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Mitigating potential and antioxidant properties of aqueous seed extract of *Leea guineensis* against dichlorovos-induced toxicity in Wistar rats

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This work was carried out to access the mitigating potential of *Leea guineensis* aqueous seed extract against dichlorovos (DDVP)-induced toxicity in Wistar rats for ten days. Twenty Wistar albino rats (weighing 90 to 106 g) were divided into four groups (Normal, DDVP-induced untreated, DDVP-induced treated with 200 mg/kg of *L. guineensis* seed and DDVP-induced treated with 400 mg/kg *L. guineensis* seed by oral gavage). DDVP was induced in the rats as a source of the main drinking water (5% v/v). The levels of malondialdehyde (MDA), total protein, albumin, bilirubin and the activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined. The results revealed that exposure of rats to the pesticide water (DDVP) resulted in significant increase ($p<0.05$) in the levels of MDA and bilirubin with significant decrease ($p<0.05$) in the levels of total protein, albumin and the activities of GPx, CAT, SOD, ALT and AST, while administration with *L. guineensis* seed showed ameliorative effects in all biochemical parameters evaluated. This showed that treatment with aqueous seed extract of *L. guineensis* (200 and 400 mg/kg), most especially 400 mg/kg could ameliorate the biochemical indices related to liver toxicity in the animals.

Key words: *Leea guineensis,* dichlorovos, pesticide, antioxidant enzymes, oxidative stress.

INTRODUCTION

Pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors (Prakasam et al., 2001). The use of pesticides causes severe environmental and health hazards to organisms (Abdollahi et al., 2004). Organophosphate compounds are widely used and high insecticidal activity, low environmental persistence, and moderate toxicity, organophosphate compounds are the most common insecticides. They are widely used in agriculture, medicine, industry and have caused severe include some of the toxic chemical agents. Due to their environmental pollution (Al-Saleh, 1994; Storm et al.,

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2000).

Recently, more than 100 different organophosphate compounds have been synthesized and are extensively used worldwide (Buyukokuroglu et al., 2008*).* Organophosphate pesticides (e.g. 2, 2-dichlorovinyl dimethyl phosphate [DDVP]) are known to cause inhibition of acetyl cholinesterase (AChE) activity in the target tissues (Jayaratnam and Maroni, 1994) which accumulates acetylcholine and may prevent the smooth transmission of nerve function leading to convulsions and death. Exposure of organophosphate pesticides is associated with toxic effects on humans and animals (De-Bleeckeer et al., 1993; Betrosian et al., 1995; Tsatsakis et al., 1998; Hagar et al., 2002). Toxicity of organophosphate pesticides results in negative effects on many organs and systems such as the liver, kidney, nervous system, immune system and reproductive system (Aly and El-Gendy, 2000). Pesticides are used daily and internationally on a massive scale. They have conferred immense benefits to human kind by improving health and nutrition. Pesticides fall into numerous chemical classes, which have widely differing biological activities and thus differing potential to produce adverse effects in living organisms, including humans (Timothy and Ballantyne, 2004).

Pesticides are known to increase the production of reactive oxygen species (ROS), which in turn generate oxidative stress in different tissues (Heikal et al., 2010; Rai and Sharma, 2007). Many studies have implicated oxidative damage as the central mechanism of toxicity (Kalender et al*.,* 2010). Oxidative damage primarily occurs through production of ROS, including hydroxyl radicals and hydrogen peroxide that are generated during the reaction and react with biological molecules, eventually damaging membranes and other tissues. Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipids bilayers (Ogutcu et al*.,* 2008) and they may damage membranes by inducing lipid peroxidation (LPO) (Heikal et al*.,* 2011).

Leea guineensis (botanical name) is a genus of plants that are distributed throughout Northern and Eastern Australia, New Guinea, South and Southeast Asia and parts of Africa including Nigeria. *Leea* genus contains approximately seventy species (Stevens, 2001). It has an English name called Red tree vine or Hansid hapan. The genus was named by Linnaeus after James Lee, the Scottish nurseryman based in Hammersmith, London, who introduced many new plant discoveries to England at the end of the 18th century (Shephard, 2003). It belongs to subfamily Vitacea and family Leeaceae with the local name(s) Ahugbokita in Yoruba Language and Okatakyi in Twi Language. It is an evergreen shrub or small tree native to tropical Africa. Leea trees are vigorous growers and need quite a lot of space. It is an understory species that grows in shady locations under the cover of taller trees. The leaves have 2 to 3 pinnates emerge in light green, but mature to a glossy dark green. *L. guineensis* is propagated by stem cutting or by seed. It is best grown in rich, evenly moist, but well-drained soils in part shade. Seed germinate in 14 to 21 days at 70°F and outdoors; it can grow to 6 to 20' tall. The plant is native to moist intermediate temperate zones in tropical Africa including Cote d'Ivoire, Liberia, Sierra Leone, Ghana, Cameroun, and Nigeria. The plant is used in the treatment of enlarged spleen in children, pregnancy detection, purgative, toothache, gonorrhea, general weakness, skin lesions, skin rash, ulcer, diarrhea, dysentery as a diuretic, oral treatment, as a pain killer, paralysis, epileptic fits (juice of fresh leaves used as an enema), convulsions, spasm, stomach troubles, herpes and boils (Molina, 2009). Therefore, the aim of this study is to evaluate mitigating potential and antioxidant properties of aqueous seed extract of *L. guineensis* against dichlorovos-induced toxicity in Wistar rats.

MATERIALS AND METHODS

L. guineensis (Plate 1) were purchased from evergreen forest in Osin-Ekiti in Ekiti State, Nigeria. Authentication was carried out at Plant Biology, Ekiti State University, Ado-Ekiti.

L. guineensis seed processing and preparation of its aqueous extract

L. guineensis seeds were oven dried at 60°C for three days, thereafter the seeds were blended in a blender, to obtain a powder form, which were then soaked in water for 24 h (1:10 w/v), after which it was sieved and freeze-dried to obtain a constant weight.

Experimental animals

Albino rats (20 *Rattus norvegicus*) with an average weight of 99.43 to 122.40 g were obtained from the animal house of the Department of Biochemistry, Afe Babalola University, Ado-Ekiti, Ekiti State.

Methods used for *in vitro* aqueous extract determinations

Vitamins and minerals in the sample were determined using AOAC (2000). Hydroxyl radical scavenging activity of sample was determined according to the method of Wang et al. (2009). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging assay was determined using the method of Sun et al. (2002) and Shimada et al. (1992). The chelating activity of ferrous ions of the sample extract was determined by the method of Decker and Welch (1990).

