Combined Administration of *Telfairia occidentalis* and *Vernonia amygdalina* Leaf Powders Ameliorates Garlic-induced Hepatotoxicity in Wistar Rats

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ABSTRACT

**Background:** Recently, combined administration of two or more plant extracts has been advocated for effective therapeutic efficacy. This study was carried out to assess the effect of combined administration of *Telfairia occidentalis* (TO) and *Vernonia amygdalina* (VA) leaf powders in ameliorating garlic-induced hepatotoxicity in rats.

**Method:** The leaf powders of VA and TO were combined in different proportions (20, 20, 30, 10 and 10, 30%), respectively and used in diet formulation which was fed to hepatotoxic rats for 28 days.

**Results:** We observed that the significantly increased (p<0.05) activities of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and serum total bilirubin concentration in rats induced with hepatotoxicity was effectively attenuated following combined administration of varied proportions of TO and VA leaf powders.

**Conclusion:** These observations are indicative of hepatoprotective attributes of the two herbs and the effect compared favourably with the normal rats. This was further substantiated and confirmed by the results of histological analysis. Thus, the complementary effects of TO and VA leaf powders proved to be capable of preventing garlic-induced hepatotoxicity and may serve as potential source of natural products for the management of liver injuries or diseases.

Key words: *Allium sativum*, liver injury, free radicals, antioxidants, histopathology


INTRODUCTION

Traditionally, the use of plant parts as sources of herbal preparations for treatment of various ailments are based on the experience passed from generation to generation, virtually by oral tradition and through practice that forms part of the indigenous knowledge of people of any locality (Sofowora, 1993). This has led to the use of herbs for therapeutic purposes ranging from headaches, cancer to liver diseases (Yuan et al., 2010). In fact, it has been reported that more than 80% of the populations in developing nations rely on traditional systems of medicine (WHO, 2002). Though infusions and decoctions have historically been the traditional forms for orally administered medicinal plants, powdered formulations have also enjoyed global acceptance over the years in many quarters. The powder is mainly orally administered with caution to avoid inhalation into the lungs. For this reason, powdered formulations are usually added to food, taken in capsules or made into pills.

The medicinal part of a plant may be administered either solely or in combination with part(s) of other plants to exercise their full medicinal effects. Synergistic effects of plant extracts have long been studied and advocated for use in medicinal practice. Sivaraj et al. (2009) reported the anti-hyperglycemic and anti-hyperlipidemic effects of combined administration of *Cassia auriculata* and *Aegle marmelos* leaf extracts in streptozotocin–induced diabetic rats. Despite the inherent medicinal potentials of many plants, the efficacy of their combined administration is yet to be fully explored and scientifically validated.

*Allium sativum* (Garlic) is a species in the onion family Alliaceae with the bulb made of cloves instead of layers. Studies have revealed that garlic can lower blood cholesterol, prevent dangerous blood clots, reduce blood
pressure, prevent cancer and protect against fungal and bacterial infections (Jelodar et al., 2005). Despite these good attributes, it has also been confirmed that unregulated consumption of garlic can increase free radical load, causing a reduction in the degree of protection given to body cells against oxidative damage (Bogin et al., 1984; Umar et al., 1998; Oboh, 2005).

*Telfairia occidentalis* is one of the green leafy vegetables widely consumed in Nigeria (Akoroda, 1990). The leaves of this plant are rich in proteins, vitamins (β-complex), minerals, fatty acids and fibers which prompt their use in soup making (Odoemenma and Onyenekwe, 1998). The plant is also rich in glycosides that yield curcurbitacins and glucose on hydrolysis. The vitamin C content of this plant is about 148 mg/100 g of dry matter (Adaramoye et al., 2007). *T. occidentalis* has been reported to protect against cancers of the esophagus, oral cavity and stomach as well as maintain blood vessel flexibility and improve circulation in the arteries of smokers (Ajayi et al., 2000). Its potency as anti-inflammatory (Oluwole et al., 2003), antibacterial (Odoemenma and Essien, 1995), hypoglycemic (Nwozo et al., 2004; Eseyin et al., 2005a), anti-cholesterolemic agent and involvement in enhanced production of β and γ-globulin (Eseyin et al., 2005b) have been reported.

