

HEPATOPROTECTIVE EFFECT OF METHANOLIC LEAVE EXTRACTS OF *V. ALBUM* ON PARACETAMOL-INDUCED LABORATORY ANIMALS

Yusuf, L*. Oladunmoye, M.K and Ogundare, A.O.

Department of Microbiology, Federal university of Technology Akure, Nigeria.

*Corresponding author. E-mail: yusuflam@yahoo.com.

Keywords: Hepatoprotective, *Viscum album*, paracetamol, hepatotoxicity, photomicrograms.

Abstract. Hepatoprotective assay was carried out on laboratory animals in which paracetamol (oral dose, 2 g/kg /body weight) was used to induce hepatotoxicity. The animals were orogastrically fed with the *Viscum album* (growing on cocoa and cola host trees) methanolic leaf extracts. The activities of serum enzyme biomarkers showed no significant difference ($P < 0.05$) for *V. album* growing on cocoa (1000-5000mg/kg) but there were corresponding significant increase in the serum enzymes ($P < 0.05$) for *V. album* growing on cola at 4000 and 5000 mg/kg doses. Similarly, the photomicrograms of the animals' vital organs (liver, stomach, small intestine and kidneys) studied revealed that *V. album* extract growing on cocoa was more hepatoprotective efficacious than that growing on cola tree on all the biochemical parameters that were screened for when compared with sylimarin (a standard hepatoprotective drug).

1.0 Introduction

The mistletoe (*Viscum album*) plant is an obligate parasite that depends partly on its host to obtain water and minerals but can carry out photosynthesis (Griggs, 1991). It is an evergreen semi-parasitic plant that can grow in most parts of the globe. Mistletoe can grow on either edible or non-edible trees, while only those that grow on edible plants are used for medicinal purposes (Evans, 2005). The growth of mistletoe on different kinds of plants, are of disease curing specificity, for example, mistletoe grown on guava, cola nuts and citrus are specific for curing diseases like cancer, hypertension, nervousness and insomnia, while those grown on cocoa is best used for curing diabetes (Ekhaise *et al.*, 2010). Mistletoe therefore is one of such plant, reported to possess several medicinal properties (Yusuf, 2015). Its use as an anti-cancer, anti-diabetic, anti-hypertensive, and indeed as 'all-purpose herb' has been reported (Yusuf, 2015 and Kafaru, 1993). Mistletoe is used traditionally and indiscriminately by the people in Akure North, Nigeria as topical and atypical antibiotics for the treatment of infectious diseases without considering its pharmacological and toxicological effects (Yusuf *et al.*, 2014). Some of these folkloric uses have already been investigated (Obatomi *et al.*, 1996, Osadebe and Ukwueze, 2008). During recent years considerable work has been done to investigate the pharmacological importance of some mistletoes species but not much work has been reported so far on comparative pharmacological importance of the plant growing on different host trees. Therefore, it was considered worthy to investigate and compare the hepatoprotective activity of mistletoes growing on two different host plants in Akure North, Nigeria.

2.0 Materials and Methods

2.1 Collection and Preparation of Samples

The leaves of Mistletoe (*Viscum album*) were collected from Odo-orokuta camp at Ayede Ogbese (Latitude; 7°14' 03.00" Longitude; 5°22'57.7" Vegetation; Tropical rain forest, and elevation; 304.5 m), Akure North Local Government, Nigeria from two different host plants; cocoa (*Theobroma cacao*) and cola (*Cola nitida*). The samples were identified and authenticated at the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure. The leaves were destalked, washed and air dried at a room temperature ($28^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for six weeks until a constant weight was achieved. They were thereafter pulverized and kept in an airtight container before

analysis. Some of the leaves were also ground fresh using electric blender and were stored (at 4°C) in refrigerator in a well labeled air-tight containers for analysis. A 100 g of each sample was weighed (using electric weighing balances) into different beakers and 1000 ml of 60% methanol was added separately. This was homogenized in a warring blender until a homogenate results and then concentrated *in vacuo* using a rotary evaporator. It was reconstituted using 35 % dimethyl sulphoxide (DMSO) 1:50(w/v) for the analysis. The extracts were sterilized using 0.45 µm millipore membrane filter.

2.2 Hepatoprotective studies

Animals divided into five groups of six rats (albino rats, Wister's strain) each were used for the study using the method of Setty *et al.* (2007). Groups 1 and 2 were maintained only on the basal diet and sterile water for 7 days. Groups 3, 4 and 5 were orogastrically dosed, each with 1000 mg/kg of silymarin, *V.album* extracts grown on cocoa and that grown on cola respectively, once a day for 7 days. On the fifth day, after the administration of the respective treatments, all the animals in groups 2, 3, 4, and 5 were administered 2 g/kg paracetamol (PCM) orally. On the seventh day, the blood samples were collected via cervical dislocation for the estimation of biochemical marker enzymes. The percentage of the protection is calculated as $100 \times (\text{values of PCM control} - \text{values of test sample}) \div (\text{values of PCM control} - \text{values of normal control})$.

