Comparative evaluation of the protective effect of leaf extracts of *Vernonia amygdalina* (bitter leaf) and *Ocimum canum* (curry) on acetaminophen induced acute liver toxicity

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The quest for alternate medicine in preventing and combating hepatotoxicity led to the screening of aqueous and ethanol extracts of dried leaves of *Vernonia amygdalina* Delile (bitter leaf) and *Ocimum canum* Linn (curry leaf) for phytochemicals and in vitro antioxidant properties. Some rats were pretreated with the extracts and later administered with 2g/kg of body weight single dose of acetaminophen and the hepatoprotective effect of the extracts was determined by assessing the liver function, antioxidant enzyme activities and histological status of their livers, using standard biochemical methods. The extracts were rich in bioactive compounds and showed concentration-dependent variation in *in vitro* free radical (DPPH) scavenging activity. A 400mg/kg of body weight per day pretreatment (for seven days) with the extracts gave hepatoprotection to the rats. This was evidenced in the reduction of the activities of alanine aminotransferase (ALT) by 43% in aqueous extract of *O. canum* to 92% in *V. amygdalina* and marked increase of the serum albumin concentration. Aqueous leaf extract of *V. amygdalina* caused the highest increase in GPx activity while ethanol leaf extract of *O. canum* gave the highest (350%) increase in superoxide dismutase (SOD) activity, compared to the negative control. Also the lobular architecture of the hepatocytes was preserved. *V. amygdalina* and *O. canum* have important role in medicine as they contain substances that scavenge free radicals, stimulate activities of antioxidant enzymes and preserve the liver architecture in occasion of acetaminophen-induced liver toxicity.

**Key words:** *Vernonia amygdalina, Ocimum canum*, hepatoprotection, oxidative stress, free radical-scavenging, hepatocytes.

INTRODUCTION

Acetaminophen (APAP) is an analgesic and antipyretic substance used in the production of the drug paracetamol. Although safe at therapeutic doses, APAP had been found to cause severe liver injury (Vidhya and Bai, 2012; Erica and Emily, 2014). It is metabolically activated by cytochrome P₄₅₀.
enzymes to a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), that depletes glutathione (GSH) and covalently bonded to protein, and repletion of glutathione (GSH) prevents the toxicity (James et al., 2009).

NAPQI is formed by a direct two-electron oxidation of acetaminophen and can be detoxified by glutathione (GSH) to form an acetaminophen-GSH conjugate (Dahlin et al., 1984). After a toxic dose of acetaminophen, total hepatic GSH is depleted by as much as 90%, and as a result, the metabolite covalently binds to cysteine groups on protein, forming acetaminophen-protein adducts (Mitchell et al., 1973; Jack et al., 2009). Depletion of GSH which is an intrinsic antioxidant is capable of regeneration of reactive oxygen free radicals and hepatocellular fatty regeneration with centrilobular necrosis of the liver. Over production of reactive oxygen species (ROS) can damage cellular biomolecules like nucleic acids, proteins, lipids, carbohydrates and enzymes resulting in several diseases (Halliwell and Gutteridge 1999). Living systems have specific pathways to overcome the adverse effects of these damages but sometimes these repair mechanisms 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), the antioxidant drugs such as silymarin can have beneficial effect (Feher and Lengyey, 2017). Silymarin is the active ingredient in the branded drug, Sylibon 140 (a known hepatoprotective drug), manufactured by Micro Laboratory Ltd, India. Silymarin has cytoprotective activity mediated by its antioxidative and radical-scavenging properties, (Kfena and Walterovab, 2005).

Focus on plant research has increased all over the world and 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). Their use in ethnomedicine for the management of ailments stem from the presence of phytochemicals (Aja et al., 2010). In Nigeria, leafy vegetables contribute greatly to the nutritional and medicinal needs of the people as staples, flavours, condiments, spices, drinks and beverages (Mbang et al., 2008; Chevellier 1996).

