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Full Length Research Paper

Toxicological evaluation of *Zanthoxylum zanthoxyloides* (Lam) Zepernick & Timler root bark used as biopesticide and medicine

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Toxicological studies were carried out to investigate the effect of consumption of *Zanthoxylum zanthoxyloides* root bark widely used in traditional medical practices and as protectant of stored cereal products in Nigeria, using albino rats. Serum, kidney and liver were tested for oxidation stress and tissue damage markers; aspartate amino transferase (AST), alanine amino transferase (ALT), urea, bilirubin and creatinine contents, and Kidney and liver glutathione peroxidase (GPX), glutathione transferase (GST), non-protein sulphadryl (NP-SH), thiobarbituric acid reactive substances (TBARS), total sulphadryl (T-SH) and superoxide dismutase (SOD). These tests were carried out using commercially available kits. Results obtained for all doses (1, 5 and 10%) of the tested parameters did not significantly vary with control (p>0.05), indicating normal liver and kidney function (even in the face of variation in liver GPX and SOD. This study has proven that the plant is safe for human consumption at the rates or dosages tested.

Key words: *Zanthoxylum zanthoxyloides,* albino rats, toxicology, biochemical parameters.

INTRODUCTION

Zanthoxylum is the most widely distributed Rutaceae genus in the world with about 200 species identified; found in Africa, North and South America, Asia and Australia (Groppo et al., 2012). Africa has 35 species distributed throughout West Africa to the Cameroons. *Zanthoxylum zanthoxyloides* is a shrub or small tree, spiny, about 6-12m tall. The plant has been widely used in ethno-medicine and thoroughly investigated as anti-tumor, anti-leukemic, (de Moura et al., 1997; Nissanka et al., 2001), antimicrobial (de Abreu et al., 2003; Dongmo et al., 2009; Larsen et al., 2015), anti-HIV (Cheng et al., 2005), antimalarial (Jullian et al., 2006), anthelminthic (Ferreira et al., 2007; Barnabas et al., 2011), treatment of sickle cell disease (Ouattara et al., 2004), aphrodisiac, analgesic (Mann et al., 2003) antioxidant (Adekunle et al., 2012) and as biopesticide (Ogunwolu et al., 1998; Gbate and Alhassan, 2004; Gbate and Fasoranti, 2008; Udo, 2011; Ileke and Ogungbite, 2014; Zhang et al., 2017; Buxton et al., 2017; Osabutey et al., 2015, 2018).

Any plant with proven medicinal or pesticidal use must also be investigated for its toxicological effects, so that its direct or indirect consumption does not cause adverse

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side effect in the consumer. It is against this background that medicinal and pesticidal plants in recent times have become subject of toxicological studies (Sathya et al. , 2012; Adeyemo-Salami and Makinde, 2013; Adebiyi and Abatan, 2013; Ileke et al., 2014; Nwosu et al., 2017; Alelign et al., 2020). The widespread use of *Z. zanthoxyloides* in ethno-medicine and crop protection with acclaimed successes without equal interest in direct toxicological effect of its consumption by multitude of Nigerians is responsible for our current interest in the plant and this study.

MATERIALS AND METHODS

Root bark of *Z. zanthoxyloides* was obtained from Bida, Nigeria (latitude 9.6° north and longitude 6.1° east) in June 2018 and was dully identified in National Institute of Pharmaceutical Research and Development (NIPRD) Abuja with voucher number NIPRD/H/7101. The plant was dried under laboratory conditions: ambient temperature of 34±6°C and relative humidity of 41±5% and was grounded into fine powder for use as part of the feed. Adult female albino rats were obtained from Biochemistry Department of Federal University of Technology, Akure, Nigeria, with average weight between 150-160 g. They were acclimatized for two weeks prior to commencement of the experiment at ambient temperature of 27°C and relative humidity of 70% and diurnal cycle of 12 h.