2.3 Biochemical Assay of Blood Serum

The blood samples were allowed to coagulate for 30 min and the clear serum was separated by centrifuging at 2500 rpm for 10 min and was then used for the analysis of biochemical hepatic markers-total bilirubin (Jendrassik *et al.*,1938),aspartateaminotransferase (AST) (Reitman and Frnakel, 1957), alanine aminotransferase (ALT) (Reitman and Frnakel, 1957) and alkaline phosphatase (ALP) (Roy, 1970). Reflotron (Boehringer Marnherm Company, Germany) was used for the analysis of some major serum biochemical markers that can reveal the effect of the extracts on the internal organs of rats. Serum total protein and Albumin/Globulin ratio level of the serum were also determined. The general procedure involves pipetting standardized amount of sample that was then applied on the test zone of the appropriate test strip. The strips were inserted into the test chamber and the flap closed. The results were then displayed after some seconds on the frame computer monitor. Test was carried out at 25°C.

2.4 Histopathological studies of selected organs

Post-mortem examination was carried out on section of the organs; liver, kidney, stomach and small intestine of albino rats. The blood was rinsed in normal saline and preserved in 10 % formalin solution. Thereafter, sub-sections were taken from each selected organ, fixed in 10% formol saline and dehydrated in ascending grades of ethanol (60%, 70%, 80% and 90%). The specimens were cleared in xylene and embedded in paraffin. The tissues were then processed into 4-5 µm thick sections with the aid of a microtome and thereafter stained with hematoxylin and Eosin, viewed under the light microscope, and the photomicrograms were taken (Carleton, 2007).

Table 1: Hepatoprotective effect of the *V. album* extracts on biochemical parameters in paracetamol-induced hepatotoxicity in albino rats.

Treatment groups	TB mg/dl	AST U/L	ALP U/L	ALT U/L
1	0.83±0.47 ^a	63.00±0.58 ^a	54.60±0.61 ^a	17.23±0.42 ^a
2	2.61±0.14 ^e	108.43±0.41 ^e	100.88±1.14 ^e	36.83±0.69 ^d
3	1.33±0.02 ^b (71.91%)	66.25±0.46 ^b (92.85%)	62.65±0.54 ^b (82.61%)	18.28±0.44 ^{ab} (94.64%)
4	1.62±0.04 ^c (55.62%)	70.15±0.47 ^c (84.26%)	66.83±0.50 ^c (73.57%)	19.42±0.67 ^b (88.83%)
5	1.95±0.03 ^d (37.08%)	80.55±1.03 ^d (61.37%)	71.00±0.49 ^d (64.56%)	22.37±0.41 ^c (73.78%)

Group 1=rats + feed only

Group 2= rats +feed +PCM.

Group 3=rats +1000mg/kg sylimarin+feed + PCM

Group 4=rats + 1000mg/kg extract grown on cocoa+feed + PCM.

Group 5=rats+ 1000mg/kg extract grown on cola+feed + PCM.

(Percentage hepatoprotection is shown in parenthesis).

Figures on the same row with the same superscript are not significantly different at $P \leq 0.05$ significant level.

Table 2: Effect of the administration of *V. album* grown on cocoa host plant on the biochemical indices of albino rat serum.

Treatment(mg/kg/wt)	AST (U/L)	ALP (U/L)	ALT (U/L)
0.00	63.00 ± 0.707 ^a	54.80 ± 0.860 ^a	16.60 ± 0.510 ^a
1000.00	62.80 ± 1.113 ^a	53.60 ± 1.077 ^a	17.00 ± 0.949 ^a
2000.00	62.80 ± 1.068 ^a	54.20 ± 1.463 ^a	17.00 ± 0.837 ^a
3000.00	63.40 ± 1.167 ^a	53.60 ± 0.927 ^a	16.60 ± 0.678 ^a
4000.00	63.20 ± 1.157 ^a	55.00 ± 1.140 ^a	17.00 ± 1.049 ^a
5000.00	63.00 ± 1.304 ^a	54.40 ± 1.364 ^a	17.20 ± 0.860 ^a
2 g/kg(paracetamol)	112.00± 0.949 ^b	99.60± 2.73 ^b	34.20± 1.428 ^b

AST = Aspartateaminotransferase.

ALP =Alkaline-phosphatase.

ALT =Alanine-aminotransferase.