Methanol extracts (Saud et al., 2013) and ethanol extracts (Ladipo et al., 2010) of vegetables could be used in treating diseases caused by microorganisms and they boost the concentration of Red Blood Cell in experimental animals,(Ezekwe et al., 2013). Pharmacological studies have demonstrated hepatoprotection, antioxidant and anti-inflammatory activities supporting the traditional uses of some leafy vegetables (Perez, 2016; Ahmed, 2016). These effects have been attributed to polyphenols and flavonoids in plant extracts which improve the functionality of the antioxidant system of the test rats (Rice-Evan et al., 1996; Edeoga et al., 2005; Sudha et al., 2011; Imaga and Bamigbetan 2013; Alamgir et al., 2016).

Vernonia amygdalina, a member of the Asteraceae family, is a small shrub that grows in tropical Africa. It typically grows to a height of 2 to 5 m (6.6 to 16.4 ft). The leaves are elliptical and up to 20 cm (7.9 in) long. Its bark is rough (Ijeh and Ejike, 2011). V. amygdalina is commonly called bitter leaf in English because of its bitter taste. The cooked leaves are a staple vegetable in soups and stews of various cultures throughout equatorial Africa (Egedigwe, 2010; Kokwaro, 2009). African common names include; Grawa (Amharic), Ewuro (Yoruba), Etidot (Ibibio), Onugbu (Igbo), Iyuna (Tiv), Oriwo (Edo), Etidot (Cross River State of Nigeria) Chusar-doki (Hausa), Mululuza (Luganda), Labwori (Acholi), Olusia (Luo) and Andndoleh (Cameroon) (Egedigwe et al., 2010; Kokwaro et al., 2009).

Ocimum canum belongs to the Lamiaceae family and it is native to the African continent and grows to a height of 2 feet. It is also known as the African basil, commonly known as curry among the native communities of Nigeria, with a distinct mint flavour and scented flowers. O. canum is grown for its medicinal and culinary value and it is highly useful in treating various types of diseases and in lowering blood glucose levels, especially in type 2 diabetes and treat corks (Kokwaro et al., 2009).

Huge quantities of these plants O. canum Linn (curry leaf) and V. amygdalina Delile (bitter leaf) are consumed all over Nigeria for flavouring and spicing of various types of food and as medicine for different ailments. Available information on their hepatoprotective property is scanty. Therefore, it is expedient to establish their hepatoprotective potentials of aqueous and the ethanol leaf extracts of these plants against injuries that may be caused by xenobiotics especially the commonly abused drug, paracetamol. This will clarify if their use can aid specific pathways, which work to overcome the adverse effects of damages caused by these xenobiotics, to keep pace with such deleterious effects.

MATERIALS AND METHODS

Plant materials, silymarin and acetaminophen

The plant materials were leaves of V. amygdalina and O. canum, the drug, Acetaminophen was a research support from Emzor Pharmaceutical Ltd, Lagos while silymarin is a branded drug (Sylibon 140) from Micro Laboratory Ltd, India.

Sample collection and preparation

Plant materials were harvested from two vegetable farms in Keffi, Nasarawa state, North Central Zone of Nigeria. The plants were identified by a Taxonomist Dr. B. O. Aziagba of the Department of Botany, Nnamdi Azikwe University, Awka (NAUH). The V. amygdalina was assigned voucher number NAUH 47A and the O. canum was assigned NAUH 144A and both were deposited at the University herbarium.

The leaves 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. Ethanol and water were separately used to extract the bioactive ingredients from the leaves.

**Preparation of extracts**

Bioactive compounds were extracted by soaking 100 g of the plant materials in 500 mL of absolute ethanol (that is, ratio 1:5; weight to volume) for 48 h. The extract was filtered using muslin cloth and then concentrated by heating at 30°C in a water bath and stored in airtight containers at 4°C. The aqueous extracts of the plant samples were prepared by soaking 100g of the powdered samples in 500mL of distilled water (that is, ratio 1:5; weight to volume) for 48 h. The extracts were filtered using muslin cloth and then concentrated by freeze drying and stored in airtight containers at 4°C.

**Animal models**

Forty two male Wister albino rats weighing between 120 to 140 g 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 h light/dark cycling. They were allowed access to unlimited food and water supply and allowed to acclimatize for three (3) weeks before the commencement of the study. All the animals were marked for identification, and their respective weights recorded. The animals were first fed with the chow (feeds) and intubated with the plant material.

