RESEARCH ARTICLE

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HISTOMORPHOLOGICAL COMPARATIVE STUDIES OF LIVOLIN FORTE AND VERNONIA AMGDALINA ON ETHANOL INDUCED HEPATOTOXICITY IN ADULT WISTAR RAT.

Olatoye Adeniyi Michael[1], Olayemi Olaniyi Taiwo2, Olatoye Adesola Adenike3

Department of Anatomy and Cell Biology, Obafemi Awolowo University Ile Ife, Osun-State, Nigeria.

Anatomy Department, Faculty of Basic Medical Sciences PMB 4000, Lautech Ogbomoso, Oyo State, Nigeria.

Department of Nursing Sciences Seventh-day Adventist College Lagere, Ile Ife, Osun State, Nigeria.

ARTICLE ABSTRACT

Introduction: Ethanol is one of the hepatotoxic substances that cause various hepato-degenerative disorders which is a major concern in medical science and researches as man is regularly exposed to it via consumption.

Methods: The aim of this study is to investigate and compare the protective effects of livolin forte with methanolic extract of *vernonia Amgdalina* on the hepatic cells of ethanol induced hepatotoxicity, 25 healthy male wistar rats weight ranging between 150-200g were allotted into 5 groups: 4 experimental groups (B,C,D and E) of five animals each and group A served as the control group received standard rat pellet and water *ad libitum* throughout; The experimental group B,C,D, and E were per orally administered with 60% ethanol/ Kg, 60% ethanol/ kg b.w + 30mg/kg of liovlin+ 400mg/kg extract of *Vernonia Amgdalina*, and 60% ethanol/kg + 400mg/kg b.w respectively throughout the period of administration:

Results: The macroscopic change such as nodular and abscesses were observed on the liver of experimental animals (B,C,D and D) with regenerating changes in groups C,D and E. while the liver architecture for the control animals appeal normal. Toxicant group B had significant higher AST, ALT and ALP compared with the normal control group A. Similar, significant increase in serum activities of AST, ALT and ALP were observed in group D&E compare to group C.

Conclusion: The result of this study indicated that livolin forte was better combine with *vernonia amgdalina* as a curative agent rather than livolin forte or *vernonia amgdalina* alone in rats model.

KEYWORDS; hepatotoxicity, Livolin forte, *Vernonia amgdalina*, Ethanol, Alanine-aminotransferase(ALT), Aspartate aminotransferase(AST), Alkaline- phosphates(ALP), liver, wistar rat.

INTRODUCTION

In 1989, a panel of 12 European and American expert by consensus defined liver hepatotoxicity as an increase of more than twice the upper limit of the normal range in the level of serum alanine aminotransferase or conjugate bilirubin or a combined increase in the level of aspartate aminotransferase, alkaline phosphatase and total bilirubin, provided that one of these was more than twice the upper limit of the normal range [Benson *et al*., 2001]. The clinical patterns of liver injury were further characterized as hepatocellular, with a predominant initial elevation of the alanine aminotransferase level, or cholestactic, in which the serum alkaline phosphatase level is first elevated. It was later suggested, before a February 2001 conference cosponsored by the FDA (Federal Drug Agency) center for drug evaluation and research, the pharmaceutical research and manufacturers of American Association for study of liver Diseases, that an alanine aminotransferase level of more than three times the upper limit of normal and a total bilirubin level of more than twice the upper limit be used as a combined tests to define clinically significant abnormalities on liver tests, with further verification through the analysis of additional clinical data. Elevations in serum enzyme level (alanine-aminotransferase, aspartate aminotransferase and alkaline phosphate) were taken as indicator of liver injury, whereas increase in both total and conjugated bilirubin level were measure of overall liver functions. It is important to recognize the pattern of liver injury, since certain drugs tend to create injury predominantly according to one pattern or the other [Bilzer *et al*., 1995]. The concept of combining the measures of liver injury and function was derived from the observation of the late Hyman Zimmerman that ”drug–induced hepatocellular jaundice is a serious lesion, the mortality rate ranges from 10-50 percent”. This observation, referred to by Dr. Robert Temple as “Hy’s law” has shown notable consistency, and it’s continue to be used by the Federal Drug Agency (FDA) to initiate close evaluation of patients with elevated liver tests. Two recent surveys, from Sweden and Spain, provide support for the observation that drug- induced hepatocellular injury with jaundice is associated with greater mortality or the need for transplantation. [Baranisrinivasan *et al*., 2009]. However,