Figures on the same row with the same superscript are not significantly different at $P \leq 0.05$ significant level.

Table 3: Effect of the administration of *V. album* grown on cola host plant on the biochemical indices of albino rat serum.

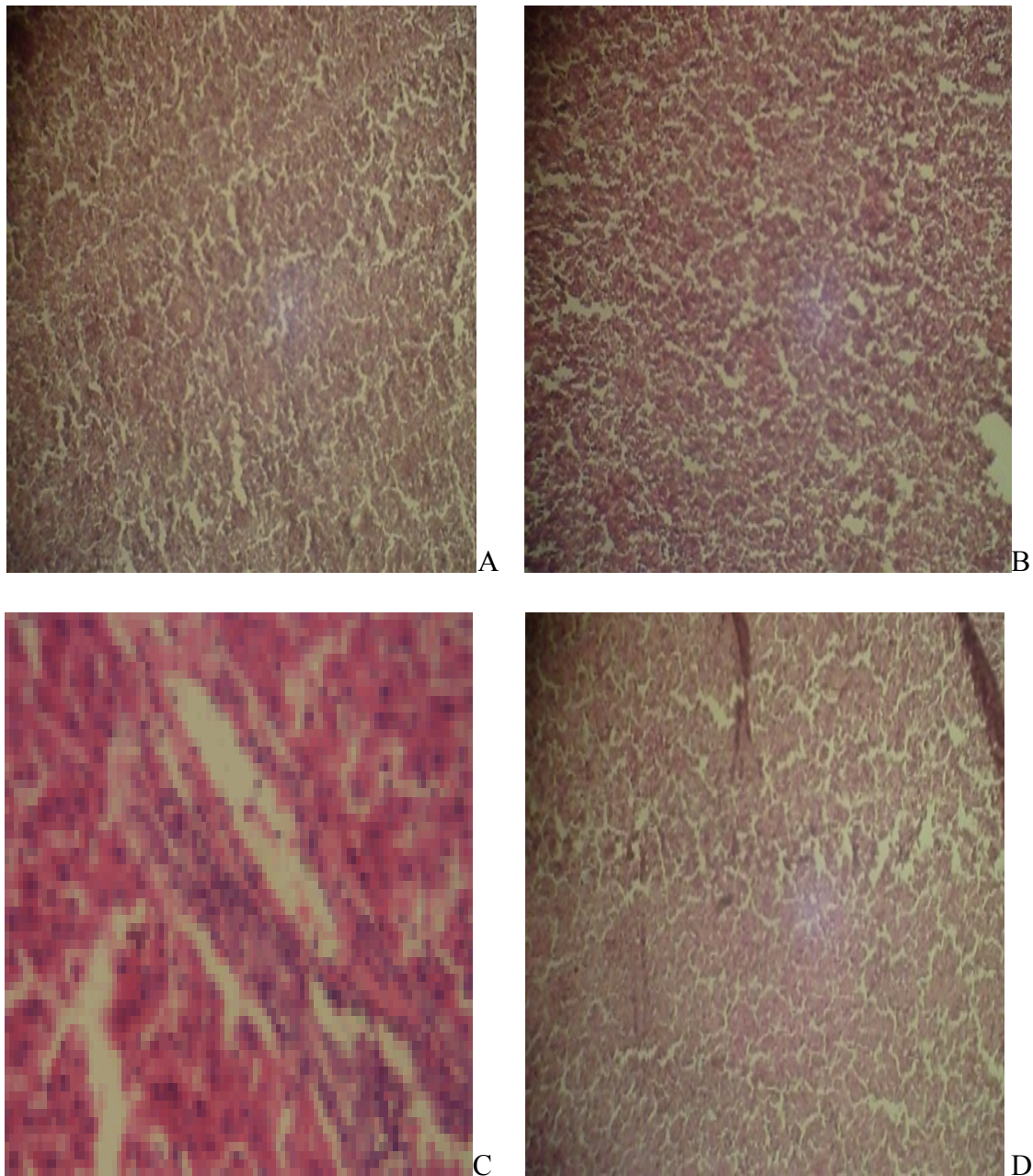
Treatment(mg/kg/wt)	AST (U/L)	ALP (U/L)	ALT (U/L)
0.00	63.00 ± 0.707 ^a	54.80 ± 0.860 ^a	16.60 ± 0.510 ^a
1000.00	62.60 ± 0.924 ^a	53.40 ± 1.029 ^a	16.20 ± 0.883 ^a
2000.00	62.40 ± 0.812 ^a	54.40 ± 1.029 ^a	16.60 ± 0.812 ^a
3000.00	63.00 ± 0.707 ^a	55.00 ± 1.303 ^a	17.00 ± 0.707 ^a
4000.00	82.40 ± 0.927 ^b	68.20 ± 2.596 ^b	21.40 ± 0.509 ^b
5000.00	95.00 ± 1.703 ^c	82.20 ± 2.517 ^c	25.20 ± 1.392 ^c
2 g/kg(paracetamol)	112.00± 0.949 ^d	99.60± 2.73 ^d	34.20± 1.428 ^d

AST = Aspartateaminotransferase.