**Administration of extracts and the drugs to the animals:**

The albino rats were divided into seven (7) groups of six (6) animals each. The group 1 (untreated group) received feed and water only, group 2 (the standard control) received feed, water and a pretreatment with 400mg/kg of body weight of Silymarin. Group 3 (negative control) received feed and water, while the groups 4-7 (test groups) received feed, water and pretreatment with 400mg/kg of body weight of either ethanol or aqueous leaf extracts of *V. amygdalina* or *O. canum* for seven days. On the eighth (8) day, the animals were fasted for up to seven hours, followed by the induction of hepatotoxicity in the animals of groups 2 to 7 by oral administration of 2g/kg of body weight acetaminophen.

**Animals sacrifice, collection and preparation of samples**

9 h after intoxication, the animals were made unconscious by exposure to chloroform in an enclosed container, as described by Ekor et al. (2006). Incisions were quickly made into the animals' cervical region with the aid of sterile blades and blood samples collected into plain tubes. Serum was collected as supernatant after centrifuging the clotted blood in a HSC (1000 to 4000rpm) bench centrifuge at 3000 rpm for 10 min.

**Preparation of liver homogenate**

After bleeding, the livers were carefully removed, trimmed of extraneous tissues and rinsed in ice-cold 1.15% KCl. The livers were then blotted dry, two grams (2 g) was weighed and homogenized in 8 ml of ice-cold phosphate buffer (100mM, pH 7.4).

The homogenate was then centrifuged first at 6,000 rpm for six minutes (6 mins) to remove nuclear debris after which the supernatant were centrifuged at 10,000 rpm for twenty minutes (20 mins) to obtain the post-mitochondrial supernatant (PMS), using a refrigerated centrifuge. This was used for the assay of the antioxidant enzymes (Super Oxide Dismutase, Catalase and Glutathione Peroxidase).

**Biochemical analysis**

The biochemical analyses were carried out using standard methods. Portions of the concentrated extracts were used for phytochemical screening using standard procedures of the Association of Analytical Chemist (2006) to identify the constituents as also described by Odebiyi and Sofowora (1990 1978), Fadey (1983), Sofowara (1993), Trease and Evans (1989) and Harborne (1973). The evaluation of free radical scavenging activity of the plant extracts against DPPH radical was done by a slightly modified spectrophotometric method previously described by Afolayan et al. (2014). The serum Alkaline Phosphatase activities of the experimental animals were estimated using the method of King (1965b) while the activities of Aspartate aminotransferase and Alanine aminotransferase were carried out using the King (1965a) method. The total protein was estimated using the colorimetric method of Lowry et al. (1951) where the peptide bonds of proteins react with Cu⁺ in alkaline solution to form a coloured complex. Malloy and Evelyn (1937) method which has a principle that states that in an aqueous solution only direct bilirubin (DB) reacts, was used to determine total bilirubin. Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Sun and Zigma (1978). The catalase activity was determined according to the method of Beers and Sizer as described by Usah et al. (2005) by measuring the decrease in absorbance at 240nm due to the decomposition of H₂O₂ using the ultra violet (UV) spectrophotometer. The method of method of Lawrence and Burk (1976) was used for the determination of glutathione peroxidase (GPx) activity. Finally, the histological study on the liver tissues was carried out using the Haematoxylin and Eosin stain as described by Bancroft et al. (2013).

**Statistical analysis**

Data were expressed as means ± standard deviation (SD). The statistical tools used for the analysis was one way analysis of variance (ANOVA) and the post hoc Newmann Keul's multiple comparisons test. The computer software utilized were Microsoft excel 2016 edition and statistical package for social sciences (SPSS) 16.0 for windows. Differences between means were considered significant at p<0.05.

**RESULTS AND DISCUSSION**

The extracts contain varying types of phytochemicals. All the extracts contain tannin and glycosides; while only ethanol leaf extract of *V. amygdalina* contained alkaloid. Saponins are contained in all the extracts, but the ethanol leaf extract of *O. canum* contain phenols (Table 1).