in each case, additional clinical information is required to determine whether the elevated values were drug-induce or disease-induced. Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug or another noninfectious agent. The human body identifies almost all drugs as foreign substances (i.e xenobiolism) and subjects to various chemical processes (i.e metabolism) to make them suitable for elimination. This involves chemical transformations to (a) reduce fat solubility and (b) to change biological activity. Although almost all tissue in the body have some ability to metabolize chemicals, smooth endoplasmic reticulum in the liver is the principal “metabolic clearing house” for both endogenous chemicals (e.g cholesterol, steroid hormone, fatty acids and protein) drug and ethanol [Mitchell *et al.*, 1973]. The central role played by the liver in the clearance and transformation of chemicals also makes it susceptible to drug induced injury [Cohen *et al.*, 1997]. Drug metabolism is usually divided into two phases: phase 1 and phase 2. Phase one reaction is thought to prepare a drug for phase two. However many compounds can be metabolized by phase two directly. Phase one re-action involves oxidation, reduction, hydrolysis and hydration and many other rare chemical reactions. These processes tend to increase water solubility of the drug and can generate metabolites which are more chemically active and potentially toxic [Bai *et al.,* 2001]. Most of phase 2 reaction takes place in cytosol and involve conjugation with endogenous compounds via transferase enzymes. Chemically active phase 1 products are rendered relatively inert and suitable for elimination by this step. A group of enzymes located in the endoplasmic reticulum known as cytochrome p-450, is the most important family of metabolizing enzymes in the liver [Haouzi *et al.*, 2000]. Cytochrome p-450 is the terminal oxidase component of an electron transport chain. It is not a single enzyme, rather consist of a family of closely related 50-isoforms, 6 of them metabolize 90% of drugs [Haouzi *et al.*, 2000]. There is a tremendous diversity of individual P-450 gene product and this heterogenetic allows the liver to perform oxidation on a vast array of chemicals (including almost all drugs) in phase 1.

Drugs continue to be taken off the market due to late discovery of hepatotoxicity. 75% of blood coming from the liver arrives directly from gastrointestinal organs and spleen via portal vein which brings drugs and xenobiotics in concentrated form [Hinson *et al.*, 2000]. Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. It dysfunction releases excessive amounts of oxidant which injures hepatics cells. Activations of some enzymes in the cytochrome P-450 system such as CYP2E1 also leads to oxidative stress [Dianzani *et al.*, 1994].

Injury to hepatocyte leads to accumulation of bile acids inside the liver, all these promote further liver damage. Non- parenchyma cells such as kupffer cells, fat storing stealate cells and leucocyte (i.e neutrophil and monocyte) also have roles in the mechanism [Chen *et al.*, 1998].

Ethanol

Ethanol has been a part of the human diet for centuries. However, its consumption in excess can result in a number of health problems, most notably liver damaged. Ethanol cannot be excreted and must be metabolized, primarily by the liver. This metabolism occurs by two part-ways. The first partway comprises of two steps, the first step catalyzed by the enzyme alcohol dehydrogenase, takes place in the cytoplasm. The second step, catalyzed by aldehyde dehydrogenase takes place in the mitochondria: Note that ethanol consumption leads to an accumulation of NADH. This high concentration of NADH inhibits gluconeogenesis by preventing the oxidation of lactate to pyruvate [Becman *et al.*, 1996]. In fact, the high concentration of NADH we cause the reverse reaction to predominate and lactate will accumulate. The consequences may be hypoglycemia and lactic acidosis. The NADH glutamate also inhibits fatty acid oxidation, the metabolic purpose of fatty acid oxidation is to generate NADH for ATP generation by oxidative phosphorylation, but an alcohol consumer's NADH needs are met by ethanol metabolism. In fact, the excess NADH signals that conditions are right for fatty acid synthesis [Brook *et al.*, 1986]. Hence, triacylglycerols accumulates in the liver leading to a condition known as 'fatty liver' the second path way for ethanol metabolism is called the ethanol inducible microsomal ethanol oxidizing system (MEOS). This cytochrome P-450 dependent pathway generate acetaldehyde and subsequently acetate while oxidizing biosynthetic reducing power, NADPH, to NADP because it uses oxygen, this path way generate radicals that damaged tissues. Moreover, because the system consumes NADPH, the antioxidant glutathione cannot be generated exacerbating the oxidative stress [Botla *et al.*, 1995]. Liver mitochondria can convert acetate into acetyl CoA in a reaction requiring ATP. The enzyme is the thiokinase that normally activate short chain fatty acid, however further processing of acetyl CoA by the citric acid circle is blocked because NADH inhibits two important regulatory enzymes-isocitrate dehydrogenase and ketoglutarate dehydrogenase. The accumulation of acetyl CoA has several consequences. First, ketone body will be formed and be realized in the blood, exacerbating the acidic condition already resulting from the high lactate concentration [Bradham *et al.*, 1998]. The processing of the acetate in the liver becomes inefficient, leading to a buildup of acetaldehyde. This very reactive compound form covalent bonds with many important functional groups in proteins, impairing protein function. If ethanol is consistently consumed at high levels, the acetaldehyde can significantly damage the liver, eventually leading to cell death. [Michael *et al.*, 2001] Liver damage from excessive ethanol consumption occurs in three stages. First stage is the development of fatty