ALP =Alkaline-phosphatase.

ALT =Alanine-aminotransferase.

Figures on the same row with the same superscript are not significantly different at $P \leq 0.05$ significant level.



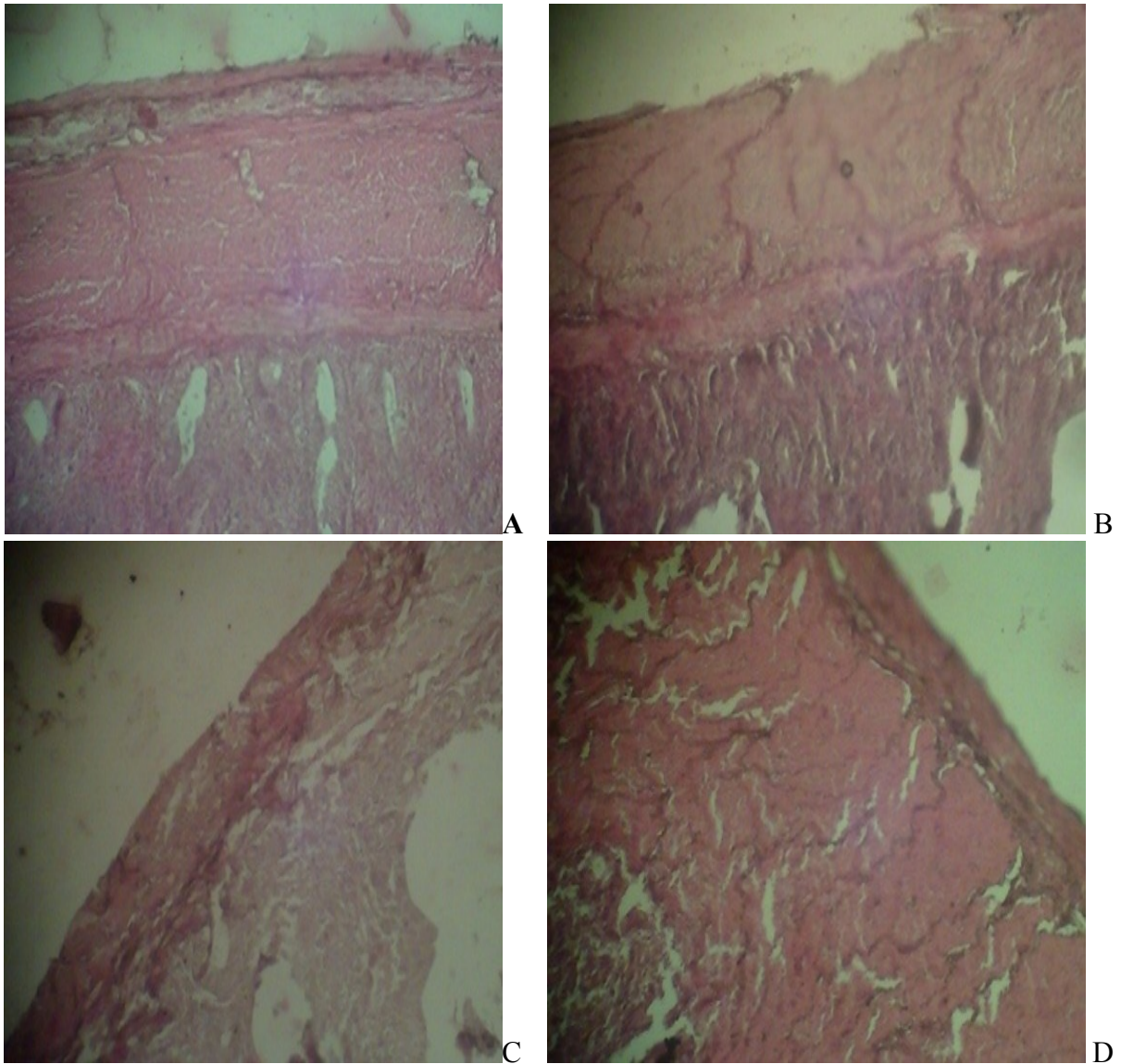
A Control (untreated animal): section of the liver showing normal architecture.

B (Sylimarin treated animal): section of the liver showing normal morphology.