Animal study design

Female and male albino rats (*Rattus norvegicus*) were used for the experiment. The environment were kept cleaned and disinfected, the rats used were twenty (20) in number. The rats were initially weighed upon arrival after which they were acclimatized for one week giving them the standard rat pellet (obtained from Ladokun Feed Mill Nigeria Limited, Ibadan, Nigeria) and water.

Pesticide water preparation

DDVP (50 ml) was diluted in 1 L of distilled water. This 5% solution was used into different experimental groups.

Animals grouping

The rats were randomly distributed into four treatment groups of five rats each. The groups were: Group 1: Consist of animals fed with the standard rat pellet and water (control); Group 2: Consist of animals fed with standard rat pellet and pesticide water (DDVP 5% v/v solution) (Negative control); Group 3: Consist of animals fed with standard rat pellet + pesticide water (DDVP 5% v/v solution) + 200 mg/kg of *L. guineensis* seed aqueous extract; and Group 4: Consist of animals fed with standard rat pellet + pesticide water (DDVP 5% v/v solution) + 400 mg/kg of *L. guineensis* seed aqueous extract.

Rats in each group were weighed individually at the beginning and at the end of the feeding period, which lasted for ten days.

Collection and treatment of blood samples

After ten days of feeding, the animals were sacrificed by simply incising the jugular vein; the blood samples were collected into plain sample tubes for serum analysis, respectively, which were allowed to stand at room temperature for 30 min to form clot after which it was centrifuged at 1000 g (gravity) for 15 min. After centrifugation, the clot forms sediment at the bottom of the centrifuge and the supernatant which is the serum was collected using a Pasteur's pipette. The serum, thus obtained were appropriately labeled and stored in a freezer at -5°C until required for further analysis.

Preparation of tissues for biochemical analyses

Following the daily exposure for 10 days, the animals were sacrificed 24 h after the last dose. The liver was excised and washed in ice-cold 1.15% KCl solution, dried using filter paper and weighed. They were then homogenized in 5 volumes of 50 nm Tris-HCl buffer (pH 7.4) containing 1.15% KCl, and centrifuged at 10,000 for 15 min.

Biochemical analysis

Alanine aminotransferase (ALT) was determined using the method of Thefeld et al. (1974) and Wallhpofer (1974) for aspartate aminotransferase (AST). Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation (Adam-Vizi and Seregi, 1982). Catalase activity was determined according to the method of Sinha (1972). The level of superoxide dismutase activity in microsomes was determined by the method of Fridovich (1989). Glutathione peroxidase (GPx) activity in the sample was determined according to the method of Rotruck et al. (1973).

In addition, histopathologies of the tissues were carried out by fixing the tissues in 10% formalin dehydrated in 95% ethanol and then cleared in xylene before being set in paraffin. Sections (about 4 µm) were prepared and stained with hematoxylin and eosin (H&E), and was examined under a light microscope with magnification of 400.

Statistical analysis

All values were expressed as the mean ± standard deviation (SD) of five animals in each groups. Data analyzed using one-way analysis of variance (ANOVA) followed by the Post-Hoc Duncan Multiple Range test for analysis of biochemical data using Statistical Package of Social Sciences (SPSS, 16.0). Values were considered statistically significant at $p<0.05$.

RESULTS

Table 1 shows the vitamins composition of *L. guineensis*. *L. guineensis* is composed of four vitamins; vitamin A (1264.4 Ug/100 g), Vitamin E (16.54 Ug/100 g), Vitamin D (9.72 Ug/100 g) and having low concentration of Vitamin C (8.29 Ug/100 g). Table 2 shows the mineral composition of *L. guineensis* seed and reveals the presence of selenium, sodium, calcium, manganese and zinc. High concentration of manganese (156.87 kg) and selenium (2.63 µg/kg) was observed.

Figure 1 shows the percent of OH scavenging activity of *L. guineensis* aqueous seed extract. The curve shows that an increase in the activity of OH results in an increase in the concentration of the extract. Figure 2 shows the percent of DPPH scavenging activity in dichlorovos-induced toxicity in rats treated with *L. guineensis* aqueous seed extract for 10 days. The curve shows that an increase in the activity of DPPH results in an increase in the concentration of the extract. Figure 3 shows the percent of iron chelation effect of *L. guineensis* aqueous seed extract. The curve shows that an increase in the effect of iron chelation results in an increase in the concentration of the extract. Figure 4 shows the percent of Ferric Reducing Antioxidant Power (FRAP) of *L. guineensis* aqueous seed extract. The curve shows that an increase in the concentration of the extract results in an increase in FRAP.

Figure 5 shows the percent NO scavenging activity of *L. guineensis* aqueous seed extract. The curve shows that an increase in the concentration of the extract results in an increase in the activity of percent of NO scavenging activity.

Table 3 shows the effect of aqueous seed extract of *L. guineensis* on the body weight of dichlorovos induced Wistar rats. There was a significant decrease in the final weight of the negative group as compared to the control group, and significant increase in the final weight of Groups 3 and 4.

In Table 4, there were significant increase ($p<0.05$) in serum and liver of the negative control group (which is the DDVP-induced group) when compared with the control group, while those treated with aqueous extract of *L. guineensis* (200 and 400 mg/kg) significantly reduced the activities of the transaminases (AST and ALT) of the serum and liver in the dichlorovos induced rats to values that was statistically similar ($p<0.05$) to the control group. All the changes induced by dichlorovos intoxication were significantly ($p<0.05$) restored to near normal levels on administration of *L. guineensis*. In Table 4, there were significant decrease ($p<0.05$) in the negative group when

Table 1. Vitamins composition of *Leea guineensis* seed (Ug/100 g).

Vitamin	Composition
A	1264.40±0.12
C	8.29±0.15
D	9.72±0.05
E	16.54±0.12

Each value is a mean of three determinations ± SEM.

Table 2. Minerals composition of *Leea guineensis* seed.

Mineral	Composition
Se (µg/kg)	2.63±0.12
Na (%)	0.08±0.01
Ca (%)	0.16±1.20
Mn (mg/kg)	156.87±0.01
Zinc (mg/kg)	48.67±0.01

Each value is a mean of three determinations ± SEM.

compared with control group while those treated with aqueous extract of *L. guineensis* (200 and 400 mg/kg) significantly increased the activity of serum total protein and albumin of the liver of the dichlorovos induced rats to values that was statistically similar ($p<0.05$) to the control group, while there was a significant increase ($p<0.05$) in the negative group when compared with the control group, while treatment with aqueous extract of *L. guineensis* (200 and 400 mg/kg) significantly increased the activity of total bilirubin and direct bilirubin of the serum of the negative group to values that was statistically similar ($p<0.05$) to the control. All the changes induced by dichlorovos intoxication were significantly ($p<0.05$) restored to near normal levels on administration of *L. guineensis*.