Feed formulation and experimental groups

The diet was prepared according to Ileke et al. (2014) and Nwosu et al. (2017). Basal diet was made up of skimmed milk (44%), corn starch (42%), mineral and vitamin premix (4%) and vegetable oil (10%). The animals were grouped into four groups made up of:

Group I: Conventional feed plus 1% Z. *zanthoxyloides* for 30 days
Group II: Conventional feed plus 5% Z. *zanthoxyloides* for 30 days
Group III: Conventional feed plus 10% Z. *zanthoxyloides* for 30 days
Group IV: Control group was given conventional feed only for 30 days.

Collection of blood serum and tissue homogenates

At the end of the 30 day experimental period 5ml blood was collected from each rat and was centrifuged to obtain serum for biochemical assay of liver and kidney function. Thereafter the animals were dissected to harvest the liver and kidney. These tissues were rinsed in normal saline solution (1:3 W/V) and then homogenised in sodium phosphate buffer (pH 6.9). The homogenate was then centrifuged to obtain clear supernatants for biochemical assays of the kidney and liver (Nwosu et al., 2017).

Bioassay

Commercially available kits (Agappe Diagnostics, Switzerland) were used for analysis of plasma aspartate amino transferase (AST), alanine amino transferase (ALT), urea, bilirubin and creatinine contents, and Kidney and liver glutathione peroxidase (GPX), glutathione transferase (GST), non-protein sulphadryl (NP-SH), thiobarbituric acid reactive substances (TBARS), total sulphadryl (T-SH) and superoxide dismutase (SOD)

Data analysis

Graph Pad Prism version 8 Software (Graph Pad Software, San Diego, CA, USA) was used for statistical analysis. One way ANOVA was followed by Brown-Forsythe test, Bartlett's test and Tukey's multiple comparisons test (p-value < 0.05).

RESULTS

Effect of plant on serum indices

Figures 1 to 5 show result of serum analysis. There was initial decrease in serum ALT activity from 23.12 U/l in 1% treatment when compared to control but this increased to 34.0 U/l in 5% treatment to 38 U/l in 10% treatment. These were not significantly different at p>0.05. Serum AST activity also showed similar variation; from 128 U/l in 1% treatment to 154 U/l in 10% treatment against 175U/l in control. Serum bilirubin, creatinine and Urea all showed no significant difference between treatments.

Effect of plant on kidney indices

The results of biochemical assay of the kidney GPX, GST, NP-SH, T-SH and TBARS are shown in Figures 6 to 10 respectively. There was no significant difference between all the three treatments and the control in each of the enzyme activity.

Effect of plant on liver indices

Figure 11 shows the liver GPX variation among treatments; control, 1 and 5% have values of 0.0043, 0.0049 and 0.005 U/l respectively, not significantly different at p>0.05; at 10% treatment the liver GPX value was higher at 0.006 U/l which was significantly different at p>0.05. Figure 14 shows liver SOD levels among treatments; control and 1% treatment gave values of 78.83 and 79.53 U/l respectively which are higher than values obtained for 5 and 10% treatment (73.33 and 68.23 U/l) and significantly different at p>0.05. Figures 12 to 16 show value obtained for liver GST, NP-SH, TBARS and T-SH respectively. All the treatments in each of these groups did not vary significantly.

DISCUSSION

Biochemical indices provide much needed parameters for determining the level of damage or effect of foreign compounds (plant materials) on the blood and tissues of living animals (Odeyemi, 2008). It has been established that there is a relationship between serum biochemical indices, and liver and kidney functions of experimental