The aqueous leaf extract of the *V. amygdalina* had a direct variation of in vitro free radical (DPPH) scavenging property with increase in concentration of the extract from 0.03 to 0.5mg/l (26.39 to 69.31%) while *O. canum*...
Table 1. The qualitative phytochemical compositions of aqueous and ethanol leaf extracts of *Vernonia amygdalina* and *Ocimum canum*.

| Parameters          | *Vernonia amygdalina* | *Ocimum canum* |
|---------------------|------------------------|----------------|
|                     | Aqueous | Ethanol | Aqueous | Ethanol |
| Alkaloid            | -       | +       | -       | -       |
| Tannin              | +       | +       | +       | +       |
| Phenols             | +       | +       | -       | +       |
| Cardiac glycosides  | +       | -       | -       | +       |
| Triterpenoids       | +       | +       | -       | +       |
| Sterols             | +       | -       | -       | -       |
| Terpenoids          | -       | +       | +       | +       |
| Saponins            | +       | +       | +       | -       |
| Balsam              | -       | +       | -       | +       |
| Cardenolides        | -       | -       | -       | -       |
| Resins              | -       | -       | -       | -       |
| Phlobatanins        | +       | -       | +       | +       |
| Glycosides          | +       | +       | +       | +       |

*+... indicates presence; -... indicates absence.*

Figure 1. Comparing the free radical, (DPPH), scavenging activity of aqueous extracts of *V. amygdalina* and *O. canum* using ascorbic acid as positive control. Values are means of triplicate results of the inhibition.

Increase from 64.86% at 0.03mg/100ml to 74.31% at 0.06mg/100ml, and gradually dropped 70.56% at a concentration of 0.5mg/100ml. Based on the concentrations used in the study, *O. canum* had its highest *in vitro* antioxidant activity at 0.06mg/100ml, *V. amygdalina* had its highest activity at 0.5 mg/100ml (Figure 1). The ethanol leaf extracts of the two vegetables showed inverse relationship between the increase in concentrations and the *in vitro* antioxidant activities with the ethanol leaf extract of *O. canum* having higher activity than the *V. amygdalina* at low concentrations and vice versa at higher concentrations as seen in Figure 2. However, ascorbic acid had more DPPH-scavenging (*in vitro antioxidant*) potential than the leaf extracts of the plants at the concentrations considered in this work (Figure 1 and 2).
Figure 2. Comparing the free radical, (DPPH), scavenging activity of ethanol extracts of *V. amygdalina* and *O. canum* using ascorbic acid as a positive control. Values are means of triplicate results of the inhibition.

Table 2. The effect of pretreatment with 400gm/kg of body weight aqueous leaf extracts of *V. amygdalina* and *O. canum* on the serum activities of AST, ALT and ALP of rats administered with 2 g/kg of body weight single dose of acetaminophen.

| Treatment               | AST (u/l)     | ALT (u/l)     | ALP (u/l)     |
|-------------------------|---------------|---------------|---------------|
| Untreated group         | 41.90±3.30    | 27.90±8.20    | 86.30±58.80   |
| Standard control        | 42.80±10.80   | 17.90±3.10    | 167.30±107.70 |
| Negative control        | 67.40±26.40** | 117.30±57.50**| 209.80±67.00**|
| *Vernonia amygdalina*   | 118.00±13.00* | 9.05±3.76**   | 104.00±56.00**|
| *Ocimum canum*          | 147.00±4.48*  | 66.33±14.58** | 201.00±51.88  |

Values are mean ± 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 Untreated group.

2 g per kilogram body weight (2g/kg of body weight), single dose acetaminophen caused significant rise in activities of ALT, AST and ALP of the animals administered with the drug without pretreatment with either the extracts or the standard hepatoprotective drug (Silymarin) when compared to the untreated group. Pretreatment with the extracts significantly reduced the activities of ALT and ALP in the serum of the rats. The activity of AST in the serum was however, raised significantly in the extract pretreated rats (Table 2 and 3). There was significant (p<0.05) decrease in the concentration of albumin of the animals administered with a single 2g/kg of body weight of acetaminophen without pretreatment with either the extracts or the standard
The effect of pretreatment with 400 gm/kg of body weight ethanol leaf extracts of *V. amygda* *lina* and *O. canum* on the serum activities of AST, ALT and ALP of rats administered with 2g/kg of body weight single dose of acetaminophen.

| Treatment       | AST (u/l) | ALT (u/l) | ALP (u/l) |
|-----------------|-----------|-----------|-----------|
| Untreated group | 41.90±3.30| 27.90±8.20| 86.30±58.80|
| Standard control| 42.80±10.80| 17.90±3.10| 167.30±107.70|
| Negative control| 67.40±26.40**| 117.30±57.50**| 209.80±67.00**|
| *Vernonia amygda* *lina* | 74.73±11.97 | 29.27±13.59** | 104.67±25.01** |
| *Ocimum canum*   | 142.60±51.81* | 57.70±25.08** | 129.00±25.36 |

Values are mean ± 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 untreated group.