liver. In the second stage – alcohol hepatis- groups of cell death and inflammation results. This stage can itself be fatal. In stage three cirrhosis– fibrous structure and scar tissue are produced around the dead cells. Cirrhotics impairs many of the liver's biochemical functions. The cirrhotic liver is unable to convert ammonia into urea, and blood levels of ammonia rise. Ammonia is toxic to the nervous system and can cause coma and death. Cirrhosis of the liver arises in about 25% of alcoholics, and about 75% of all cases of liver cirrhosis are the result of alcoholism (Morimoto *et al*., 1994).

Vernonia amdalina

Medicinal plants have been used for years immemorial before the advent of modern medicine. The use of herbs in treating certain diseases is now given a little more concentration than before, this is because they are cheaper in cost, and they have a saver alternative source of antimicrobials [Morton *et al*,. 1987]. According to world health organization, a medicinal plants is any plant in which one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drugs. According to [Nwafor *et al*., 1995] most of the world's medicinal plants are located in the tropics where about two third (2/3) of all plants species are found. Out of which about 12-14 percent are estimated to have medicinal value. Plants as sources of raw materials for developing new drugs has been increasing in interest, this is confirmed by the fact that approximately 119 pure chemical substances extracted from higher plants are used in medicine throughout the world, there is an increasing consumer preference for plants -derived medicine [Abatan *et al*., 1997] it is a well-known fact that as many as 80% of the world's population depend on the traditional medicines for their primary health care (WHO, 2000). In this regards traditional medicines may be said to have been source of inspiration to the world in general. This is sequence to the fact that medicines derived from plants have made large contributions to human health and well-being [Iwu *et al*., 1993] According to Morton [2007], the reason for this prevalence is due to the facts that traditional medicine has remained the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities. Consequently, the report that the use of natural plant products for therapeutic purposes, is growing and largely embraced by the general population [Osifo *et al*,. 2011] and thus, the shift of attention from synthetic drugs to natural plant products reported by [Ikuerowo *et al*., 2009]. According to available literature, every drug is associated with hepatotoxicity almost certainly due to its ability to generate free radicals and to cause disturbance in hepatocytes biochemistry [Fernandez *et al* 2005]. Hence, *Vernonia amgdalina* like other drugs are metabolized by the liver and if this be the case, *Vernonia amgdalina* promotes fat combustion, restricts fat accumulation in the liver by increasing adiponectin receptor gene expressions and improves insulin resistance. Because there are few side effects of long term treatment of hepatotoxicity using herbal remedy comparing to modern medicine which has lot of side effects, whereas Liver diseases are a major global concern and this type of disease has extremely poor

prognosis and high mortality because of the lack of effective treatment options [Morton *et al*,. 1987]. Despite the remarkable advances in the field of Medicine, hepatic diseases remains a major public health problem, thus the search for new effective medicines without side effects is still ongoing, Hence the need for this research work.

Materials and Methods

25 health wistar rats of the weight ranging between 150-200g were randomly allotted into five groups; 5 animals in a group (n=5). The experimental animals were cared for and they were acclimatized in the animal housing of the department of Anatomy, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology Ogbomoso for two weeks. The animals were maintained on standard laboratory rat pellet and were given water *ad libitum* bought from Bova Feed Limited, Orita naira, Ogbomoso. The rats were kept under an ambient temperature between 28-30c throughout the experimental period. The pelletized feed consists of the following: Oyster shell, Fish meal, Maize, Yeast, Brewers, wheat meal, Died grains, Groundnuts cake and salt. The aggregate ingredient of the wistar rat pellet supplies about 21% Protein, 4.5% Fat, 6.0% fibre, 0.8% Phosphorus and 0.8% Calcium. The 25 healthy male adult wistar rats of the weight ranging between 150- 200g were allotted into five groups: Normal Control (**Group A**), Ethanol Control (**Group B**), Combine Therapy Control (**Group C**), Livolin Forte Control (**Group D)**, and *Vernonia amgdalina* Control (**Group E**) of five animals each. All Animals were weighted a day before the administrations of drugs and a day after the last administration of drugs.