C (Paracetamol treated animal): section of the liver showing haemorrhagic hepatitis and leucocytic infiltration.

D (*V. album* treated animal): section of the liver showing normal morphology.

Plates 1: Comparative photomicrographs of the laboratory animals' liver (post treatment).



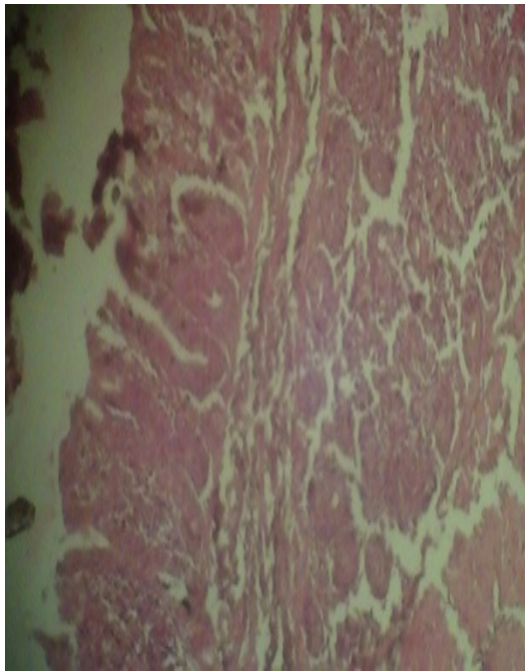
A Control (untreated animal): section of the stomach (fundus) with normal morphology

B (Sylimar treated animal): section of the stomach appearing normal

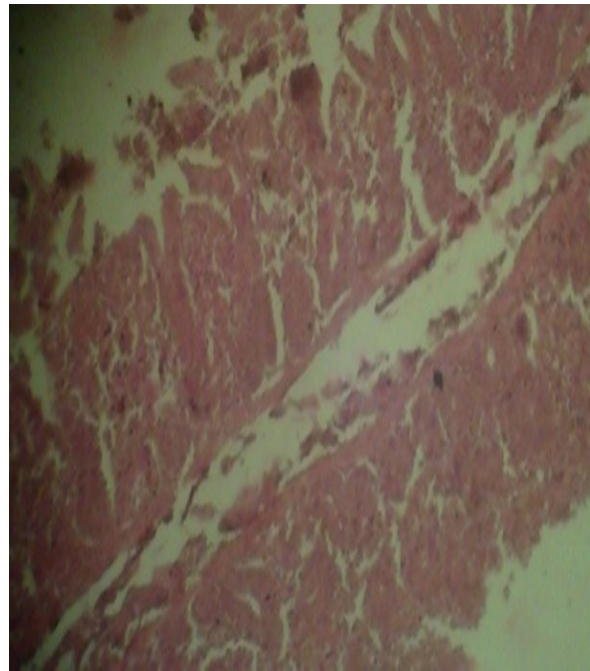
C (Paracetamol treated animal): Stomach showing gastritis.

D (*V.album* treated animal): section of the stomach showing normal morphology

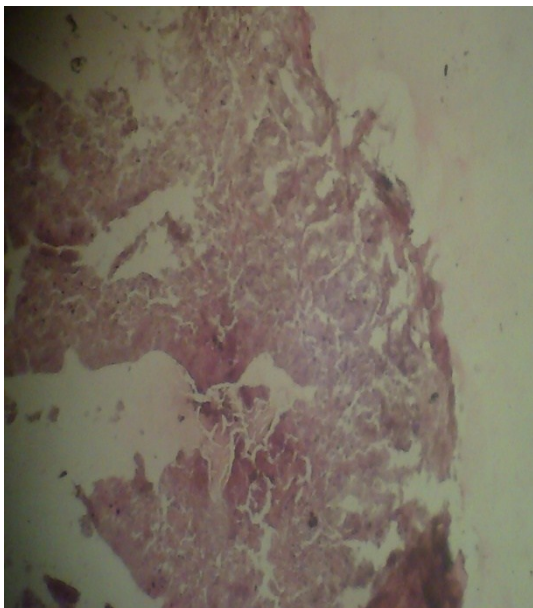
Plates 2: Comparative photomicrographs of the section of laboratory animals' stomach (post treatment).