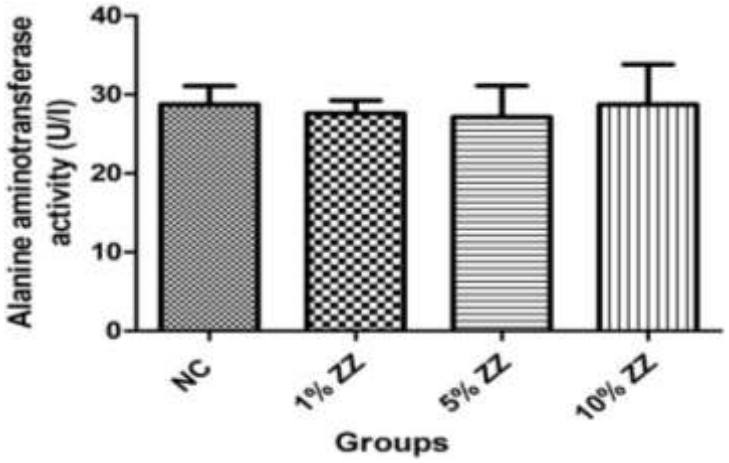

Figure 1. Serum ALT among treatments. NC: Control; ZZ: *Z. zanthoxyloides;* ALT: Alanine amino transferase.

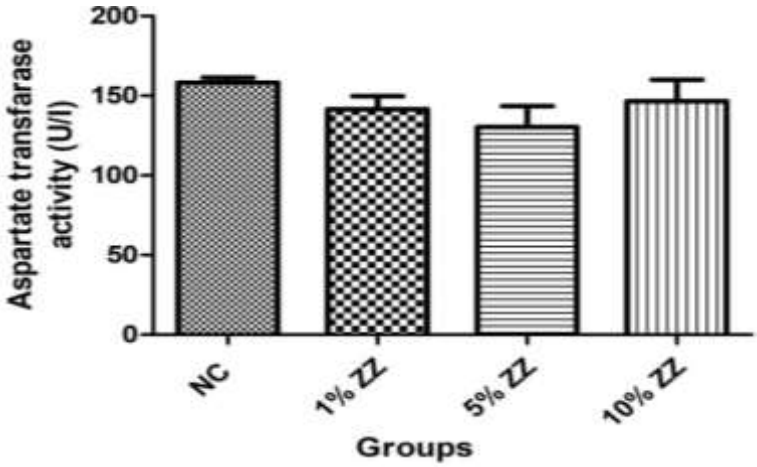

Figure 2. Serum AST among treatments. NC: Control; ZZ: *Z. zanthoxyloides;* AST: Aspartate amino transferase.