Acute toxicity (LD_{50}) study

The acute oral toxicity study showed that the extract is absolutely save with **LD50** 1000mg/kg/oral route (Ijeh et al., 2011)

Experimental design

Group A: (Consist of five male wistar rats) received distilled water p.o throughout the experimental period. This is the normal control group. **Group B**: (Consist of five male adult wistar rats) received 60% ethanol /kg body weight p.o for 21 days without treatment. This is the negative control group. **Group C** (Consist of five male adult wistar rats) received 60% ethanol /kg p.o for 21 days followed by 400mg/kg p.o doses of Methanolic extract of *Vernonia amdalina* and 30mg/kg of standard livolin forte p.o respectively, this serves as the combine therapy group. **Group D** (Consist of five male adult wistar rats) received 60% ethanol/kg for 21 days followed by 30mg/kg livolin forte p.o for 21 days, this group served as the livolin control group. **Group E** (consist of five adult wistar rats) received kg of 60% ethanol/kg p.o for 21 days, followed by 400mg/kg doses of *Vernonia amgdalina* p.o, this serves as the methanolic extract of *Vernonia amgdalina* control group.

Methanolic Extract *Vernonia amgdalina*

The Sharma 2011 was adopted, the plant leaves gotten in March, 2016 from Adenike area of Ladoke Akintola University of Technology Campus, Ogbomoso, oyo state. These leaves were identified in the botany department

located around Ladoke Akintola University Agric. Farm, and later taken to the department of biology for authenticity. The leaves of Vernonia amgdalina were air dried for three weeks blended with blending machine and sieved into powder form. The 300g of the *Vernonia amgdalina* powder were dissolved and soaked in 1000mls of 60% methanol for 72 hours with constant stirring and latter sieved and the filtrate was concentrated to dryness in a vacuum rotary evaporator at 40-50 c after which 400mg of its was processed twice and the fractions were placed in a freezer, and used for the experimental purposes. All the experimental animals were sacrificed 24 hours after the last administration, through cervical dislocation. They were then dissected and quickly fixed in 10% formal saline for histological studies. Digital photomicrography of the liver sections were obtained to show the morphological changes that occurred in the treatment.

Biochemical analysis

Levels of aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), alkaline phosphatase (**ALP**) were determined.

Histological & Histochemical analysis

Harvested Liver tissue was fixed in 10% formal saline subjected to histological and histochemical procedures.

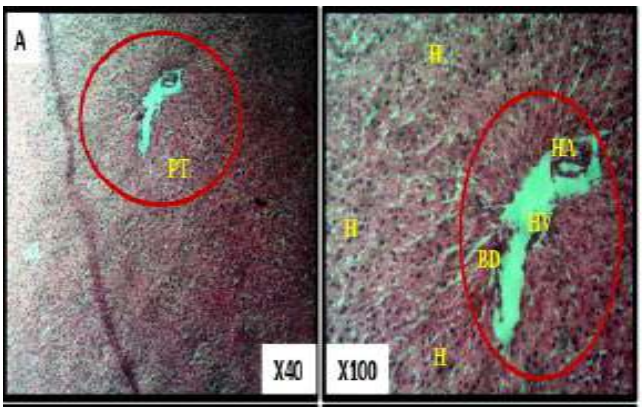
General histoarchitecture studies was demonstrated using haematoxylin and eosin stain.

Statistical analysis

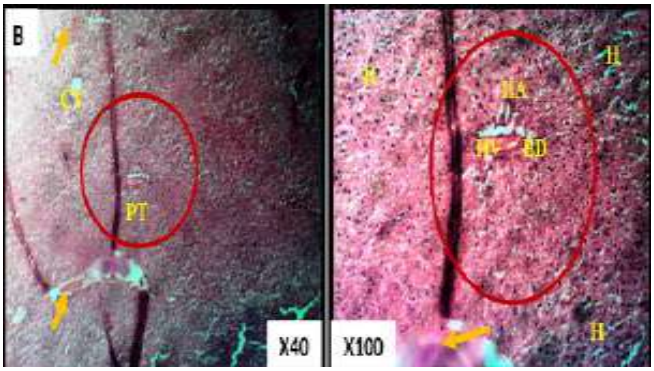
Data were presented as mean and standard error of mean (mean± SEM) *(P <0.05)- statistical significant difference when compared across groups.