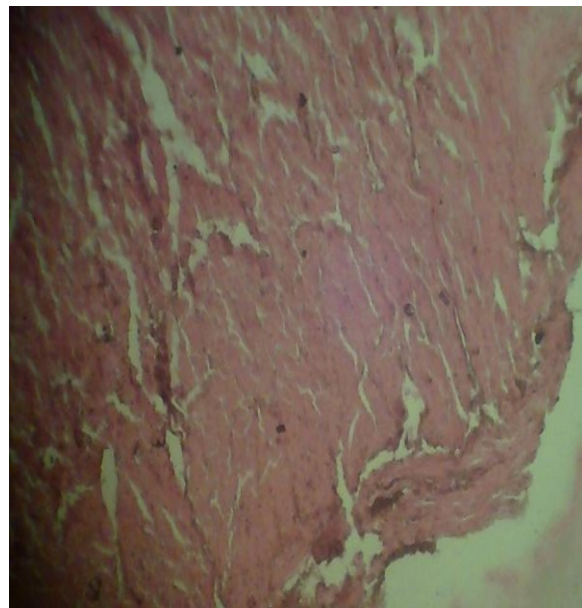
A



B



C



D

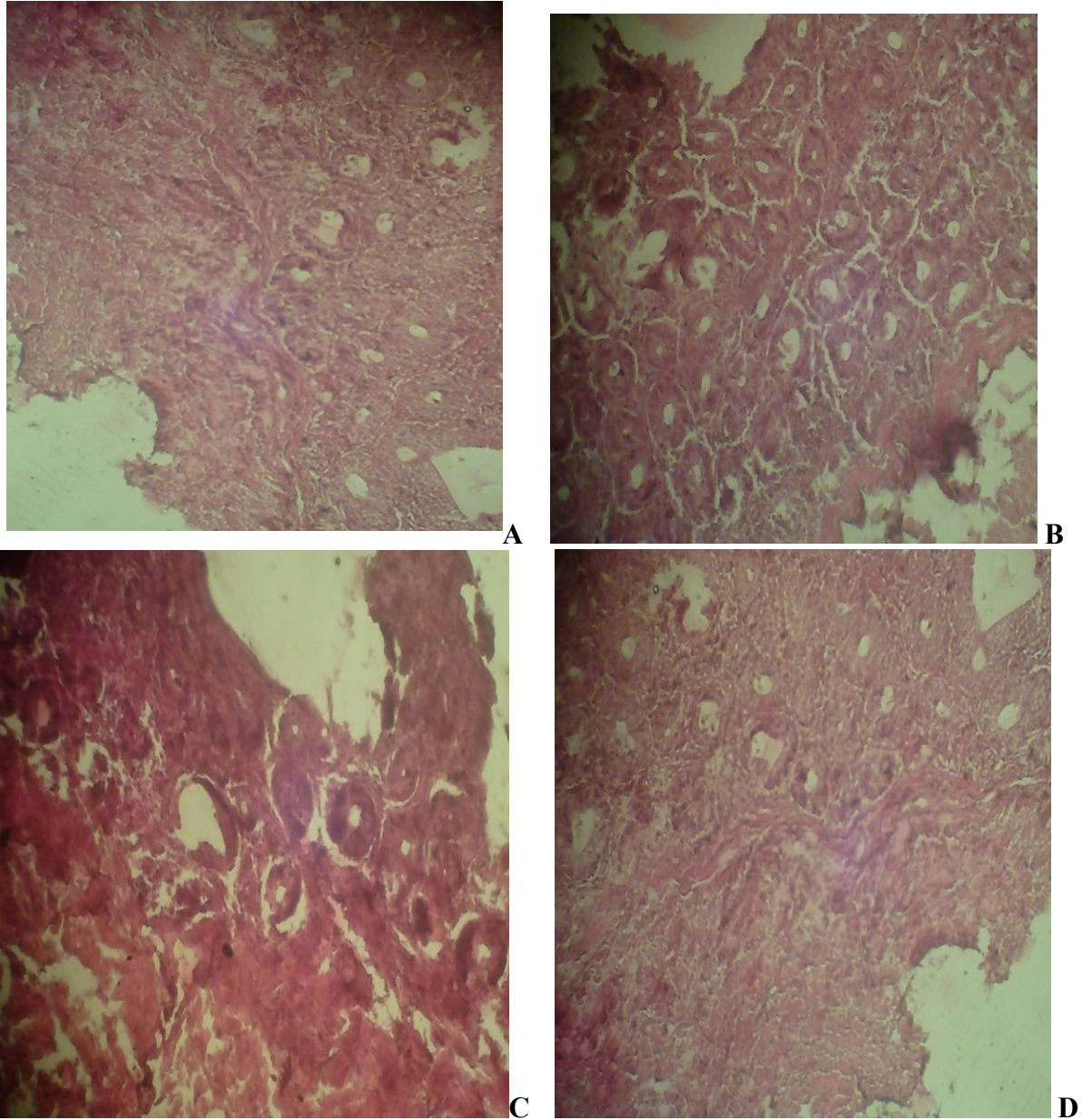
A Control (untreated animal): Section of the intestine (jejunum) with normal villi projections (arrow)

B (Sylimarin treated animal): Section of the intestine (jejunum) with atrophy

C (Paracetamol treated animal): Section of the intestine (jejunum) with ulceration and infiltration of cells

D (*V.album* treated animal): Section of the intestine (jejunum) appearing normal.