In Table 6, there were significant increase ($p<0.05$) in serum and liver of the negative group when compared with control group, while when treated with aqueous extract of *L. guineensis* (200 and 400 mg/kg) significantly reduced the activity of malondialdehyde (MDA) of the serum and liver of the negative group to values that was statistically similar ($p<0.05$) to the control. All the changes induced by dichlorovos intoxication were significantly ($p<0.05$) restored to near normal levels on administration of *L. guineensis*.

In Table 7, there were significant decrease ($p<0.05$) in serum and liver of the negative group when compared with control group while those treated with aqueous extract of *L. guineensis* (200 and 400mg/kg) significantly increased the activity of GPx, SOD and CAT of the serum and liver of the negative group to values that was statistically similar ($p<0.05$) to the control. All the changes induced by dichlorovos intoxication were significantly ($p<0.05$) restored to near normal levels on administration of *L. guineensis*.

Histology results of dichlorovos-induced toxicity in rats treated with *L. guineensis* seed extract

Histopathological studies of the liver of control Wistar rats showed normal histology (Figure 6a). For rats to which dichlorovos only was administered, portal congestion, periportal cellular infiltration, and vacuolar degeneration of hepatocytes were observed (Figure 6b). The group to which DDVP was simultaneously administered with 200 mg/kg of aqueous extract of *L. guineensis* also showed almost normal liver histology (Figure 6c), while the group to which DDVP was simultaneously administered with 400 mg/kg of aqueous extract of *L. guineensis* also showed normal liver histology (Figure 6d).

DISCUSSION

Toxicity in human is a threatening truth and much more than any disease caused by organism as toxic substances are everywhere in air, in water and in food (Paliwal et al., 2009). Many compounds which are essential to use for human welfare are at the same time injurious when viewed from safety point. Some compounds are not directly used by humans but indirectly they enter human (through food chain) and induce injuries.

Pesticides are examples of compounds that are used against various pests for human welfare, but are also harmful to humans as they eventually find themselves within human body via food chain. Liver is the primary organ that handles toxic substances in the body and as such it suffers the hazardous effects of these substances first. According to Williams et al. (2005), any change in liver systematic will definitely affect complete metabolism of an animal. It was shown that the levels of 'marker' enzymes in tissues and biological fluids may be altered following the administration of foreign agents and as such alterations can be used to assess the assault inflicted on the tissue cellular system of experimental animals, hence, their use in this study (Akanji et al., 1993; Shahjahan et al., 2004; Yakubu et al., 2006).

The results show that the *L. guineensis* seed has appreciable amounts of vitamin A, C, D and E. The presence of these minerals coupled with zinc, manganese, and selenium (Tables 1 and 2) could be responsible for the protective properties observed in this study (Imafidon, 2012).

The effect of the administration of aqueous extract of *L. guineensis* seed on body weight of dichlorovos-induced Wistar rats is as shown in Table 3. The administration of the pesticides bring about a significant decrease in the final weight of the negative control group and a significant increase was observed in the final weight of the groups treated with 200 and 400 mg/kg after administration of

Table 3. Effect of aqueous seed extract of *Leea guineensis* on body weight of dichlorovos-induced Wistar rats.

Group	Initial weight (g)	Final weight (g)
Control	126.00±8.51[a]	140.50±6.50[a]
Negative control	121.00±4.31[a]	61.50±5.50[d]
Dichlorovos + 200 mg/kg of *Leea guineensis*	122.00±2.67[a]	71.00±3.00[c]
Dichlorovos + 400 mg/kg of *Leea guineensis*	100.00±1.11[b]	90.00±1.00[b]

Each value is a mean of five determinations ±SEM. Values with different superscript (a, b, c and d) across each column are significantly different ($p<0.05$).

Table 4. Effect of *Leea guineensis* on dichlorovos-induced rats on serum and liver AST and ALT activities.

Group	AST (U/L)		ALT (U/L)	
	Serum	Liver	Serum	Liver
Control	2.01±0.01[a]	57.80±5.42[a]	9.37±1.45[a]	150.10±2.01[a]
Negative control	21.00±2.10[d]	100.04±4.62[d]	22.30±5.56[c]	200.00±1.21[d]
Dichlorovos + 200mg/kg of *Leea guineensis*	10.31±1.01[c]	74.20±5.32[c]	11.00±1.91[b]	174.00±2.10[c]
Dichlorovos + 400mg/kg of *Leea guineensis*	6.20±1.21[b]	64.20±11.64[b]	9.27±5.61[a]	168.00±3.01[b]

Each value is a mean of five determinations ±SEM. Values with different superscripts (a, b, c and d) across each column are significantly different ($p<0.05$).

the pesticide in comparison to negative control. This implies that the toxic effect of the pesticides resulted in a noticeable weight reduction and the administration of the extract tends to normalize the loss in body weight. This could be attributed to the interaction of pesticides with biological tissue resulting in a reduction in the body mass after administration of the toxicant.

The measurement of the activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation and diagnosis (Fishman, 2006) and to a reasonable extent, the toxicity of drugs including plant extract (Wroblewski et al., 1956). Tissue enzyme assay can also indicate tissue cellular damage long before structural damage can be picked up by conventional histological techniques (Szasz, 1969). Enzymes do not usually originate from the serum, but rather are derived from the disintegration, metabolism, and turn-over of tissues and blood cells. Therefore, enzymes from diseased tissues and organs may become manifested in the serum resulting in increased activity (Mahajan, 1997). Aminotransferase which include alanine aminotransferase (ALT) otherwise referred to as glutamate pyruvate transaminase (GPT) and aspartate aminotransferase (AST) otherwise referred to as glutamate oxaloacetate transaminase (GOT) are enzymes located in the cytosol and mitonchondria where they are involved in the transfer of amino group from α-amino to α-keto acids. They are also involved in the biochemical regulation of intracellular amino acid pool (Chapatwala et al., 1982). These aminotransferase belong to the plasma non-functional enzymes which are normally localized within the cells of liver, heart, kidney, and muscles. Their presence in serum may give information on tissue injury or organ dysfunction (Wells et al., 1986). Blood and tissues levels of ALT and AST can be used to assess the toxic impact of chemical compound.

They are present in hepatocytes, leaks into the blood with liver cell damage (Wroblewski et al., 1956). The increase in transaminase activity in the liver is an indication of hepatocellular injury that occurs due to formation of reactive oxygen species and reactive intermediates after the treatment of the pesticide (Bandyopadhyay et al., 1999).