The effect of pretreatment with 400 gm/kg of body weight aqueous leaf extracts of *V. amygda* *lina* and *O. canum* on the serum concentrations of total protein, albumin, and bilirubin of rats administered with 2g/kg of body weight single dose of acetaminophen.

| Treatment       | Total protein (g/dl) | Albumin (g/dL) | Total bilirubin (mg/dl) | Direct bilirubin (mg/dl) | Indirect bilirubin (mg/dl) |
|-----------------|----------------------|----------------|------------------------|--------------------------|--------------------------|
| Untreated group | 75.10±5.70           | 18.67±5.00     | 12.50±2.70              | 5.30±1.40                | 5.90±0.70                |
| Standard control| 76.80±10.50          | 4.70±2.30      | 9.00±5.20               | 6.50±2.90                | 4.00±3.30                |
| Negative control| 62.80±1.20           | 4.00±1.42      | 16.40±4.20              | 4.30±2.50                | 7.20±1.90                |
| *V. amygda* *lina* | 61.16±11.94         | 6.00±2.00      | 11.80±2.00              | 2.65±0.75                | 9.15±2.75                |
| *O. canum*      | 77.57±12.48          | 10.67±2.31*    | 8.87±1.96               | 1.20±0.35                | 4.67±0.83                |

Values are mean ± SD of six (6) results, * and ** show values with significant increase and decrease respectively, compared to the Untreated group.

The effect of pretreatment with 400mg/kg of body weight ethanol leaf extracts of *Vernonia amygda* *lina* and *Ocimum canum* on the serum concentrations of total protein, albumin, and bilirubin of rats administered with 2g/kg of body weight single dose of acetaminophen.

| Treatment       | Total protein (g/dl) | Albumin (g/dL) | Total bilirubin (mg/dl) | Direct bilirubin (mg/dl) | Indirect bilirubin (mg/dl) |
|-----------------|----------------------|----------------|------------------------|--------------------------|--------------------------|
| Untreated group | 75.10±5.70           | 18.67±5.00     | 12.50±2.70              | 5.30±1.40                | 5.90±0.70                |
| Standard control| 76.80±10.50          | 4.70±2.30      | 9.00±5.20               | 6.50±2.90                | 4.00±3.30                |
| Negative control| 62.80±1.20           | 4.00±1.42      | 16.40±4.20              | 4.30±2.50                | 7.20±1.90                |
| *V. amygda* *lina* | 78.48±11.29         | 12.00±6.93*    | 18.43±5.40              | 6.13±4.52                | 8.97±6.81                |
| *O. canum*      | 75.00±2.65           | 13.33±2.31*    | 3.63±1.85**             | 2.33±1.60                | 0.83±0.58**               |

Values are mean ± SD of six (6) results, * and ** show values with significant increase and decrease respectively, compared to the Untreated group.

2 g per kilogram body weight (2g/kg of body weight), single dose acetaminophen caused the perturbation of the liver as evidenced in the significantly raised activities of ALT, AST and ALP in the negative control as compared to the untreated group. The liver injury suggested here is in consonance with the work of Prabu *et al.*, 2011 and Ekor *et al.*, 2006, which reported liver damage as a result of the administration of 2g/kg of body weight of acetaminophen in albino rats. The activities of the liver antioxidant enzymes, SOD and GPx were significantly reduced in the homogenate of the negative control, compared to the untreated group. The activity of hepatoprotective drug (Silymarin) when compared to the untreated group. Pretreatment with the extracts raised the serum concentrations of the rats as seen in Tables 4 and 5.