Plates 3: Comparative photomicrogram of the section of laboratory animals' small intestine (post treatment)



A Control (untreated animal): section of kidney showing normal architecture

B (Sylimarin treated animal): section of kidney with normal mophology

C (Paracetamol treated animal): section of kidney nephron with interstitial dialation and congested medulla

D (*V.album* treated animal): section of kidney appearing normal.

Plates 4: Comparative photomicrogram of the section of laboratory animals' kidneys (post treatment).

3.0 Discussion

Paracetamol is a known antipyretic, analgesic drug which produces hepatic necrosis at high doses and normally eliminated as sulfate and glucuronide conjugate. Results (Table 1) obtained from the hepatoprotective profile of the extract grown on cocoa, on biochemical parameters in albino rats show no significant difference ($P < 0.05$) when compared with the control at all concentration used (1000 – 5000 mg/kg). However, there was a significant difference ($P < 0.05$) at concentrations 4000 and 5000 mg/ml of the extracts grown on cola. This is an indication that extracts from cola might create mild hepatic injury at a very high dose or if it is used

indiscriminately (Yusuf, 2015). Eno *et al.* (2004) had also reported LD50 of 4170.5 mg/kg on albino rats injected with *V. album* leaves extract intraperitoneally. Though, they did not document the host plant.

In administration of toxic doses of paracetamol, the sulfation and glucuronidation routes become saturated and hence, higher percentages of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquinimine by cytochrome-450 enzymes. The Semiquinone radicals, obtained by one electron reduction of N-acetyl-p-benzoquinimine, can covalently binds to macromolecules of cellular membrane which increase the lipid peroxidation resulting in the tissue damage. Higher doses of PCM and N-acetyl-p-benzoquinimine can alkylate, oxidise intracellular glutathione (GSH), results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation thereby causes liver damage. In the assessment of liver damage by PCM, the determination of enzyme markers; Aspartateaminotransferase (AST), Alanineaminotransferase (ALT), Alkalinephosphatase (ALP) and total bilirubin were largely used. The results are shown in Tables 2 and 3. Liver necrosis or membrane damage releases the enzyme into circulation which can be measured in serum. A high level of AST indicates liver damage, as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes were indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Oyetayo and Osho, 2004). Serum ALP and bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure. It is evident that several phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of the hepatotoxin or by inhibition of lipid peroxidation induced by it (Mehta *et al.*, 1999). Phytoconstituents like flavonoids (Baek *et al.*, 1996) and (Pandit *et al.*, 2004), triterpenes (Xiong *et al.*, 2003), saponins (Tran *et al.*, 2001) and alkaloids (Vijyan *et al.*, 2003) are known to possess hepatoprotective activities. These phytochemicals had been reported to be present in the extracts (Yusuf, 2013b).

The results of the comparative histopathology of the livers, stomachs, intestines and kidneys of the experimental animals are shown in the photomicrograms (Plates 1-4). It was observed that the *V. album* treated liver showed normal morphology without necrosis when compared with the control groups. In contrast, paracetamol treated liver section showed haemorrhagic hepatitis, leucocytic infiltration and contrasting features. Similarly, the stomach showed gastritis, section of the intestine (jejunum) showed ulceratic and infiltration of cells and section of kidney nephron showed interstitial dialation and congested medulla. Results from this finding further give insight to the therapeutic effect of *V. album* leaves extracts. The plant extracts was able to improve the health of the experimental animals and found to be toxicologically safe (Yusuf *et al.*, 2013).

V. album leaves extract demonstrated significant ($P < 0.05$) liver protection against the hepatic injuries caused by the paracetamol hepatotoxins.

References

- [1] Baek, N. L., Kim, Y. S., Kyung, J. S. and Par, K. H. (1996). Isolation of anti-hepatotoxic agent from roots of *Astragalus membranaceus*. *Korean J Pharmacognosy*, 27:111-6.
- [2] Carleton, H. (2007). *Carleton's Histological technique*. The 4th ed, London, Oxford University, pp. 23-27.
- [3] Ekhaise, F. O., Ofoezie, V. G. and Enobakhare, D. A. (2010). Antibacterial Properties and Preliminary Phytochemical Analysis of Methanolic Extract of Mistletoe (*Tapinanthus bangwensis*). *Bayero Journal of Pure and Applied Sciences*, 3(2):65-68.
- [4] Eno, A. E., Owo, O. I., Itam, E. H. and Konya, R. S. (2004). Blood pressure depression by the *V. album* in Renal and DOCA-induced hypertension in rats. *Phytother. Res.* 14: 234-239.
- [5] Evans, J. (2005). Mistletoe: Good for more than free kisses. *Journal of American Botanical Council*, 68: 50 – 59.
- [6] Griggs, P. (1991). Mistletoe, myth, magic and medicine. *The Biochemist*, 13:3-4.