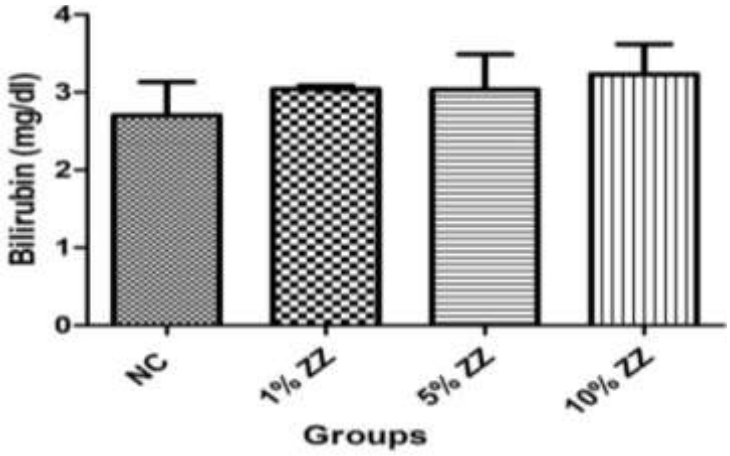

Figure 3. Serum Bilirubin among treatments. NC: Control; ZZ: *Z. zanthoxyloides*

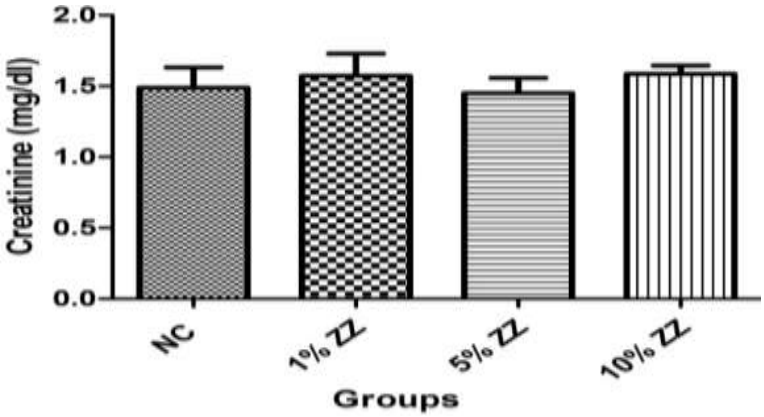

Figure 4. Serum Creatinine among treatments. NC: Control; ZZ: *Z. zanthoxyloides*

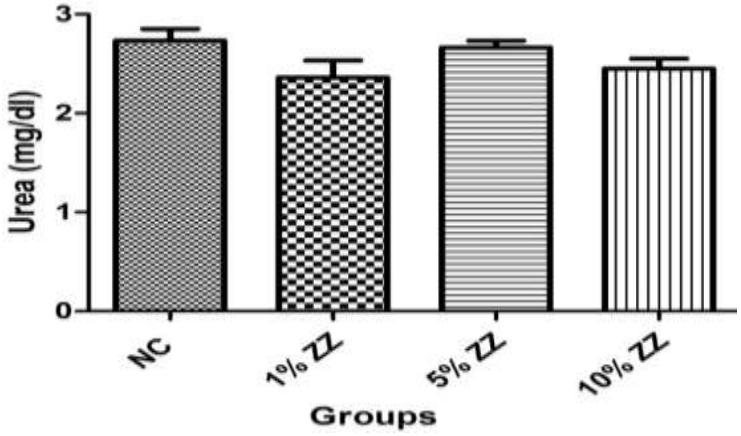

Figure 5. Serum Urea among treatments. NC: Control; ZZ: *Z. zanthoxyloides.*

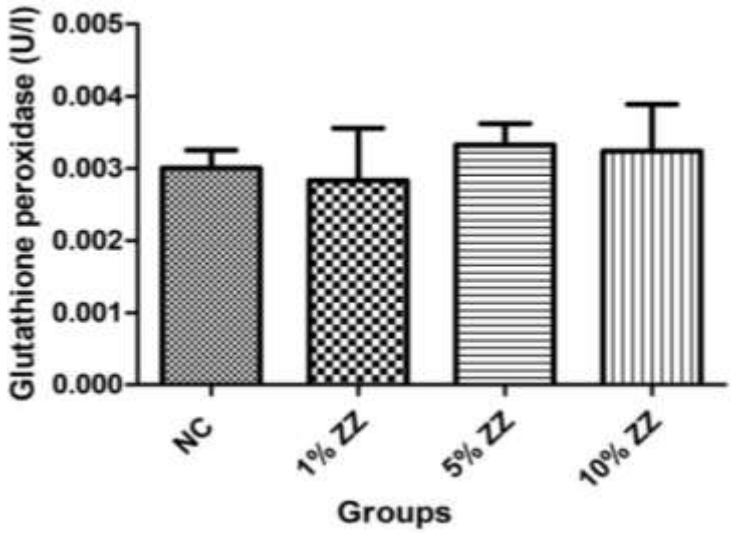

Figure 6. Kidney GPX among treatments. NC: Control; ZZ: *Z. zanthoxyloides;* GPX: Glutathione peroxidase

