Effect of *Xylopia aethiopica*, *Fiscus mucuso* and *Anthocleista vogelli* extracts on some Biochemical Parameters following ethanol-Induced Toxicity.

*O. A Ayoka[1], R. E Okonji[2], D. A Ofusori[3], O. A Komolafe[3], K.D.S Bamitale[4], J. B Fakunle[5]

1. Department of Physiological Sciences, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
2. Department of Biochemistry, Faculty of Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
3. Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
4. Department of Medical Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
5. Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

*Corresponding author: Dr. O. A Ayoka, Department of Physiological Sciences, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

ABSTRACT

The objective of the study was to comparatively verify the effects of aqueous extracts of three plants on some biochemical parameters following ethanol administration with a view to ascertaining the role of the extracts in ameliorating ethanol toxicity.

A total of forty rats were divided into eight groups (n=5). Group A were control rats; Group B were administered with absolute ethanol; Group C were ethanol administered rats treated with *Xylopia aethiopica*; Groups D were ethanol administered rats treated with *Fiscus mucuso,* Group E were ethanol administered rats treated with *Anthocleista vogelli*; Group F were normal rats administered orally with *Xylopia aethiopica*; Group G were normal rats administered orally with *Fiscus mucuso*; Group H were normal rats administered orally with *Anthocleista vogelli*. At the end of the experimental period, the animals were sacrificed and serum was obtained for total protein, uric acid, creatinin, urea, Aspartate aminotrasferase (AST) and Alanine aminotransferase (ALT) analysis using respective research kits.

The result showed that *Xylopia aethiopica* had protective effect on the kidney as compared with *Fiscus mucuso* and *Anthocleista vogelli* treated rats. Also, The AST and ALT was lowered with the start of *Xylopia aethiopia* treatment. The total protein, creatinin and urea were slightly ($p>0.05$) affected with ethanol, an effect which was normalized with the start of extract treatment.

It can be concluded that *Xylopia aethiopica* had a better reno-protective and hepatoprotective effect than *Anthocleista vogelli* and *Fiscus mucuso* extract as evidenced in its role in normalizing the negative influence of ethanol toxicity.

Key Words: Ethanol, *Xylopia aethiopica, Fiscus mucuso, Anthocleista vogelli*

Comment [I1]: aspartate

Comment [I2]: alanine

INTRODUCTION

Natural compounds have been adopted as protective and therapeutic agents against various toxicities caused by necrotizing agents such as ethanol. Some of the extracts of plants are very beneficial due to the antioxidant properties; others have cytotoxic effects [1]. Although there is gradual decline in the use of medicinal plants due to the introduction of modern synthetic medicine, information has it that traditional medicine still accounts for about 80% of the health needs of the rural populace in most regions of Africa. Despite the huge benefits attached to medicinal herbs, it is not recommended to use it without adequate knowledge of its toxicity, dosage and purity.

Xylopia aethiopica, Fiscus mucuso and *Anthocleista vogelli* are among the many medicinal plants valued in many countries of Africa. *Xylopia aethiopica* is predominant in West African and commonly referred to as "pepper tree", "African guinea pepper" or "Ethiopian pepper" [2]. It is wide spread in tropical Africa, Zambia, Mozam-bique and Nigeria [3]. Investigations have shown that owing to its antiseptic and antioxidant properties, the aqueous extract is usually administered after child birth [3-5]. *X. aethiopica* is also well known for its anti-hypertensive and diuretic effects [3].

The genu *Ficus* is made up of about 1000 species across tropical and warm temperate regions with greatest diversity in Asia, Malaysia and tropical South America. The tree is large and up to 21m in height. In Latin, ficus means fig, which is derived from the Persian 'fica'. The common name for *Ficus mucuso* is fig. It is a semi-deciduous spreading savannah tree with greenish flowers. The seeds are very tiny and numerous [6]. Because of the high nutritive value, apes [7, 8] and indeed humans [9] depends so much on *Ficus* as part of their diet.

The antioxidant status and beneficial effects of *Ficus sycomorus* have been documented [6, 10].

Comment [I3]: genus

Comment [I4]: 21 m

Anthocleista vogelli is predominantly found in swampy areas, river banks and Raphia grooves [11, 12]. It is about 20m in height. It is a medicinal plant that is widely used in West Africa [11, 12]. It is used to manage constipation and also regulate menstruation. It acts as a strong purgative and diuretic. In some countries such as Sierra leone, it is used in the treatment of jaundice and hepatitis [11]. In Nigeria and Congo, the back and seed of this promising plant is used in the treatment of ovarian problem, bronchitis, hernia and fever. *Anthocleista vogelli* contains compound such as 1,7-dihydroxy-3,8-dimathoxy-xanthrone and 1,8-dihydroxy-3,7-dimathoxy-xanthrone. These compounds are responsible for is anti-malaria and anti-ulcer potential.

Antioxidants occur naturally in some plants which constitute part of human daily diet [6]. The intake of such nutritious plants display antioxidant properties which mop up free radicals thus preventing oxidative stress and maintaining good health. The most common antioxidants present in diets are vitamin E, vitamin C and carotenoids. Other non-nutrient food substances, including, phenolic and polyphenolic compounds also exhibit antioxidant properties [6, 13]. Based on the documented antioxidant evidences and nutritive value of these three plants, it is convenient to evaluate their basic role against ethanol toxicity.