2 g per kilogram body weight, (2g/kgbw) of acetaminophen reduced significantly the activities of SOD and GPx when compared to the negative control (Tables 6 and 7). The pretreatment of the rats with 400 mg/kg of body weight of the aqueous and ethanol leaf extracts of *V. amygda* *lina* and *O. canum* all led to significant increase in the activities of these enzymes (SOD and GPx).
CAT was also reduced in the negative control, although the reduction was not statistically found to be significant when compared to the untreated group. This is an indication of the induction of oxidative stress in the liver. Disrupted hepatic lobular architecture of the rats was also observed, all these alterations were seen in the negative control (group 3) as compared to the untreated group (group 1).

Acetaminophen also altered the concentration of protein (total protein and albumin) in the serum of the rats, which could be as a result of the binding of NAPQI to proteins (Mitchell et al., 1973) or the effect of NAPQI on the protein synthesizing/metabolizing ability of the liver. The aminotransferase are abundant in the liver and are released into the bloodstream following hepatocellular damage, making them sensitive markers of liver damage (Al-Mamary, 2002; Sarvesh, 2012).

Therefore, marked increases in the serum ALT and AST activities reported in the present study is an indication of liver damage. Serum activity levels of aminotransferases is 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 Mathew, 2013; Olorunnisola et al., 2011; Martin and Friedman, 1992).

*N-acetyl-p-benzoquinone imine (NAPQI), is an oxidative product of acetaminophen metabolism that binds covalently to the sulfhydryl groups of proteins resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver causing hepatotoxicity (Kanchana and Mohammed, 2011). Results from the present study provides evidence of the induction of oxidative stress nine hours (9 h), following 2g/kg of body weight acetaminophen (APAP), administration.

The induced oxidative stress as found in the study is evident in the significantly decreased activities of the SOD, CAT and the GPx of the animals in the negative control group as compared to the untreated group, resulting in decrease in cellular defence against oxidative damage. The increased formation of superoxide may have led to hydrogen peroxide formation as found in James et al. (2003).

| Table 6 | The effect of pretreatment with 400mg/kg of body weight of aqueous leaf extracts of *V. amygdalina* and *O. canum* on the activities of the liver antioxidant enzymes of rats administered with a 2g/kg of body weight single dose of acetaminophen. |
| Samples | SOD (U/mg) | CAT (U/mg) | GPx (µ/mg) |
|---------|------------|------------|-------------|
| Untreated group | 0.05±0.01 | 3.00±2.30 | 397.00±100.00 |
| Standard Control | 0.20±0.40 | 20.90±14.90 | 184.60±23.60 |
| Negative Control | 0.02±0.01 | 3.00±2.60 | 115.60±10.03 |
| *V. amygdalina* | 0.04±0.03* | 13.56±11.42 | 329.41±87.25* |
| *O. canum* | 0.01±0.00** | 2.74±1.87 | 233.49±30.00* |

Values are mean ± SD of six (6) results, * and ** show values with significant increase and decreases respectively, compared to the Negative control while """" show values with significant increase and decreases respectively compared to the untreated group.

| Table 7 | The effect of pretreatment with 400mg/kg of body weight of aqueous leaf extracts of *V. amygdalina* and *O. canum* on the activities of the liver antioxidant enzymes of rats administered with a 2g/kg of body weight single dose of acetaminophen. |
| Samples | SOD (U/mg) | CAT (U/mg) | GPx (µ/mg) |
|---------|------------|------------|-------------|
| Untreated group | 0.05±0.01 | 3.00±2.30 | 397.00±100.00 |
| Standard Control | 0.20±0.40 | 20.90±14.90 | 184.60±23.60 |
| Negative Control | 0.02±0.01 | 3.00±2.60 | 115.60±10.03 |
| *V. amygdalina* | 0.03±0.01* | 11.25±4.86* | 249.12±98.50* |
| *O. canum* | 0.09±0.06* | 6.54±1.95* | 203.83±63.93* |

Values are mean ± SD of six (6) results, * and ** show values with significant increase and decreases respectively, compared to the Negative control while """""" show values with significant increase and decreases respectively compared to the untreated group.
Figure 3. The effect of pretreatment with 400mg/kg of body weight of leaf extracts of *V. amygdalina* and *O. canum* on the histopathology of livers of rats administered with a 2g/kg of body weight single dose of acetaminophen

Ekor et al. (2006) reported that after 7 h, following paracetamol (PCM) administration, there was a rise in GST activity, indicating increased GST-catalysed conjugation of NAPQI with GST that led to the depletion of cellular GSH level. Histological profile of the livers of the rats in the negative control group showed a poorly preserved hepatic lobular architecture, sharply demarcated hepatocyte, necrosis and exhibiting periportal sinusoidal congestion, which is a confirmation of liver injury.