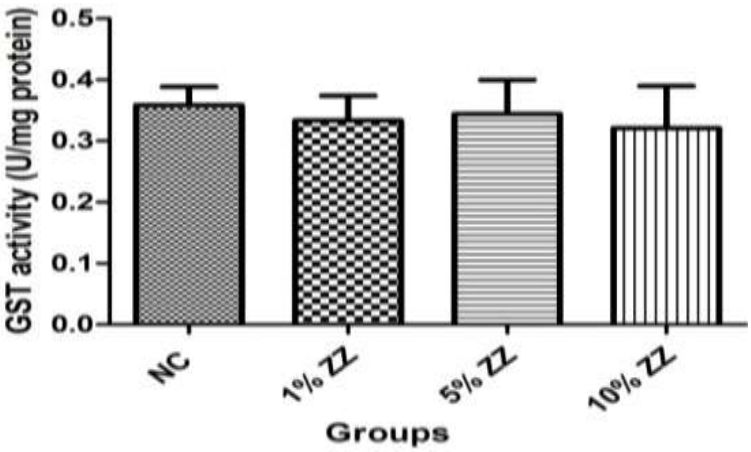

Figure 7. Kidney GST among treatments. NC: Control; ZZ: *Z. zanthoxyloides;* GST : glutathione transferase

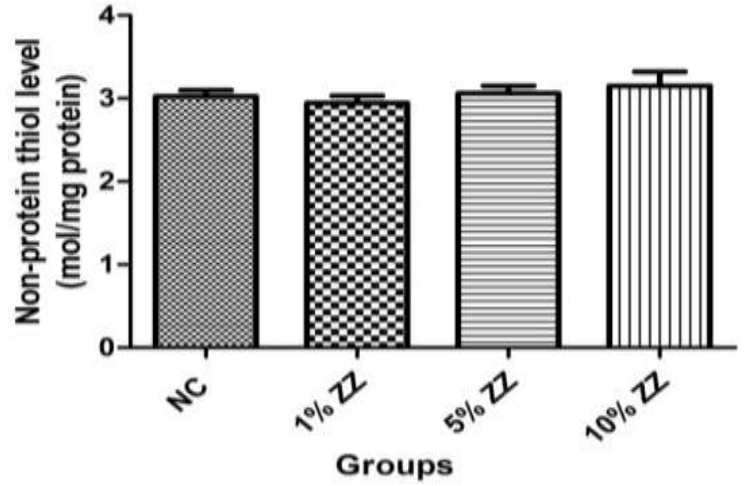

Figure 8. Kidney NP-SH among treatments. NC: Control; ZZ: *Z. zanthoxyloides;* NP-SH: Non-protein sulphadryl

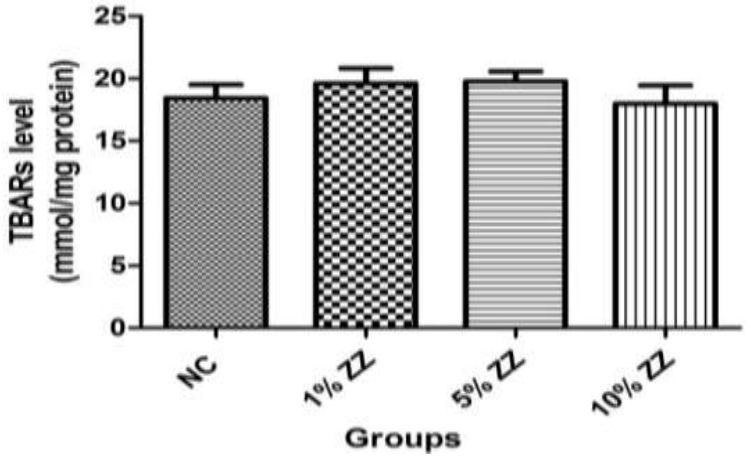

Figure 9. Kidney TBARS among treatments. NC: Control; ZZ: *Z. zanthoxyloides;* TBARS: Thiobarbituric acid reactive substances.

