers may escalate it .Nowadays, heart disease and diabetes provide the first and second leading cause of death in the world, respectively and people are suffering from these diseases are likely to be more prone to kidney disease. There is great urgency for a nonconventional, affordable therapy for patients who cannot afford expensive dialysis or kidney transplant to keep them alive.

Recent research work of this same laboratory undertaken on Nutraceutical like alpha lipoicacid and probiotic (Mandalet al., 2013) therapy have shown excellent nephroprotective activity against acetaminophen induced renal failed male rats. Acetaminophen is used to induce uremia and renal failure in our laboratory. Acetaminophen is a commonly used antipyretic agent which, in high doses, causes renal tubular damage and uremia. An acute acetaminophen (paracetamol, N-acetyl p-aminophenol; APAP) overdose may result in a potentially fatal hepatic and renal necrosis in humans and experimental animals.

Recently, natural plants like Vernonia amygdalina have been reported that it contains some antioxidant properties. which are associated with its chemical constituents. Several researchers have previously established that the antioxidant activities of fruits and vegetables like Vernonia amygdalina are related to their phenolic compounds. Natural polyphenols scavenges free radicals, chelates metal catalysts, activates antioxidant enzymes, reduces α-tocopherol radicals and inhibits oxidases, all of which have chain-breaking antioxidant activities and contributing to the prevention of degenerative diseases, cancer and atherosclerosis (Roginsky, 2003). He established that the (DPPH) diphenyl-picryhydrazyl radical scavenging activities of the leaves of Vernonia amygdalina fractions were significantly higher than that of Glutathione.

Nowadays, heart disease and diabetes provide the first and second leading cause of death in the world, respectively and people are suffering from these diseases are likely to be more prone to kidney disease. There is great urgency for a nonconventional, affordable therapy for patients who cannot afford expensive dialysis or kidney transplant to keep them alive (Roy et al., 2013).The aqueous and alcoholic crude extracts of the leaves, bark, stem and roots are reported to be widely used as anti-malarial, for the treatment of eczema and as a purgative (Masaba, 2000) . The roots and the leaves of Vernonia amygdalina are used in traditional medicine to treat fever, stomach discomfort, hiccups and kidney problems (Challand, 2009). It is known as quinine substitute because it is widely used for the treatment of fevers. The wood, particularly those from the root is a tooth cleaner, an appetizer, fertility inducer and also for gastrointestinal upset (Challand, 2009).

The root infusion is taken in Nigeria for the treatment of intestinal worms as well as for enteritis and rheumatism. Wild Chimpanzees have been observed to eat both the leaves and stems of the plant as a medication for self-deparasitization. Other documented medicinal uses include the treatment of schistosomiasis, amoebic dysentery, treatment of malaria, wound healing, venereal diseases, hepatitis and diabetes. Fresh leaves of Vernonia amygdalina have been reported to have abortifacient and purgative activities. It is used in some part of Africa to prepare cough remedy. The

chopped roots of Vernonia amygdalina are used for the treatment of sexually transmitted diseases in parts of Zimbabwe. The root of Vernonia amygdalina is used for its antifertility effect and for the treatment of amenorrhea.

MATERIALS AND METHODS

Study Area

The study was carried out at the Department of Biological Sciences, Federal Polytechnic, Ado- Ekiti, Nigeria.

Experimental Design

Twenty five (25) Wistar albino rats were randomly divided into five (5) groups with each group consisting of five rats. The rats were allowed to acclimatize to the environment for 2 weeks.