Aspartate aminotransferase is primarily found in the liver mitochondrial and cytoplasm. It is also found in heart, muscles, kidney, and brain. Its serum level increases in hepatic necrosis, myocardial infarction, and muscles injury (Srinivasan and Radhakrishnamurthy, 1977).

Alanine aminotransferase is a liver cytosol enzyme more specific to the liver so that a rise only occurs with liver disease (Poli et al., 1987). Generally, decrease in ALT and AST in the serum may perhaps suggests that the experimental diet confer protection on the liver tissues against injury, damage or disease, which are often the direct cause of elevation of the enzymes in the blood stream (Sanjiv, 2002).

The transaminases activity of DDVP-induced rats treated with aqueous extract of *L. guineensis* seed is as shown in Table 5. Inducing of DDVP in the negative group was shown to bring about an increase in the excessive excretion. The induction of dichlorovos in Wistar rats has displayed such effect, as indicated by the significant decrease ($p<0.05$) in the total serum proteins of the negative group when compared with the control group and a significant increase was observed in the treatment groups (Groups 3 and 4) which were treated with 200 and 400 mg/kg of *L. guineensis* seed after administration of the pesticide (Table 6).

Table 5. Effect of *L. guineensis* on dichlorovos-induced rats on some liver function indices.

Group	Total protein (g/dl)	Albumin (g/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Control	0.76±0.01[a]	1.09±0.38[a]	2.79±0.15[a]	6.10±0.50[a]
Negative control	0.56±0.01[b]	0.18±0.08[d]	4.79±0.09[d]	10.80±0.40[d]
Dichlorovos + 200 mg/kg of *Leea guineensis*	0.65±0.29[a]	0.44±0.05[c]	4.18±0.42[c]	7.30±1.30[c]
Dichlorovos + 400 mg/kg of *Leea guineensis*	0.81±0.35[a]	0.78±0.01[b]	3.82±0.53[b]	6.30±0.10[b]

Each value is a mean of five determinations ± SEM. Values with different superscripts (a, b, c and d) are significantly different ($p<0.05$).

Table 6. Effect of *L. guineensis* on dichlorovos-induced rats on lipid peroxidation (MDA) ($\times 10^{-6}$ nmol/ml).

Group	Serum	Liver
Control	0.52±0.08[a]	3.02±0.03[a]
Negative control	2.21±0.06[d]	6.23±0.35[c]
Dichlorovos + 200 mg/kg of *Leea guineensis*	0.44±0.66[c]	4.23±0.01[b]
Dichlorovos + 400 mg/kg of *Leea guineensis*	0.21±0.58[b]	3.27±0.17[a]

Each value is a mean of five determinations ± SEM. Values with different superscripts (a, b, c and d) are significantly different ($p<0.05$).

Albumin which is manufactured by the liver can be used to assess the health status of liver. It is the major protein present within the blood (Yakubu et al., 2003). Low serum albumin has also been associated with low protein intake. Albumin serves in the maintenance of osmotic pressure of the blood and body fluids, and transport of inorganic anions, fatty acids, and drugs (Brunt, 1984). Therefore, decrease in serum albumin level would affect the metabolism of these substances that are transported by it (Pasternak, 2000). Any effect that negatively affects albumin content would be expected to have a deleterious impact on total plasma proteins as in massive hepatic necrosis, chronic cirrhosis and other disorders with significant destruction or replacement of liver cells. In these activities of ALT and AST in both liver and serum of the rats and administration of 200 and 400 mg/kg of the *L. guineensis* seed extract brought about a significant ($p<0.05$) decrease in the activities. This can imply that due to the noxious effect of DDVP, there was an increase in the activity of ALT and AST and the administration of *L. guineensis* seed aqueous extract posses a tendency to control the increased activity in transaminase. For example, administration of the toxicants has been reported to bring about an increase in the transaminase activity of the serum and hepatic tissues (Imafidon, 2012).

Total proteins and albumin are plasma proteins that measure synthetic function of the liver. They help in maintaining blood osmotic pressure. Hypoproteinaemia is the deficiency of protein in the plasma, partly due to dietary insufficiency or

In this present study, there was observable depletion of albumin in the DDVP-induced rats when compared with the control. However, the administration of *L. guineensis* seed (200 and 400 mg/kg) in Groups 3 and 4 (most especially 400 mg/kg) showed a significant ($p<0.05$) increase in the concentration level of albumin (Table 6). This implies that the toxic effect of the pesticides resulted in a noticeable decrease in the serum total proteins and albumin and the administration of the extract tends to normalize the decrease of serum total protein and albumin. Previous studies have reported that induction of toxicant have a

Table 7. Effect of *L. guineensis* on dichlorovos-induced rats On GPx, SOD And CAT activities.

Group	GPx (m/ml)		SOD (m/mg protein)		CAT (unit/mg protein)	
	Serum (×10^2)	Liver (×10^2)	Serum	Liver	Serum	Liver
Control	2.22±0.01^a	3.18±18.00^a	2.98±5.11^a	6.92±4.50^a	0.99±0.11^a	1.15±0.02^a
Negative control	0.92±1.44^b	1.05±2.10^b	0.93±6.90^c	2.20±9.50^d	0.34±0.01^c	0.18±0.01^d
Dichlorovos + 200 mg/kg of *Leea guineensis*	1.20±14.00^b	4.15±11.50^a	1.87±7.20^b	3.30±1.50^c	0.62±0.06^b	0.46±0.01^c
Dichlorovos + 400 mg/kg of *Leea guineensis*	2.18±1.21^a	5.86±70.00^a	2.46±4.30^a	4.97±1.66^b	0.89±0.01 ^{a}	0.80±0.02^b

Each value is a mean of five determinations ± SEM. Values with different superscripts (a, b, c and d) are significantly different (p<0.05).

negative effect on the serum total proteins and albumin leading to a decrease in the concentration levels (Nuhu and Aliyu, 2008).

Bilirubin is the major breakdown product that results from the destruction of old red blood cells. It is removed from the blood by the liver; hence, it is a good indication of the function of liver. Bilirubin concentration is elevated in the blood either by increased production of bilirubin or decreased liver uptake (as a result of liver disease). Chebeseborough (1992) reported that a rise in the concentration of serum bilirubin indicate or suggests liver damage since the liver serves as an excretory unit rather than a distributing unit for bilirubin. Total and conjugated bilirubin are formed through breakdown of red blood cells by hepatocytes and used to access extent of hepatocellular damage (Paliwal et al., 2011).