*Vernonia amygdalina* (bitter leaf) is a shrub that grows predominantly in the Tropical Africa. Leaves from the plant serve as vegetable food and culinary herb in soup (Abozi and Raseroka, 2003). In African herbal homes, extracts of the plant are used in the control of tick, treatment of cough, feverish condition, constipation and hypertension (Regassa, 2000; Kambizi and Afolayan, 2001; Amira and Okubadejo, 2007). Teas containing bitter leaf are also used in the management of diabetes and other metabolic diseases associated with liver (Sceff et al., 2001). Phytochemical screening of *V. amygdalina* revealed the presence of saponins, sesquiterpene and flavonoids (Igile et al., 1994). Strong antioxidant activities have been reported for flavonoids from *V. amygdalina* and its saponins have been reported to elicit antitumoral activities in leukemia cells (Jisaka et al., 1993).

The promising data generated from comparative study of the effects of the leaf powders of *T. occidentalis* and *V. amygdalina* on garlic-induced hepatotoxicity in rats motivated the present research. Therefore, this study investigated the effect of combined administration of the leaf powders of the two plants on garlic-induced hepatotoxicity in rats.

**MATERIALS AND METHODS**

**Chemicals and reagents:** Diagnostic kits for total bilirubin, conjugated bilirubin, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were purchased from Randox Laboratories Ltd., Co. Antrim, United Kingdom. All other chemicals and reagents were of analytical grade from Sigma-Aldrich Inc., St. Louis, USA.

**Plant materials and authentication:** *Allium sativum*, *Telfairia occidentalis* and *Vernonia amygdalina* were obtained from Yankuru Market in Kano metropolis, Kano State. These vegetables were authenticated in Botany Unit, Department of Biological Sciences, Bayero University, Kano, Nigeria and a voucher specimen was prepared and deposited at the University Herbarium.

**Experimental animals:** Male Wistar rats weighing between 130-150 g were obtained from the Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria, Nigeria. They were kept in cages in a room maintained at a temperature of 26-29°C with a 12 h light-dark cycle for 2 weeks to acclimatize. The animals were allowed free access to feed (VitalFeed Ltd., Ibadan, Nigeria) and water ad libitum. The protocol conforms to the guidelines of the National Institute of Health (NIH, 1985) for laboratory animal care and use and in accordance with the principles of good laboratory procedure (WHO, 1998).

**Induction of liver damage:** The procedure described by Sabiu and Wudil (2011) was adopted to induce liver damage. Briefly, rats were fed diet containing 4% garlic powder continuously for 28 days. The feed and water were given to the animals ad libitum.

**Processing of plant materials and formulation of diet:** Edible parts of the leafy vegetables were washed in distilled water and then air-dried at room temperature alongside cloves of garlic. The dried plant materials were milled into powdered form with an electric blender and subsequently used in diet formulation as presented in Table 1.

**Animal treatment:** Twenty five male Wistar rats were randomly distributed into five treatment groups of five animals each. Rats in Group 1 (control) were fed normal diet. The animals in Group 2 were placed on diet containing 4% garlic powder (hepatotoxic rats),

<table>
<thead>
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<th>Table 1: Feed formulation using the plant materials</th>
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<td>Variables (g)</td>
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<td>Normal basal diet</td>
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<td>Garlic powder</td>
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<td><em>V. amygdalina</em></td>
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<td><em>T. occidentalis</em></td>
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while those in Groups 3, 4 and 5 were given diet containing 4% garlic powder along with *V. amygala*ina and *T. occidentalis* leaf powders in ratios 1:1 (20%:20%), 3:1 (30%:10%) and 1:3 (10%:30%), respectively (as presented in Table 1) for 28 days. The feed and water were administered *ad libitum* throughout the period of the experiment.

**Collection of blood and isolation of liver:** At the end of the 28 days feeding period, the rats were fasted overnight and humanely sacrificed by cervical dislocation. Blood was collected by heart puncture into centrifuge tubes and allowed to clot for 15 min before centrifuging at 3,000 g for 15 min using a bench top centrifuge (Beckman and Hirsch, Burlington, IO, USA). The serum was carefully collected and used for liver function tests. A portion of the liver was also sliced and fixed in saline formaldehyde solution for histological examination.

**Assay of liver biochemical indices and histopathological examination:** The procedure described by Reitman and Frankel (1957) was used to assay for Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities. Total bilirubin concentration was determined by the method of Jendrassik and Grof (1938), while the determination of Alkaline Phosphatase (ALP) was done adopting the method of Rec GSCC (1972). Histopathological examination of the liver was carried out using the method of Bancroft and Stevens (1990).