Previous studies by Densie (2013), Prerona et al. (2011), Huang et al. (1992), Ekor et al. (2006), Kanchana and Mohammed (2011) and Okediran et al. (2014) had all shown extracts from plant to be potent in hepatoprotection. The present study provides evidence that the pretreatment of rats, with a 400mg/kg of body weight per day with aqueous or ethanol leaf extract of *V. amygdalina* and *O. canum* for seven days, was hepatoprotective against toxicity and oxidative stress arising from a 2g/kg of body weight oral administration.
with acetaminophen over nine hours. The pretreatment with the leaf extract led to significant decrease of the serum activities of ALT and ALP of the animals in the test groups when compared to that of those in the negative control. While Silymarin gave 85% decrease, the aqueous leaf extracts of V. amygdalina and O. canum, gave 92 and 43% decrease respectively in the activity of ALT in the serum of the rats as compared to the negative control. ALP activity in the serum was decreased by 70, 50 and 4.3% as a result of the pretreatment with silymarin, aqueous leaf extracts of V. amygdalina and O. canum respectively.

The ethanol leaf extracts of the plants (V. amygdalina and O. canum) decreased the serum ALT of the rats by 75 and 50%, respectively. While the ethanol leaf extract of V. amygdalina and O. canum led to 50 and 39% decrease in ALP activity, respectively. This suggests that the leaf extracts of the plants are hepatoprotective. The aqueous leaf extract of V. amygdalina offered the best protection, followed by Silymarin, ethanol leaf extract of V. amygdalina, ethanol leaf extract of O. canum and aqueous leaf extract of O. canum in that order. The binding of NAPQI to proteins (Kanchana and Mohammed, 2011), may have been inhibited by pretreatment with silymarin, aqueous and ethanol leaf extracts of V. amygdalina and O. canum in this study. The increase in albumin concentration in the serum was in the increasing order of silymarin (18%) < aqueous leaf extract of O. canum (167%) < ethanol leaf extract of V. amygdalina (200%) < ethanol leaf extract of O. canum (233%) < aqueous leaf extract of V. amygdalina (267%).

The activities of CAT, SOD and GPx were increased significantly at 95% confidence level by the actions of the leaf extracts. Catalase, Superoxide Dismutase and Glutathione Peroxidase are the primary intracellular defence mechanism to cope with increased oxidative stress, eliminating superoxide anion and hydrogen peroxide that may oxidise cellular substrates thereby preventing free radical chain reactions (Ekor et al., 2006).

The induction of higher activities of these antioxidant enzymes is a suggestion of the protection of the livers by reducing oxidative stress on the liver. The Silymarin showed the highest increase of CAT activity, while aqueous leaf extract of V. amygdalina led to the highest increase in GPx activity and ethanol leaf extract of O. canum gave the highest (350%) increase in SOD activity, compared to the negative control. These hepatoprotective potentials were also corroborated by the histopathological studies. All these protections may be due to the antioxidant properties of the plants, which stem from their phytochemical components. It has been found that phytochemicals have the potential to reduce oxidative damage to cells (Densie, 2013, Prerona et al., 2011; Huang et al., 1992).

In this study, the aqueous leaf extract of V. amygdalina gave the best hepatoprotective effect, even better than the standard drug, followed by the ethanol extract of the same plant material. This could stem from the fact that the aqueous and ethanol leaf extract of V. amygdalina contains phenols that were not detected in the aqueous leaf extract of O. canum. The better activity of the ethanol leaf extract over the aqueous leaf extract of O. canum can also be attributed to the presence of phenols in the ethanol extract.

Conclusion

The leaves of the plants were found to be potential antioxidants and hepatoprotective (and the compared well with the standard drug Silymarin), with the best result obtained from the aqueous leaf extract of V. amygdalina. Therefore, they can be good sources of raw materials for the production of drugs for the prevention and treatment of liver and associated diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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