Result

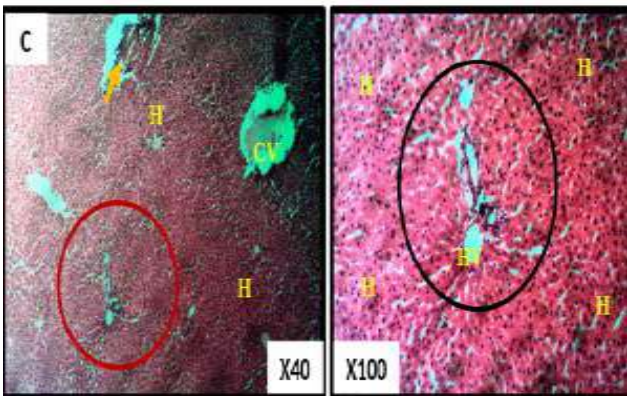
Haematoxylin and eosin staining slides for general histoarchitecture of the tissue



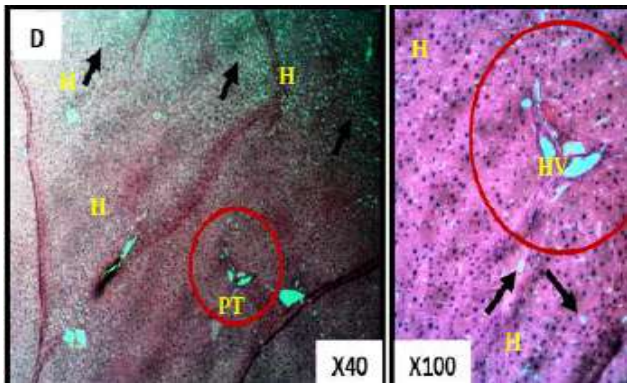
Group A showed normal liver cytoarchitecture with well distributed hepatocytes, intact portal triad and normal sinusoid.



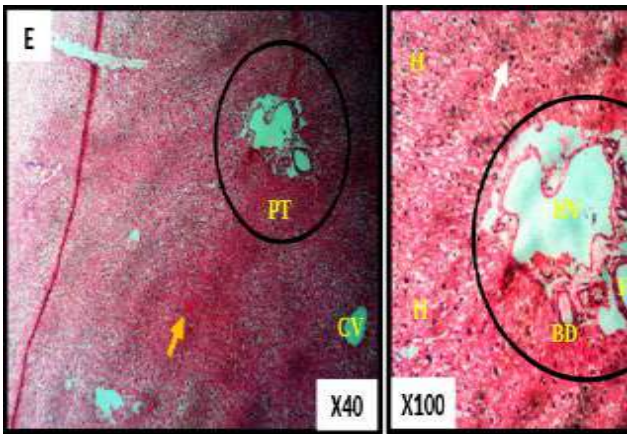
Group B showed some degenerative changes, some hemorrhage and fibrosis. the hepatocytes looks pathological with disorganized portal triad (yellow arrow)



Group C showed mild degeneratve changes (yellow arrow) but not so pathologically significant. The hepatocytes look almost similar to the control group.



Group D showed some degenerated hepatocytes (white dot at X40 and X100 magnification black arrow)



Group E showed some mild fibrosis (yellow arrow) but interestingly the hepatocytes though a bit pathological seems to be regenerating (white arrow)

Treated Groups showing the hepatocytes (**H**), portal triad (**PT**) comprised of hepatic vein (**HV)**, hepatic artery (**HA**) and the bile duct (**BD**). Also observed across the micrographs is the central vein (**CV**) and branches of blood vessels. The treated groups show mild infiltration of inflammatory cells (**YELLOW ARROW**). Red cells, hemorrhage, necrosis as well as fibrosis (**YELLOW ARROW**) with some degenerative changes occurring in the liver are seen in the treated groups. The control group shows intact cyto-architecture with intact and well distributed hepatocytes. MG-**X**40 and **X**100, H&E stain.