At the onset of the experiment, the Rats were treated as shown below:

- Group 1: Normal control (NC) - Distilled water only for 14 days (No treatment)
- Group 2: Induced untreated group (Positive Control) - 3000mg/kg acetaminophen alone for a single administration
- Group 3: 3000mg/kg acetaminophen + 50 mg/kg Vernonia amygdalina extract for 14 days
- Group 4: 3000mg/kg acetaminophen + 100 mg/kg Vernonia amygdalina extract for 14 days
- Group 5: 3000mg/kg acetaminophen + 200 mg/kg Silymarin (liver damage treatment drug) for 14 days

This can be calculated as:

- Group I Normal control (No treatment)
- Group II were given 2.40g of paracetamol dissolved in 10ml of distilled water
- Group III rats were given 2.73g of paracetamol dissolved in 10ml of distilled water
- Group IV rats were given 3.91g of paracetamol dissolved in 10ml of distilled water
- Group V rats were given 3.53g of paracetamol dissolved in 10ml of distilled water

After three days of injection of paracetamol one rat per each group were anaesthetized with chloroform and was dissect for confirmation of the liver damage. After the liver had been confirmed damaged; three groups were subjected to the following oral treatments once a day for 14days and remaining one group was used as paracetamol control i.e.

- Group II - paracetamol control
- Group III rats received 50mg/kg BW of bitter leaf (Vernonia amygdalina)
- Group IV rats received 100mg/kg BW of bitter leaf (Vernonia amygdalina)
- Group V rats received 200.mg/kg BW of Silymarin Drug day on 14th day.

Experimental Procedure

Plant Materials

Samples of Bitter leaf (*Vernonia amygdalina*) were gotten from a private farm in Ado Ekiti, air dried in the laboratory, pulverized and then stored in an airtight container.

Reagents and Chemicals

All reagents and chemicals were all of analytical grade.

Preparation of Plant Extract

Bitter leaves (*Vernonia amygdalina*) were air-dried for 30 days at room temperature. The air-dried samples were ground to fine powder using a blender. 500 g of the powdered leaves was soaked in 2000 ml of distilled water for 72 hours. It was then filtered using a cheese cloth, and freeze-dried to obtain the dried extract. The extract was kept in a closed container and kept inside the fridge at 4^0C for further studies.

Animals Protocol

Twenty five male Wistar albino rats weighing 150 kg –170 kg were gotten from a reputable farm in Ado Ekiti and housed in Animal Production unit, Department of Agricultural Technology, The Federal Polytechnic, Ado-Ekiti for research purpose. They were housed in clean wire meshed cages under standard conditions temperature (24 ± 1^oC), relative humidity, and 12 / 12-hour light and dark cycle. They were allowed to have free access to feed (commercial palletized diet from Vital Feed Mill) and drinking water *ad libitum* daily. The rat beddings were changed and replaced every day throughout the experimental period.

Measurement of the Weight of the Animals

The weights of the animals were taken using weighing scale. The results obtained were used to determine the volume of the sample to be administered to each of the animal.

Animal Treatment/Administration

The treatment samples were administered to the animals using 2ml syringe. The animals in group I (the control) were given distilled water for fourteen days; II were given Paracetamol; the animal in groups III, IV and V were given 50, 100, and 200mg/kg Silymarin according to their body weights respectively.

CHEMICAL ANALSIS

Biochemical Estimations

At the end of the treatment period, rats were sacrificed under light ether anesthesia and blood collected via the ocular vein without the use of anticoagulant. The blood was allowed to stand for 10 min before being centrifuged at 2,000 rpm for 10 min to obtain serum for analysis.

The levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were tested. The rats were dissected, the liver removed and placed in iced beakers. A 1g portion of the liver was used to prepare homogenate of the liver (10%) in ice cold KCl solution using Teflon homogenizer.The homogenate was centrifuged at 4000g for 10 min to remove debris. The supernatant was used for the total antioxidant status - TAS (Randox, Ransod Ltd, UK).

The liver samples were removed from sacrificed rats and placed in ice-cold 0.15M KCl solution in a beaker embedded in salinated ice. They were rinsed thoroughly in the saline solution and excess fluid blotted out with a paper towel before weighing the samples in chilled containers. One gram portion of the liver was homogenized in 10ml of burv.50micropipet and 250micropipet (0.5ml) was add to each sample. The lightly-stopper tube was warmed for 30min at 37^{o}C in a boiling water bath, 1.6g of sodium hydro-oxide was dissolved in 100ml of distinct water and 50micropipet was added to each sample 0.5ml. All measurements were done using Spectronic 21 digital spectrophotometer.