-
- [7] Jendrassik, L. and Grof, P. (1938). A colorimetry method for determination of serum bilirubin. *Biochemistry*, 1:297-81.
- [8] Kafaru, E. (1993). Herbal remedies, *The Guardian*, Thursday June 3, 1993, p 24.
- [9] Mehta, R. S., Shankar, M. B., Geetha, M and Saliyu, A. K. (1999). Hepatoprotective activity of *Trianthema portulacastrum*. *Indian Drugs* 36:241-4.
- [10] Obatomi, D.K., Aina, V. O. and Temple, V. J. (1996). Effect of African Mistletoe on blood pressure in spontaneously hypertensive rats. *International Journal of Pharmacognosy*, 34(2): 124-127.
- [11] Oyetayo, V. O. and Osho, B. (2004). Assessment of properties of strain of *Lactobacillus* & *Plantarum* isolated from fermentary corn slurry (Ogi), *Journal of food Agriculture and environment*, 2(1):132-134.
- [12] Pandit, S., Sur, T. K., Jana, U., Debnath, P. K. and Bhattacharyya, D. (2004). Prevention of carbon tetrachloride-induced hepatotoxicity in rats by *Adhatoda vasica* leaves, *Indian Journal of Pharmacology*, 36: 312-133.
- [13] Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic acid transaminases. *American Journal of Clinical Pathology*, 1: 56-63.
- [14] Roy, A. V. (1970). Enzymes in clinical chemistry. *Journal of Clinical Chemistry*, 16:431-9.
- [15] Setty, R. S. Q. and Ueresh, K. (2007). Hepatoprotective activity of *Calotropis procera* flowers against paracetamol-induced hepatic injury in rats. *Fitoterapia*, 78:451-4.
- [16] Tran, Q. L., Adnyana, I. K., Tezuka, Y., Nagaoka, T., Tran, Q. K. and Kadota, S. (2001). Triterpene saponins from Vietnamese ginseng (*Panax vietnamensis*) and their hepatoprotective activity. *J Nat Prod.* 64:456-61.
- [17] Ukwueze, S. E. (2008). An evaluation of the Antibacterial Activities of Leaf Extracts from *Loranthus micranthus* L, Parasitic on *Persea americana*. *Scientia Africana*, 7(1):51-55.
- [18] Vijyan, P., Prahant, H. C., Dhanaraj, S. A., Badami, S. and Suresh, B. (2003). Hepatoprotective effect of total alkaloid fraction of *Solanum pseudocapsicum* leaves. *Pharmaceutical Biology*, 41:443-8.
- [19] Xiong, X., Chen, W., Cui, J., Yi, S., Zhang, Z. and Li, K. (2003). Effects of ursolic acid on liver protection and bile secretion. *Zong Yao Cai*, 26:578-81.
- [20] Yusuf, L., Oladunmoye, M. K and Ogundare, A. O. (2013a). In-vivo Antibacterial Activities of Mistletoe (*Viscum album*) Leaves Extract Growing on Cocoa Tree in Akure North Nigeria. *European Journal of Biotechnology and Bioscience*, 1(1): 37-42.
- [21] Yusuf L, Oladunmoye M.K, Ogundare A.O, Akinyosoye F.A, Hassan G. F, and Momoh A.O. (2013b). Comparative antibacterial studies of mistletoes growing on two different host plants in Akure North, Nigeria. *International Journal of Medicine and Medical Sciences* ISSN: 2167-0404 Vol. 2 (5).
- [22] Yusuf, L., Oladunmoye, M. K., Ogundare, A. O., Mohmo, A. O. and Daudu O. A. Y. (2014). Comparative antifungal and toxicological effects of the extract of mistletoes growing on two different host plants in Akure North, Nigeria. *International Journal of Biotechnology and Food Science*, Vol. 2(2), pp. 31-34.
- [23] Yusuf L. (2015). Comparative antimicrobial and anticancer properties of mistletoe (*Viscum album* L.) growing on cocoa (*Theobroma cacao* L.) and cola (*Cola nitida* vent schott and endl.) trees in Akure, Nigeria. Ph.D thesis in the Department of Microbiology of the Federal University of Technology Akure, Ondo State, Nigeria. pp. 236.