In this study, it was observed that there was a significant increase (p<0.05) in the level of bilirubin concentration in the negative group which were induce with dichlorovos when compared with the normal and a significant decrease (p<0.05) in the concentration levels of bilirubin in the treatment groups (Groups 3 and 4) which were administered 200 and 400 mg/kg of aqueous extract of *L. guineensis* seed, respectively. It has been reported that when a toxicant is induced, it leads to hyperbilirubinaemia (high level of bilirubin) which is often the first and sometimes the only manifestation of a liver disease (Nuhu, 2008). Also, the low level of malondialdehyde (Table 6) in the treatment groups which were treated with 200 and 400 mg/kg of *L. guineensis* seed aqueous. Seed extract can be ascribed to high level of free radical scavenging compounds and enzymes (Table 7) which are known to protect cells against oxidative damage.

Studies have shown that excessive free radical production resulting in oxidative stress could be an important mechanism of organophosphate toxicity (Praassam et al., 2001). Dichlorovos, a volatile organophosphate compound with strong pesticide activity has been reported to alter the biological prooxidant-antioxidant balance in various toxicity studies (Hsu et al., 2001). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are common antioxidant enzymes which inhibit oxidative reactions.

Catalase (hydrogen peroxide/hydrogen peroxide oxidoreductase) is an important cellular antioxidant enzyme that defends against oxidative stress. It is found in the peroxisomes of most aerobic cells. It serves to protect the cell from toxic effects of high concentrations of hydrogen peroxide (H_2O_2) by catalyzing its decomposition into molecular oxygen and water, without the production of free radicals (Chelikani, 2004). Superoxide dismutase (SOD) is an antioxidant enzyme that catalyses the dismutation dismutation of superoxide (O^{-2}) into oxygen and hydrogen peroxide, thus an important defense in nearly all cells exposed to oxygen (Milani, 2011).

Glutathione peroxidase is the general name of an enzyme family with peroxidase activity whose biological role is to protect the organism from oxidative damage (Krishna, 2010). The administration of the pesticide (DDVP) brings about a significant decrease in the antioxidant enzymes level of the negative control group and a significant increase was observed in the treatment groups (Groups 3 and 4) treated with 200 and 400 mg/kg of *L. guineensis* seed, respectively after administration of the pesticide. This implies that the administration of the pesticide (DVVP) resulted in an increased rate of formation of free radicals causing reduction in the antioxidant level (Table 7) and the administration of the extract due to antioxidant properties scavenge the free radicals present in the hepatic tissues thereby leading to an increase in the level of the antioxidant enzymes. A recent study which supports this present study showed that dichlorovos induced oxidative stress in rats through abnormal production of ROS (Sharma, 2012). The histopathology evaluation of the liver

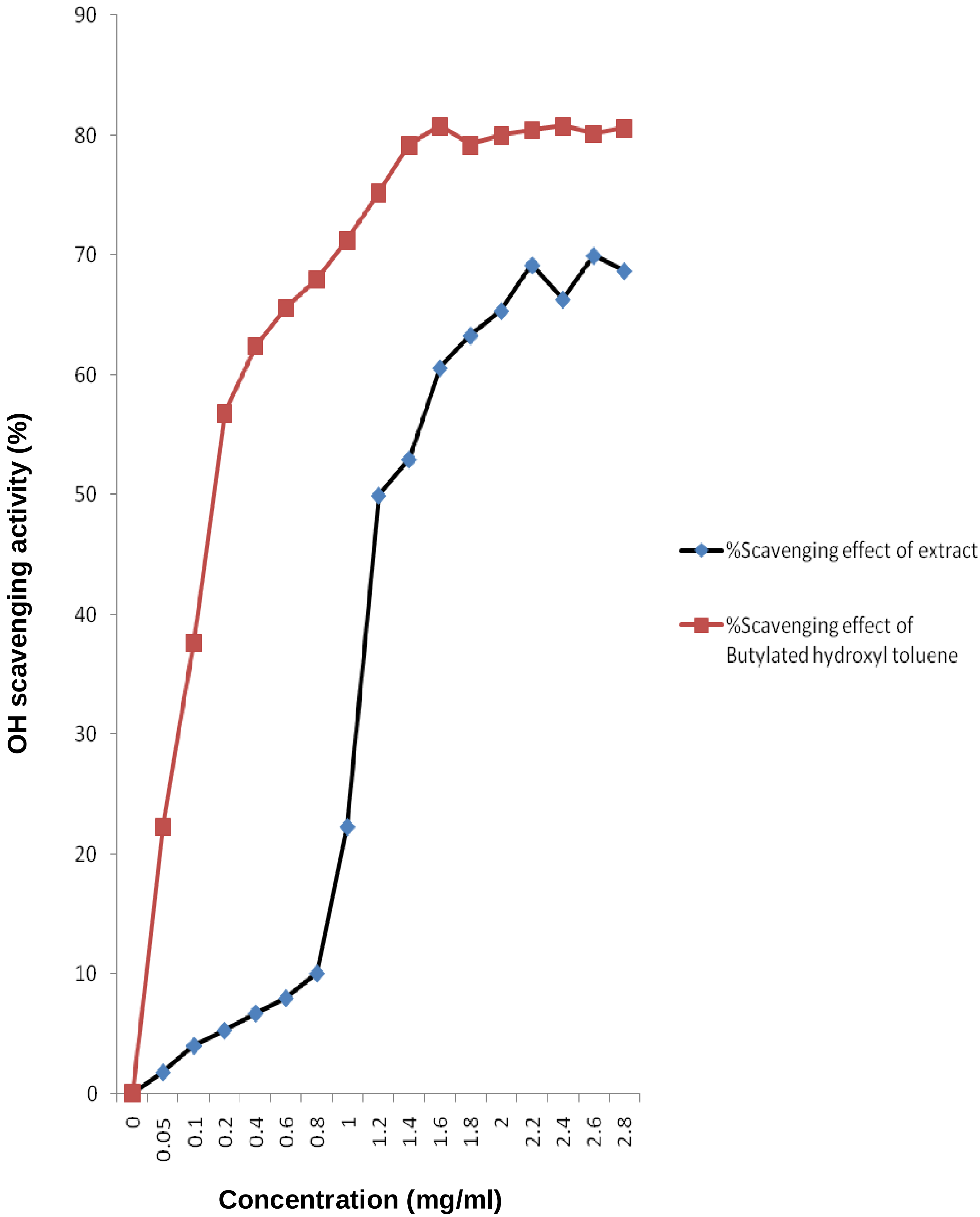

Figure 1. Percentage of OH scavenging activity of *L. guineensis* aqueous seed extract.