Enzyme Assays

Alkaline phosphatase (EC 3.1.3.1) activity was assayed using the method described by Babson, A.L; (1966). The procedure as described by Reitman and Frankel, S (1957) was employed for the assay of aspartate aminotransferase (AST) (EC 2.6.1.1) and alanine aminotransferase (ALT) (EC 2.6.1.2). Was determined using Biuret reagent as described by Plummer 11. All measurements were done using Spectronic 21 digital spectrophotometer.

Dissection of Rats

The rats were dissected and portion of blood was collected in EDTA sample bottles and allowed to stand for 1 hour. Serum was prepared by centrifugation at 3000 rpm for 15 min at 25^{o}C. The clear supernatant was collected and used for the estimation of serum biochemical parameters.

Preparation of Homogenates

The liver was excised using scissors and forceps, washed in distilled water, blotted with filter paper and weighed. It was then chopped into bits and homogenized in ten volumes of the homogenizing phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates was centrifuged at 3000 rpm at 4°C for 30 mins. The supernatant obtained was collected and stored under 4^{0}C and then used for biochemical analyses.

Determination of Biomarkers

Assay of Aspartate Aminotransferase (AST) Activity

AST activity was determined following the principle described by Reitman and Frankel (2014).

Principle

α-Oxoglutarate + L-aspartate AST $\longrightarrow$ L. glutamate + Oxaloacetate

Aspartate aminotransferase was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Procedure

Briefly, 0.1 ml of diluted sample of (serum, pancreas, liver and kidney) was mixed with phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (100 mmol/L), and α-oxoglutarate (2 mmol/L) and the mixture incubated for exactly 30 min at 37^{o}C. 0.5ml of 2, 4-dinitrphenylhydrazine (2mmol/L) was added to the reaction mixture and allowed to stand for exactly 20 min at 25^{0}C. Then 5.0 ml of NaOH (0.4 mol/L) was added and the absorbance read at 546 nm against the reagent blank after 5 min.

Assay of Alanine Amino Transferase (ALT) Activity

The principle described by Reitman and Frankel (2014) was followed in the assay of ALT using commercially available assay kit (Randox laboratories, UK) according to the instructions of the manufacturer.

Principle of Reaction

Alanine amino transferase catalyzes the transfer of L-amino groups from L-alanine to α-oxoglutarate, a reaction which produces L-glutamate and pyruvate. The unstable pyruvate is then complexed with 2, 4-dinitrophenylhydrazine (DNPH) to produce an intensely colored hydrazone on the addition of NaOH. This colored complex absorbs radiation at 530 nm -550 nm.

Assay Procedure

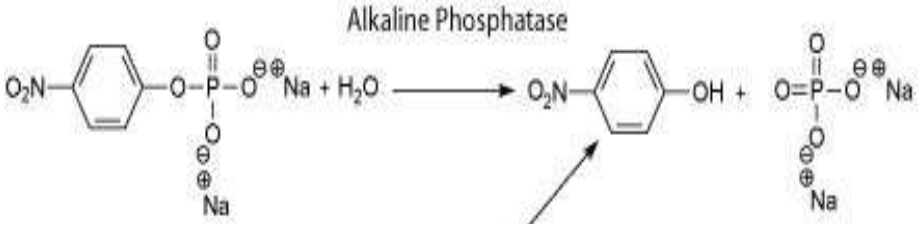
Reagent 1 (0.5 ml) containing phosphate buffer (100 mmol/l, pH 7.4), L-alanine (200 mmol/l) and α-oxoglutarate (2.0 mol/l) was added to 0.1ml of serum in a test tube and the mixture was incubated at 37^{o}C for 30 minutes. Exactly 0.5 ml of R2 containing 2, 4-dinitrophenylhydrazine (2.0 mmol/l) was added and the solution incubated again at 20^{o}C for 20 min. Finally, 5 ml of NaOH was added and the solution was allowed to stand for 5 minutes at room temperature and the absorbance was read at 546 nm.