**Statistical analysis:** Results were expressed as Mean±Standard Deviation (SD) (n = 5). The data were subjected to one way analysis of variance (ANOVA) and Duncan Multiple Range Test was employed to separate treatment means. The p values of less than 0.05 were considered statistically significant.

**RESULTS**

**Enzyme activity and bilirubin concentration:** Figure 1-4 show the effects of combined administration of leaf powders of *Telfairia occidentalis* and *Vernonia amygdalina* on the activities of serum ALP, ALT, AST and total bilirubin concentration, respectively in the experimental animals. Feeding of rats with diet containing 4% raw garlic continuously for 28 days caused a significant (p<0.05) increase in the activities of these enzymes as well as level of total bilirubin when compared with the normal control. The observed elevations in the serum assayed parameters induced by raw garlic powder were significantly attenuated (p<0.05) in the rats co-fed with both *T. occidentalis* and *V. amygdalina* leaf powders. The results showed that combined feeding of both plants at varied ratios significantly reduced the activities of the enzymes and total bilirubin level comparable to the normal control. Infact, the diets were able to reduce the levels of the studied parameters to about half when compared to the hepatotoxic control. However, there was no significant difference in the effect elicited by the different formulated diets notwithstanding the variation in quantity of the two plants.

Fig. 1: Serum activity of alkaline phosphatase (ALP) in garlic-induced hepatotoxic rats co-fed with diet containing *V. amygdalina* and *T. occidentalis* leaf powders. Results are Mean±SD (n=5). Bars with different letters are significantly different (p<0.05). G: Garlic powder, TO: *T. occidentalis*, VA: *V. amygdalina*
Fig. 2: Serum activity of alanine aminotransferase (ALT) in garlic-induced hepatotoxic rats co-fed with diet containing *V. amygdelina* and *T. occidentalis* leaf powders. Results are Mean±SD (n=5). Bars with different letters are significantly different (p<0.05). G: Garlic powder, TO: *T. occidentalis*, VA: *V. amygdelina*

Fig. 3: Serum activity of aspartate aminotransferase (AST) in garlic-induced hepatotoxic rats co-fed with diet containing *V. amygdelina* and *T. occidentalis* leaf powders. Results are Mean±SD (n=5). Bars with different letters are significantly different (p<0.05). G: Garlic powder, TO: *T. occidentalis*, VA: *V. amygdelina*

**Histopathological analysis:** The liver micrographs of the rats are presented in Fig. 5. The liver of the normal control rats showed normal architecture with well preserved cords of hepatocytes, well demarcated sinusoids and no area of infiltration by inflammatory cells (Fig. 5a). This is in sharp contrast to the features observed for animals fed 4% garlic powder (hepatotoxic rats). The animals exhibited drastic alterations in liver architecture ranging from extensive fatty change, distended hepatocytes, vacuolated cytoplasm, compressed sinusoids, fatty degeneration area of necrosis, to infiltration by inflammatory cells (Fig. 5b). However, the liver micrographs of vegetables treated animals relatively showed distinct and essentially normal cords of hepatocyte with non-prominent fatty change (Fig. 5c, d and e).
Fig. 4: Serum concentration of total bilirubin in garlic-induced hepatotoxic rats co-fed with diet containing V. amygdalina and T. occidentalis leaf powders. Results are Mean±SD (n = 5). Bars with different letters are significantly different (p<0.05). G: Garlic powder, TO: T. occidentalis, VA: V. amygdalina

Fig. 5(a-e): Light micrograph (X400, Haematoxylin and eosin stained) of the liver of (a) Control rat, (b) Hepatotoxic rat, (C) 4% garlic powder+20% V. amygdalina+20% T. occidentalis treated rat, (d) 4% garlic powder+50% V. amygdalina+10% T. occidentalis treated rat and (e) 4% garlic powder+10% V. amygdalina+30% T. occidentalis treated rat
DISCUSSION

Medicinal plants play a vital role in sustaining human health and in the prevention of many diseases, including liver damage resulting from oxidative stress (Alia et al., 2008). Their therapeutic potency could either be unitarily or synergistically exploited (Shen-Nong, 2002). In this study, the complementary hepatoprotective effect of T. occidentalis and V. amygdaлина leaf powders on garlic-induced oxidative insults in rats was elucidated.