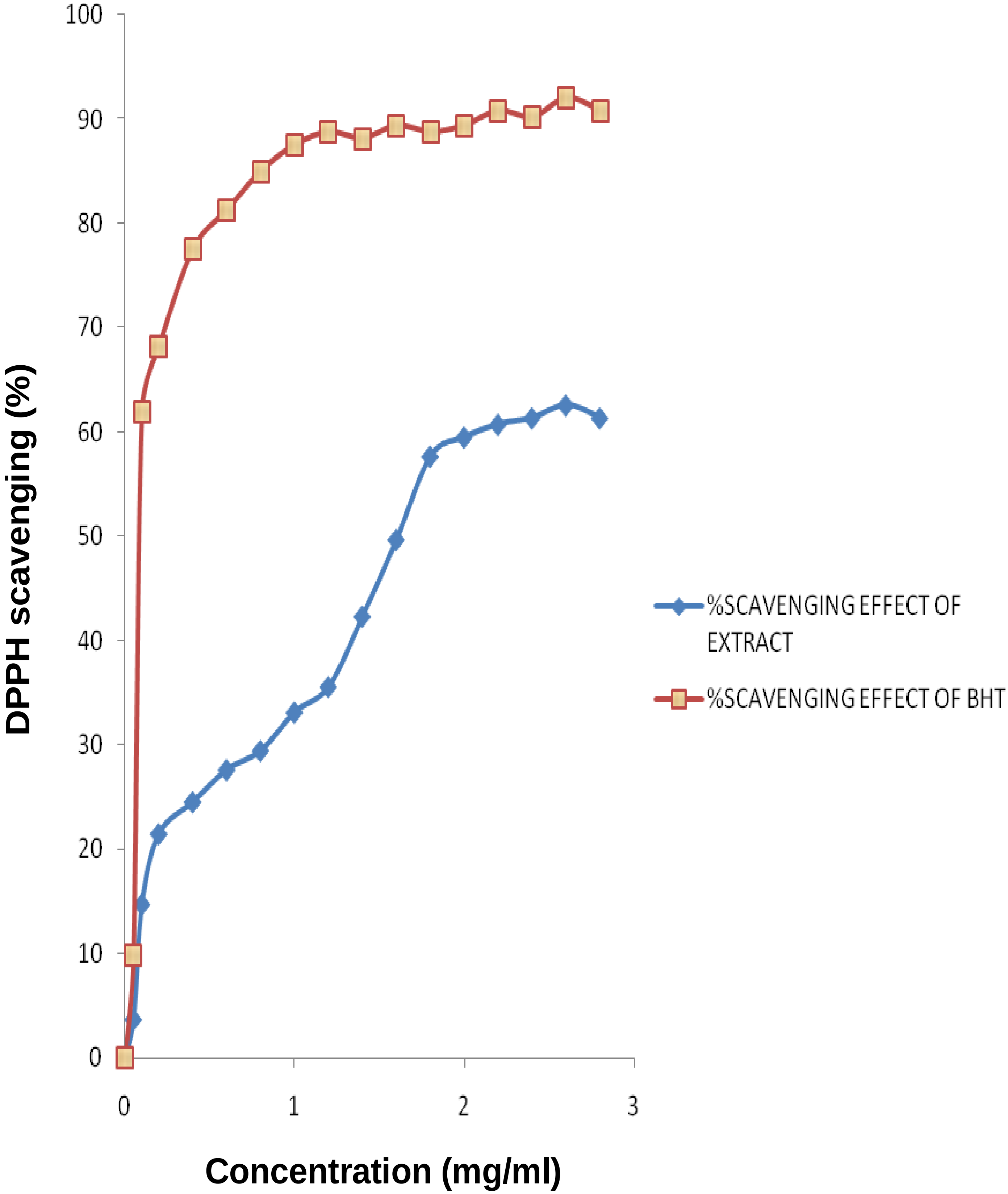

Figure 2. Percentage of DPPH scavenging activity of *L. guineensis* aqueous seed extract.