Calculation

The activity of ALT in the serum was obtained from the standard curve provided in the kit.

Assay of Alkaline Phosphatase (ALP) Activity

Assay of serum ALP was based on the method of (Englehardt *et al*., 1970) using commercial assay kits (Randox laboratories, UK) according to the instructions of the manufacturer.



P-nitrophenylphosphatewater, p-nitrophenol phosphate

Figure 1: Reaction Catalyzed by Alkaline Phosphatase.

ALP activity was measured by monitoring the concentration of p-nitrophenol formed when ALP reacted with p-nitrophenyl phosphate.

Assay Procedure

Exactly 1.0 ml of the reagent (1 mol/l diethanolamine buffer pH 9.8, 0.5 mmol/l $MgCl_2$; substrate: 10 mmol/l p-nitrophenol phosphate) was added to 0.02 ml of the serum sample and mixed. The absorbance was taken at 405 nm for 3 minutes at intervals of 1 minute.

Calculation

ALP activity was determined using the formula U/l = 2760 x A405 nm/min

Statistical Analysis

All values are expressed as mean ± SD. Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using SPSS 11.09 for windows (Anthony and Richard, 2006). The significance level was set at $p < 0.05$.

RESULTS

Tables 1 showed the effects of Bitter leaf (*Vernonia amygdalina*) extract on the liver Biomarker Enzymes in acetaminophen-induce hepatotoxicity in albino rats respectively.

Table 1: Effects of Bitter Leaf (Vernonia amygdalina) Extract on the Liver Biomarker Enzymes and Molecules in Acetaminophen Induced-Hepatotoxicity in Albino Rats

Parameters	Positive Control	Negative Control Acetaminophen (3000mg/kg)	Acetaminophen + *Vernonia amygdalina* (50mg/kg)	Acetaminophen + *Vernonia amygdalina* (100mg/kg)	Acetaminophen + Silymarin (200mg/kg)
AST	95.52±1.20	133.83±1.13^{a}	117.31±2.98^{b}	102.68±3.42^{c}	99.07±2.05
ALT	76.44±0.94	118.81±7.56^{a}	94.33±0.39^{b}	80.78±0.58^{c}	82.47±0.97
ALP	60.26±1.28	91.07±1.08^{a}	82.68±1.56^{b}	67.33±1.06^{c}	65.17±1.47
Bil	43.22±0.67	76.34±0.92^{a}	57.14±1.92^{b}	48.91±2.06^{x}	48.05±0.87

Valves with the same Superscripts in Row Differ Significantly ($p \leq 0.05$)

AST-Aspartate aminotransferase; ALT-Alanine aminotransferase;ALP-Alkaline phosphates Bil-Bilirubin

Table 2: Effects of Bitter Leaf (Vernonia amygdalina) Extract on the Kidney Biomarker Enzymes in Acetaminophen Induced-Hepatotoxicity in Albino Rats

Parameters	Positive Control	Negative Control Acetaminophen (3000mg/kg)	Acetaminophen + *Vernonia amygdalina* (50mg/kg)	Acetaminophen + *Vernonia amygdalina* (100mg/kg)	Acetaminophen + Silymarin (200mg/kg)
AST	45.37±0.29	72.96±0.33^{a}	64.02±0.98^{b}	51.32±1.20^{c}	49.29±0.21
ALT	39.08±0.22	57.34±1.02^{a}	48.13±0.56^{b}	42.43±1.02^{c}	36.37±0.48
ALP	27.25±0.82	41.61±0.50^{a}	36.22±0.40^{b}	29.15±0.47^{c}	32.13±0.37

Valves with the same Superscripts in Row Differ Significantly ($p \leq 0.05$)

AST- Aspartate aminotransferase; ALT--Alanine aminotransferase; ALP- Alkaline phosphates