Animal weight table

Groups	Before	After
A	150±3.33	170±1.67
B	170±4.54	150±4.33
C	200±5.33	185±3.33
D	200±5.34	175±1.67
E	200±5.33	180±3.33

Animal weight (g)

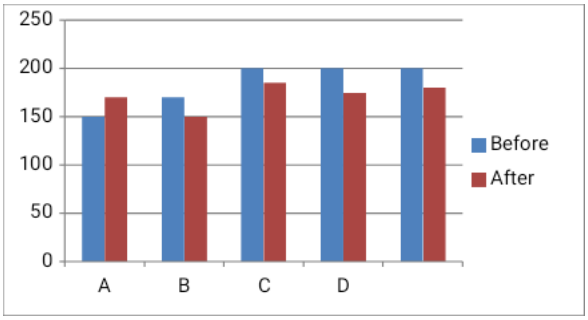


Figure 1.

Data were presented as mean and standard error of (Mean ± SEM) ($P < 0.05$) statistical significant difference when compared across groups.

There was significant increase in the average weight of the experimental animals in group A after treatment compare to the animals in the same group before treatment.

Group **B** (Ethanol Control Group) reduced in weight after ethanol induction compare to before induction.

Group **C D** and **E** weight significantly reduced after treatment compare to before treatment. Meanwhile, the average weight of the animals in group B was significantly reduced compare to other groups.

Liver function table

Groups	**AST**	**ALP**	**ALT**
A	**20±0.33**	**25±1.07**	**16±0.89**
B	**60±1.54**	**55±2.01**	**69±1.05**
C	**20±0.33**	**35±0.83**	**24±0.92**
D	**40±1.64**	**44±0.67**	**47±1.12**
E	**29±0.54**	**42±1.33**	**30±0.95**

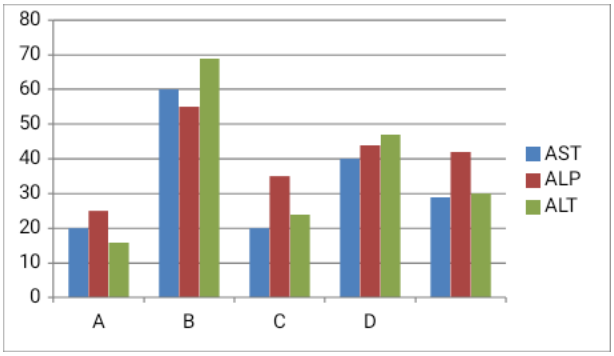


Figure 2

Liver Function Tests (IU/lL)

Group B had significantly higher AST, ALT and ALP serum activities compared to the normal control rats. Similarly, significant increase in serum activities of AST, ALT and ALP were observed in group D (**Livolin forte Control)** and E (***Vernonia amgdalina***) compared to group C (Combine treatment group). Moreover, group **D** showed little significant of increase in **AST**, **ALT** and **ALP** differences in the parameters mentioned above compared to group **E.** Group **C** and E have little or no significant differences in **ALP**, **ALT** and **AST** when compared together. The observed increase in **ALT**, **AST** and **ALP** level in the toxicant group is a sign of liver damage arising from the administration of the ethanol. This may be an indication of diminished synthetic

function of the liver which may consequently lead to enhanced retention of fluid in the tissues spaces causing generalized edema and ascites. The observed seldom decreases in the serum activities of **ALT**, **AST** and **ALP** level of treatment groups: **C**, **D** and **E** compared to group B is a sign of the protective effect combined used of Livolin forte and *Verninia amgdalina* have on recovering the histoachitecture of damaged liver.

Observable features

Group **A** animals were noticed to be agile and alert throughout the research period with no significant differences in weight. Group **B** animals were super alert at the onset of the administration of ethanol. After two weeks of administrations of ethanol, poor movements associated with Dementia, Wilson's diseases, chorea, Athethosis were observed in group **B, C, D** and **E**. But more tends in group **B**.

Completing Interest

No competing of interest.

Authors Contribution and Conclusion

The Results of this research work indicated that Livolin forte was better combine with *Vernonia amgdalina* as a curative agent rather than livolin forte alone or *Vernonia amgdalina* alone in rat. Meanwhile they both have protective effects on liver damage, but methanolic extract of *Vernonia amgdalina* proves better in ameliorating the damaged caused by ethanol. Therefore, if properly standardized, the methanolic extract of *Vernonia amgdalina* could be a good supplement in the management of hepatotoxicity.

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