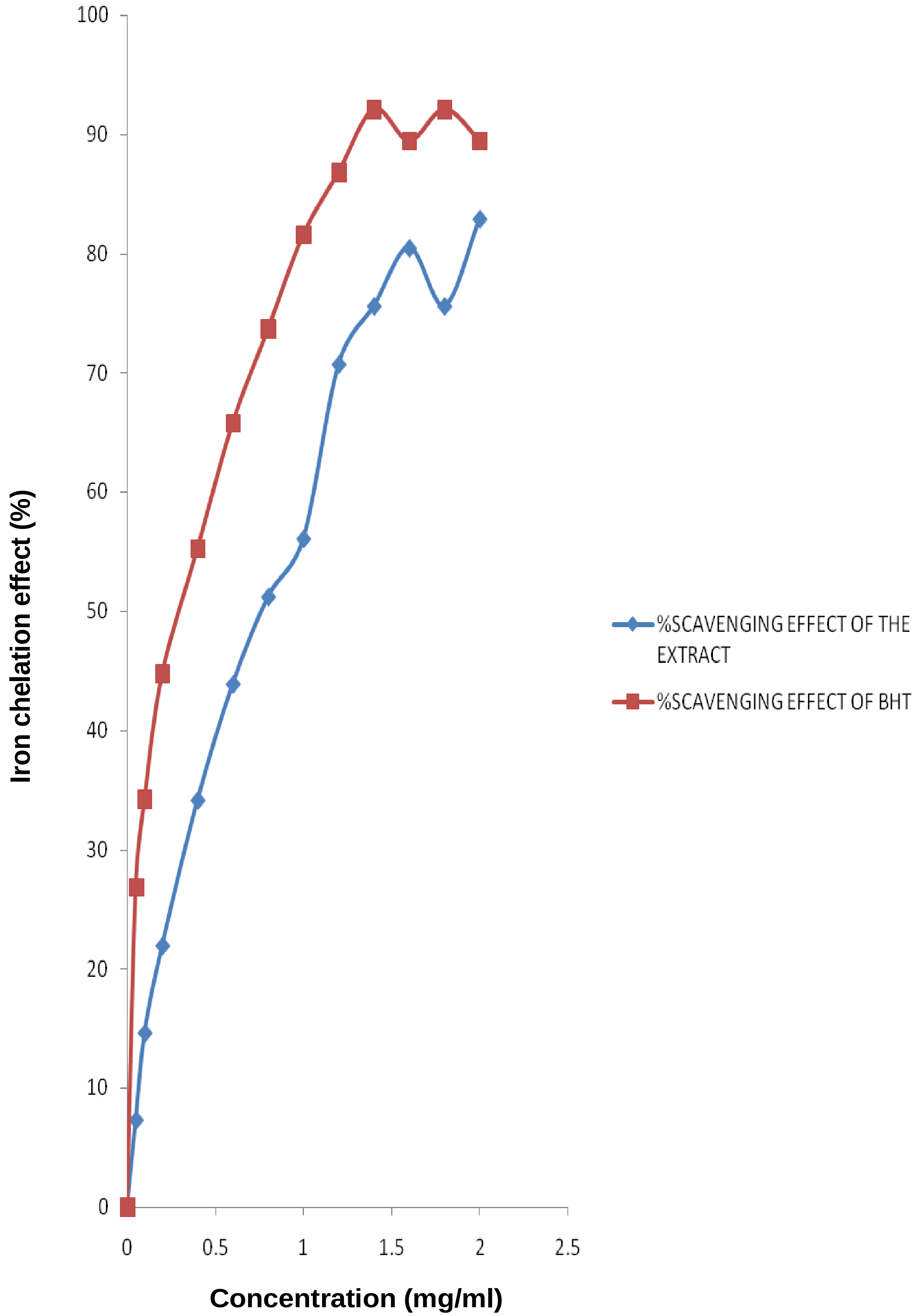

Figure 3. Percentage of iron chelation effect of *L. guineensis* aqueous seed extract.

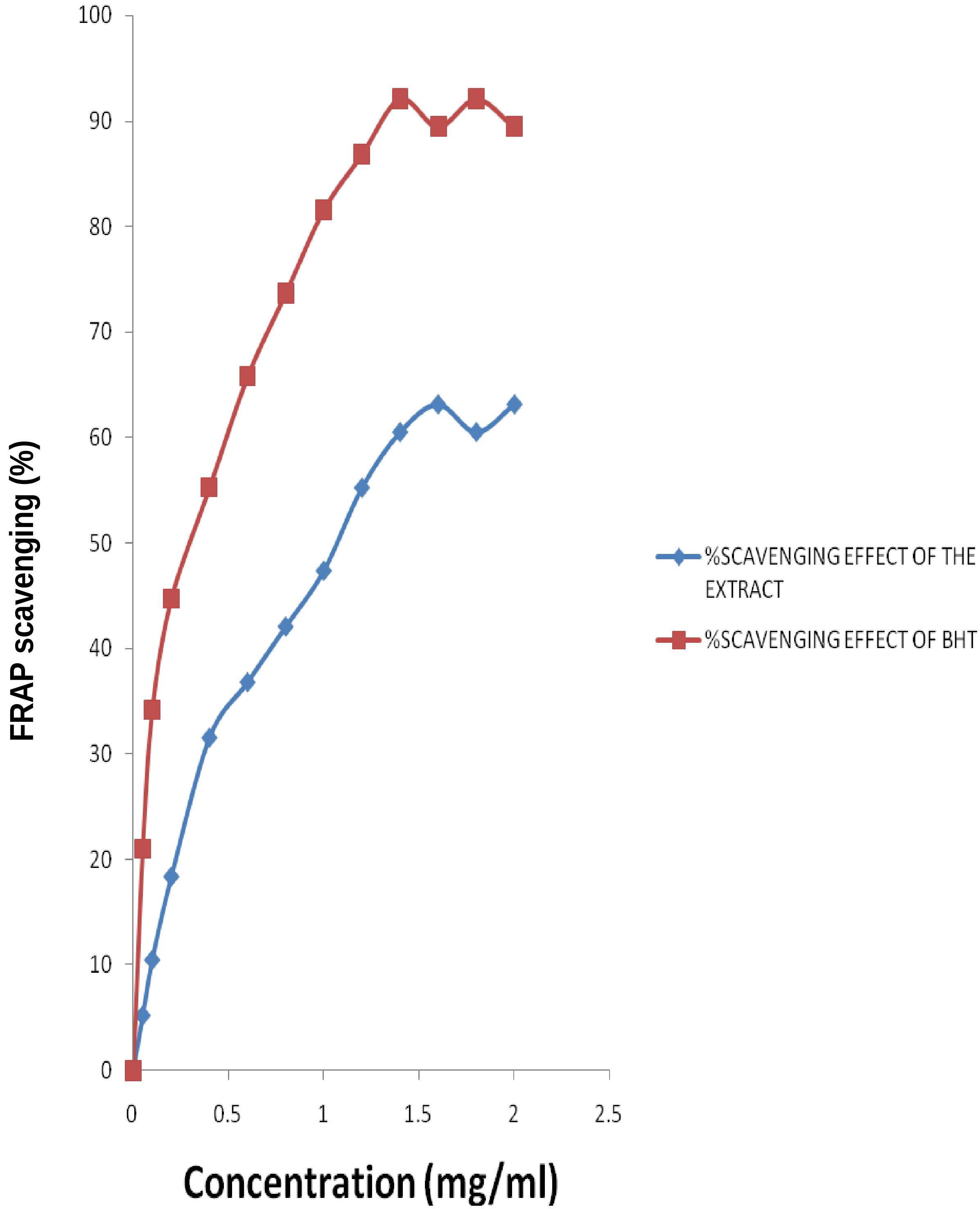

Figure 4. Percentage of FRAP Of *L. guineensis* aqueous seed extract.

liver shows that the liver of the DDVP-induced group was markedly damaged when compared with the control and the extract treated group.

The protective effect of the extract as shown in the result may be linked to the presence of antioxidant minerals, vitamins and other free radical scavenging compounds such as NO, FRAP, DDPH, OH and iron chelation (Figures 1 to 5). These have been reported that