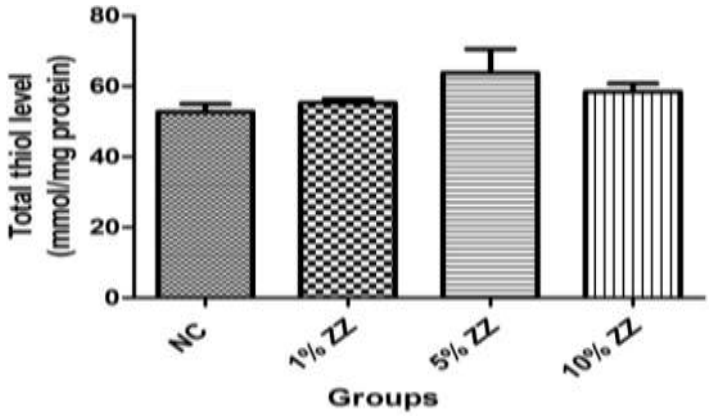

Figure 10. Kidney T-SH among treatments. NC: Control; ZZ: *Z. zanthoxyloides;* T-SH = Total sulphadryl

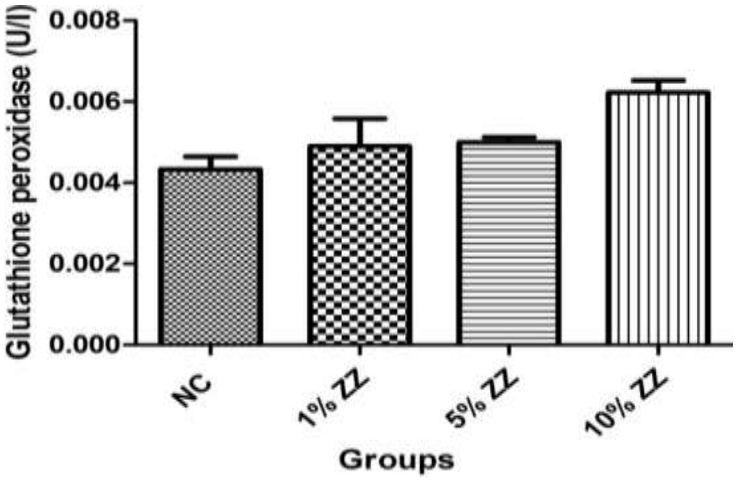

Figure 11. Liver GPX among treatments.NC: Control; ZZ: *Z. zanthoxyloides;* GPX: Glutathione peroxidase.

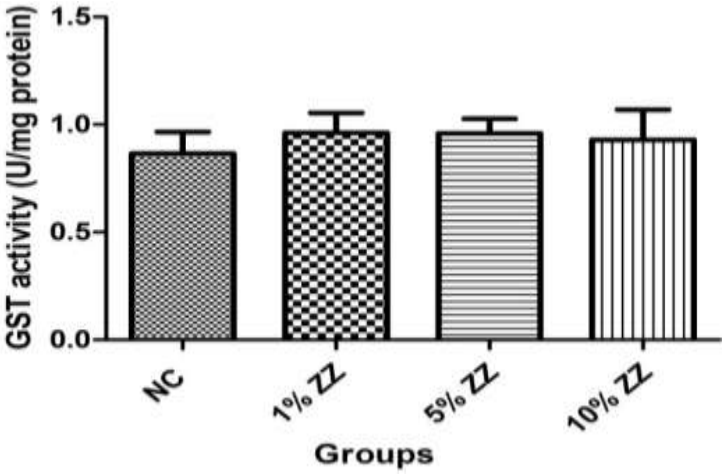

Figure 12. Liver GST among treatments. NC: Control; ZZ: *Z. zanthoxyloides;* GST: glutathione transferase

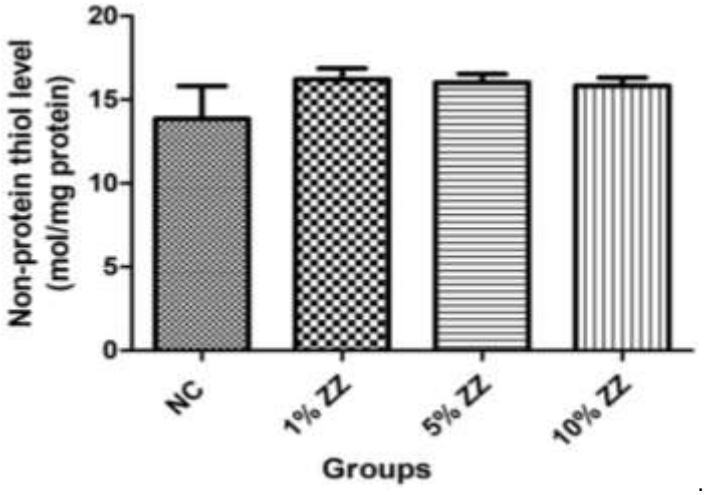

Figure 13. Liver NP-SH among treatments. NC: Control; ZZ: *Z. zanthoxyloides;* NP-SH = Non-protein sulphadryl.