Table 3: Effects of Bitter Leaf (Vernonia amygdalina) Extract on the Serum Biomarker Enzymes in Acetaminophen Induced - Hepatotoxicity in Albino Rats

Parameters	Positive Control	Negative Control Acetaminophen (3000mg/kg)	Acetaminophen + *Vernonia amygdalina* (50mg/kg)	Acetaminophen + *Vernonia amygdalina* (100mg/kg)	Acetaminophen + Silymarin (200mg/kg)
AST	45.37±0.29	72.96±0.33^{a}	64.02±0.98^{b}	51.32±1.20^{c}	49.29±0.21
ALT	39.08±0.22	57.34±1.02^{a}	48.13±0.56^{b}	42.43±1.02^{c}	36.37±0.48
ALP	27.25±0.82	41.61±0.50^{a}	36.22±0.40^{b}	29.15±0.47^{c}	32.13±0.37

Values with the same Superscripts in Row Differ Significantly ($p \leq 0.05$)

AST- Aspartate aminotransferase; ALT- Alanine aminotransferase; ALP- Alkaline phosphate

The liver is our greatest chemical factory, it builds complex molecules from simple substances absorbed from the digestive tract, it neutralizes toxins, it manufactures bile which aids fat digestion and removes toxins through the bowels (Kim *et al.*, 2014).Liver disease is worldwide problem. Conventional, drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain Table according to Olorunisola*et al.*(2012).

The hepatotoxins produced a wide variety of clinical and histopathological indicators of hepatic injury. In this study, it was observedthat Alanine aminotransferase (ALT) (57.34%) was less than the findings of Mandade, (2011) who reported (85.54%) and Hilscher*et al.* (2016) of (95.55%)ALT. Aspartate aminotransferase (AST) (72.96%) was also in agreement with Jude *et al.*(2016)who reported (78.55%) while alkaline phosphatase (ALP) (41.61%) was lesser than the findings of the Mandade (2011) who found (65.34%) ALP. Elevations in serum enzyme levels are taken as the relevant indicators of liver toxicity. An elevation in transaminase levels is considered as an ominous marker for hepatotoxicity (Jude *et al*., 2016).

An alanine aminotransferase (ALT) test measured the amount of this enzyme in the blood. ALT is found mainly in the liver, but also in smaller amount in the kidney, heart, muscles, and pancreases. ALT was measured to see if the liver is damaged or diseased. Low levels of ALT are normally found in the blood. But when the liver is damaged or diseased, it releases ALT into the blood stream. Both ALT and AST levels are reliable test for liver damage e.g. hepatitis, Cirrhosis (Mandade, 2011).

In human, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone, and the placenta. A noticeable change in the biomarker enzyme activities such as (Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) of the albino rats treated with acetaminophen were observed. Induction of acetaminophen caused significant ($P<0.05$) increase in the activities of AST, ALT and ALP when compared to the control. This finding is in agreement with Mandade (2011) who reported changes in the biomarker enzyme activities in his study.

Oral administration of the extract of Bitter leaf (*Vernonia amygdalina*) reduced the activities of AST, ALT and ALP in rats **(Table 1, 2 and 3)** when compared to the untreated group (positive control). This is an indication that the extract might prevent liver damage by maintaining the integrity of the plasma membrane and suppressing the leakage of the enzymes through the membrane. The significant ($P<0.05$) reduction in the activity of AST, ALT and ALP by Silymarin, and Bitter leaf (*Vernonia amygdalina*) extract treatment may be correlated to their hepatoprotective effects.

CONCLUSIONS

Medicinal plants are used in several countries to manage liver damage and thought to be less toxic than the synthetic drugs. Phyto-medicines are also easily available and affordable to many people. The Bitter leaf (*Vernonia amygdalina*) extracts may not have toxic effect on the liver at the employed dosage, seen in the lowered concentration of AST, ALT and ALP as biochemical enzymes markers of liver damage. Based on these findings, the results thereby lend credence to the ethno-medicinal use of the extract in the management of liver damage at evaluated dosages and their uses should be encouraged.

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