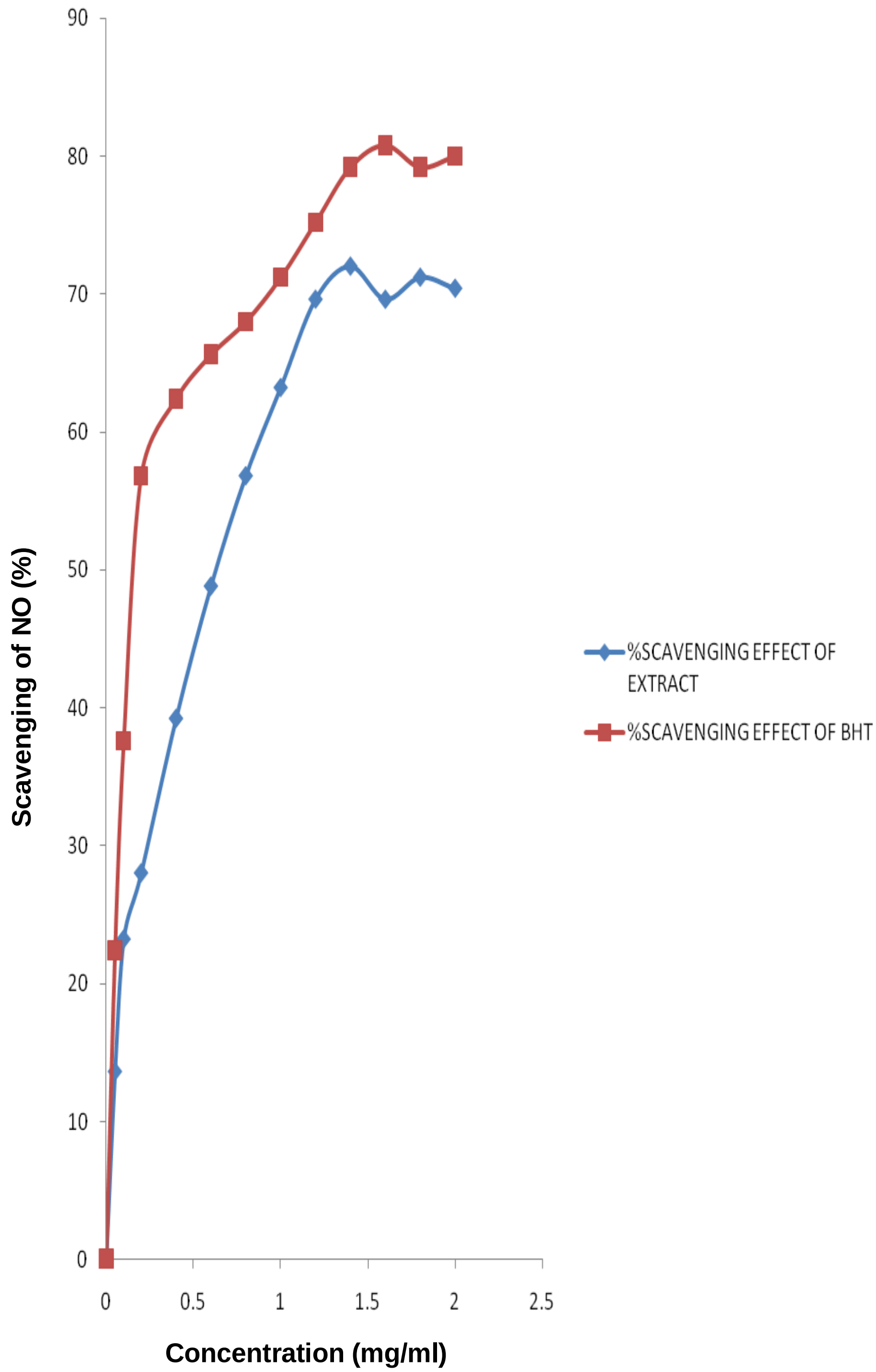

Figure 5. Percentage of NO scavenging activity of *L. guineensis* aqueous seed extract.

that they are very useful parameters in the detoxification/ neutralization of reactive oxygen species.

Conclusion

Dichlorovos pesticide is hepatotoxic in laboratory animals. *L. guineensis* seed has shown to protect liver against DDVP-induced oxidative stress by altering the levels of increased lipid peroxidation and enhancing decreased activities of CAT, SOD and GPx. Therefore, hepatoprotective effect of *L. guineensis* seed may be attributed to its antioxidant properties.

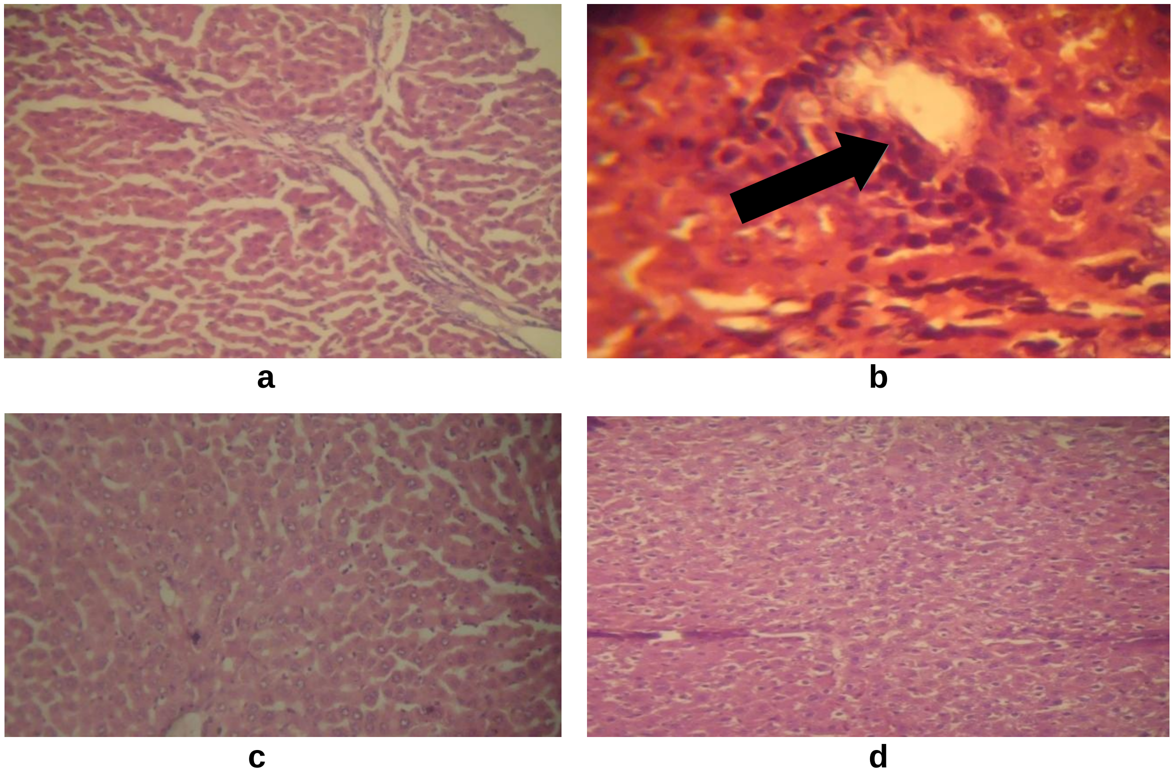

Figure 6. Changes in histology of liver samples of DDVP-induced toxicity in rats treated with *Leea guineensis* seed extract.
(a) Control, (b) DDVP induced, (c) DDVP + *L. guineensis* (200 mg/kg), (d) DDVP + *L. guineensis* (400 mg/kg). Black arrow shows portal congestion, periportal cellular infiltration, and vacuolar degeneration of hepatocytes.

Plate 1. *Leea guineensis*.

Conflicts of interest

No competing interests exist.

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