Serum analysis of biochemical indices for liver damage is often employed to ascertain its integrity following exposure to pharmacological agents (Uboh et al., 2010). The significant elevation in the activities of serum ALP, ALT and AST following ingestion of 4% raw garlic powder may be an indication of liver injury. This may be attributed to generation of free radicals which triggered chains of reactions resulting in liver damage. The increase in serum activities of these enzymes might be due to their leakage into the circulatory system following altered permeability of hepatocytes’ membranes, reflecting a severe damage to the structural architecture of the liver (Reichling and Kaplan, 1988; James et al., 2003; Teufelhofer et al., 2005). These results also agree with earlier reports by Umar et al. (1998), Oboh et al. (2006) and Sabiu and Wudil (2011). These authors opined that unregulated consumption of garlic could be toxic to erythrocytes and hepatocytes. Conversely, the significant reduction in enzyme activities of rats placed on diets containing T. occidentalis and V. amygdaлина leaf powders suggests that both plants were able to ameliorate the deleterious effects of garlic on the hepatocytes of rats.

Similarly, the observed significant increase in serum level of total bilirubin in hepatotoxic rats could be due to defect in either carrier-mediated saturable system at the sinusoidal surface of the hepatocytes, involved in bilirubin uptake or MRP-2 (multidrug-resistance-like protein 2) that sees to the active transport mediated secretion of conjugated bilirubin into bile. This might have resulted from loss of potential gradient of ions transversing hepatocytes membrane. This agrees with the earlier reports by Oboh and Akindahunsi (2004) and Sabiu and Wudil (2011) where, raw garlic was reported to have caused increase in serum total bilirubin. Excretion of serum bilirubin from the body is reduced if the liver is damaged resulting in high concentration of this metabolite in the blood. Thus, the significantly reduced level of bilirubin in the serum of rats fed with T. occidentalis and V. amygdaлина leaf powders signifies that the two plants may be rich in substances involved in maintaining the structural integrity of the hepatocyte membrane.

Studies have shown that T. occidentalis and V. amygdaлина are rich in antioxidants and phytochemicals which promote good health (Amic et al., 2003; Oboh, 2005). Therefore, the effects produced by co-feeding leaf powders of the two plants could possibly be attributed to their electron-donating capacities to form stable products and subsequently, terminating free radical chain reactions. The effect of antioxidants in ameliorating oxidative damage is believed to have reduced cancer incidence and also prevent cellular damage. Natural antioxidants have also been studied to be vitamins (carotenoids, tocopherol or ascorbic acid and their derivatives) and nitrogen containing compounds (alkaloids, amino acid derivatives, amines and chlorophyll), (Velıoğlu et al., 1998). The chlorophyll contents of T. occidentalis and V. amygdaлина are considerably high and have been reported to contribute to their therapeutic potential (Philipson et al., 1993). This factor may have contributed to the ameliorative effect experienced in rats fed with formulations containing leaf powders of the two herbs following hepatic damage. This submission conforms with the report of Oboh and Akindahunsi (2004) that chlorophyll found in most green plants lessens the toxic load on the liver by binding to various harmful substances.

Another important factor worthy of consideration in the assessment of the efficacy of therapeutic agents for hepatic injury is their effect on histology of vital organs in the body. Liver from normal control rats showed distinct architecture with well preserved cords of hepatocytes characterized by non-vacuolated cytoplasm, well demarcated sinusoids, no fatty change, no fatty degeneration and no area of infiltration by inflammatory cells. Animals that were fed diet containing 4% garlic powder (hepatotoxic rats) displayed a considerable level of liver damage, while those placed on diet containing V. amygdaлина and T. occidentalis leaf powders at varied concentrations apparently annuls the deleterious effects produced by garlic on the liver cells comparable to normal. The observed architectural features in the hepatocytes of vegetables treated groups compared favourably with normal control. Generally, the effects noticed were in consonance with the results of biochemical analysis.

CONCLUSION

The amelioration of deleterious effects posed by ingestion of 4% garlic powder through co-feeding of the leaf powders of T. occidentalis and V. amygdaлина is a pointer to their hepatoprotective potentials in rats. Thus, their complementary efficacy can be harnessed in the management of liver related disorders.

REFERENCES