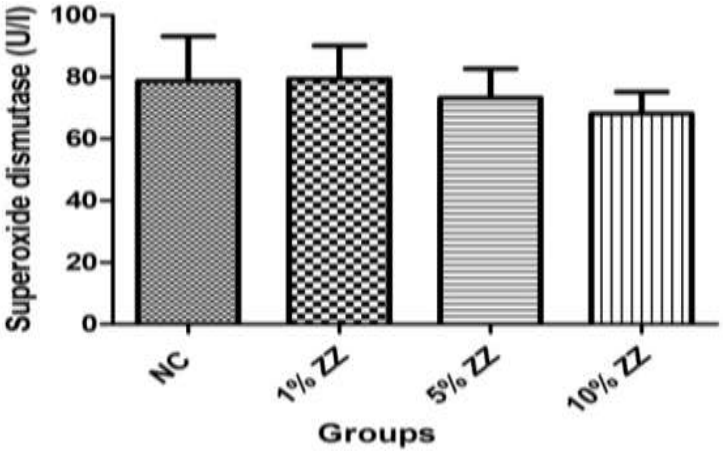

Figure 14. Liver SOD among treatments. NC: Control; ZZ: *Z. zanthoxyloides;* SOD = Superoxide dismutase

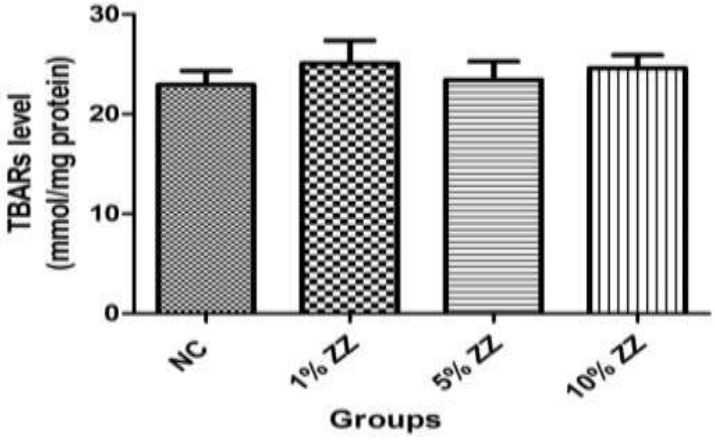

Figure 15. Liver TBARS among treatments. NC: Control; ZZ: *Z. zanthoxyloides;* TBARS: Thiobarbituric acid reactive substances.

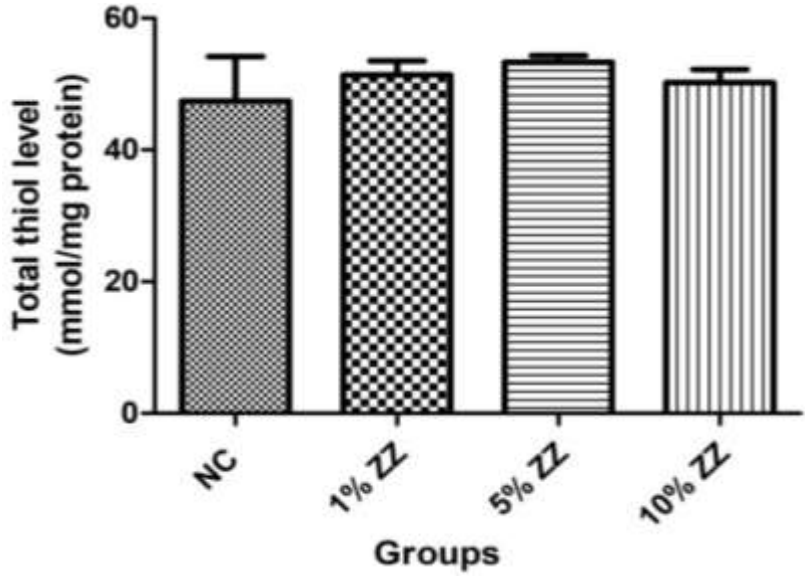

Figure 16. Liver T-SH among treatments. NC: Control; ZZ: *Z. zanthoxyloides*; T-SH = Total sulphadryl.

animals (albino rats) (Adebiyi and Obatan, 2013; Ileke et al*.,* 2014; Ojuwundu et al*.,* 2014; Nwosu et al*.,* 2017).

This study has shown that administration of *Z. zanthoxyloides* crude powder to feed each of these test animals at different doses did not change the ALT, AST, bilirubin, creatinine and urea level of their serum indicating the plant did not induce changes in the activities of serum enzymes, protein synthesis and deamination when compared to control. This is similar to findings of Nwosu et al *(*2017) on the effect of *Dennettia tripetala* seed powder on serum indices in albino rats and Sathya et al. (2012) using extract of *Acalypha indica*. Higher values of these indices in the serum will have indicated severe toxicity; damage to tissues and cell membrane in the liver or kidney leading to release of the enzymes into the serum, hence their use as markers for toxicity (Yakubu et al*.,* 2007; Odeyemi, 2008; Ileke et al., 2014; Nwosu et al*.,* 2017). ALT and AST are liver enzymes responsible for conversion of proteins and amino acids into energy for the liver cells (McGill, 2016).

All the indices measured for Kidney function showed no significant difference ($p>0.05$) which indicated that the kidney was also not affected by the different doses of plant administered. Under liver function, GST, NP-SH, TBAR and T-SH levels showed no significant difference at all doses, indicating normal liver function. Earlier researchers recorded severe damage to vital organs (kidney, liver, etc.) on application of plant extracts (Adeyemo-Salami and Makinde, 2013; Adebiyi and Abatan, 2013 and Alelign et al., 2020). However liver GPX and SOD varied significantly with doses, these differences may be due to increased oxidative stress on the liver and the response of antioxidant enzyme complex to cope with the stress (Li et al*.,* 2005). The dosages used in this study confirms the findings of Zahoui et al*.* (2010) that revealed median lethal concentration (LC50) value for *Fagara zanthoxyloides* was found to be between 0.5 - 5.0 g/kg indicating overdose of the plant is non-fatal but victims suffer from gastrointestinal disorders when taken in excess (Anokbonggo et al*.,* 1990). Chaaib et al*.* (2003), Queiroz et al*.* (2006) and Adekunle et al*.* (2012) reported increasing antioxidant ability of *Z. zanthoxyloides* with increasing dosage by scavenging DPPH radicals and chelating iron; this is attributed to the presence of phenolic acids such as chlorogenic and caffeic acids and flavonoid compounds such as quercetin, rutin and kaempferol, confirming the non-toxicity of the plant asserted by this study. In addition Ulrichova et al*.* (1983) reported that the plant also displays acetylcholinesterase inhibitory activity. The ability of *Z. zanthoxyloides* to scavenge free oxygen radicals or reactive oxygen species (ROS) is responsible for nontoxicity of the plant applied at all dosages in this work because the plants either do not induce production of ROS in the test animals (which will lead to toxicity) or remove the ROS as soon as they are produced.

Conclusion

Z. zanthoxyloides did not show toxicity in the animals under investigation as seen in all the indices under study and had earlier been proven to have antioxidant properties. This underlines its historical use in ethno-medicine. However, purification and formulation of its active components into drugs and pesticide should be the

focus of new research.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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