The present study was thus initiated to comparatively evaluate the effects of three aqueous extracts of *Xylopia aethiopica, Fiscus mucuso* and *Anthocleista vogelli* on some biochemical parameters following ethanol administration with a view to ascertain their effect in ameliorating ethanol toxicity.

MATERIALS AND METHODS

Plant Materials

The fresh fruit of *Xylopia aethiopica* and leaves of *Fiscus mucuso and Anthocleista vogelli* were procured from the central market in Ile-Ife, Osun State, Nigeria. They were authenticated by comparison with the existing specimen deposited in the Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of Extract

The fresh fruit of *Xylopia aethiopica,* leaves of *Fiscus mucuso and Anthocleista vogelli* were air dried and powdered using a grinding and crushing machine (Daiki Rita Kogyo Co Ltd, Japan). The powders were extracted in cold water with intermittent shaking for 48 hours. The aqueous filtrate was concentrated in vacuum rotary evaporator (Buchi Ratavapour R110, Schweiz). The fruit of *Xylopia aethiopica* and leaves of *Fiscus mucuso and Anthocleista vogelli* yielded 18.39g (5.93%), 19.75g (5.34%) and 51.39g (3.06%) respectively. Extracts were dissolved in normal saline solution and administered orally at a dose of 200mg/kg to animals in groups C-H for twenty one days. The extract was administered 24hrs after ethanol toxicity was established for animals in groups C-E. Ethanol toxicity characterized by gastric heamorrhagic patches was established one hour after administration in line with previous study [14]

Comment [I5]: Lines 103 to 107 should be moved to appear below line 123. See the first review

Animals

Forty adult wistar rats were procured and acclimatized for two weeks in the Animal Holdings of the College of Health Sciences Obafemi Awolowo University, Ile Ife. Animals were allowed free access to rat chow (Caps feeds Nigeria) and water *ad libitum* throughout the study. All the animals were treated according to the recommendations of National Academy of Sciences and published by the National Institutes of Health, USA [15].

Experimental Design

The forty wistar rats were randomly divided into eight groups (n=5).

GROUP A: Control (administered with normal saline)

GROUP B: Absolute ethanol (1ml/kg b.w)

GROUP C: Absolute ethanol (1ml/kg b.w) + *Xylopia aethiopia*

GROUP D: Absolute ethanol (1ml/kg b.w) + *Fiscus mucuso*

GROUP E: Absolute ethanol (1ml/kg b.w) + *Anthocleista vogelli*

GROUP F: *Xylopia aethiopia*

GROUP G: *Fiscus mucuso*

GROUP H: *Anthocleista vogelli*

At the end of the experiment, the animals were sacrificed. Before the sacrifice, blood samples were collected via cardiac puncture after the animals were placed under slight anaestesia. Serums obtained were assayed for total protein, uric acid, creatinin, urea, Aspartate aminotrasferase (AST) and Alanine aminotransferase (ALT) using respective diagnostic kits. All biochemical analysis were carried out in the Department of Biochemistry, Obafemi Awolowo University, Nigeria.

Comment [I6]: anesthesia

Comment [I7]: aspartate

Comment [I8]: alanine

Statistical Analysis

One-way analysis of variance (ANOVA) using SPSS version 17.0 (SPSS, Cary, NC, USA) was used to analyze the data. P value <0.05 was considered as significant.

Comment [I9]: compare the means of the groups

RESULTS

In this study, uric acid levels were decreased in the serum of ethanol administered rats. Following the treatment with *Xylopia aethiopia* and *Anthocleista vogelli* (Group C and E), there was a significant ($p<0.05$) increase in the uric acid concentration (Table 1). Also, groups treated with *Xylopia aethiopia* and *Anthocleista vogelli* only (group F and H) presented similar increase ($p<0.05$) in uric acid concentration. There was a concomitant decrease in the uric acid concentration of *Fiscus mucuso* treated groups (group D and G). A significant ($p<0.05$) increase in AST and a non significant increase in ALT in the ethanol administered group was observed. The AST was lowered with the start of treatment with *Xylopia aethiopia, Fiscus mucuso* and *Anthocleista vogelli* while only *Xylopia aethiopia* had a non significant decrease on the ALT. The total protein and urea concentrations in all the groups were only slightly affected in the ethanol administered group; an effect which was normalized in the extract treated group. The creatinine concentration in all the groups was not significantly affected by the ethanol administration (Table 1).

Comment [I10]: I still insist that the authors should write up the result to show that comparison was made between the means of the groups since that is what is shown in Table 1. For example Table 1 shows that the uric acid level of groups E and F were significantly highest while groups D and G had the least uric acid level.
2. Also table 1 did not show the baseline serum enzyme readings thus writing that there was increase or decrease in the enzyme levels cannot be substantiated using what is presented in the table.

TABLE 1: Showing the Effects of *Xylopia aethiopica*, *Fiscus mucuso* and *Anthocleista vogelli* on Some Serum Enzymes Following Ethanol Administration

Groups	**Total Protein** (mg/dl)	**Uric Acid** (mg/dl)	**Creatinin** (mg/dl)	**Urea** (mg/dl)	**AST** **(U/L)**	**ALT** **(U/L)**
Group A	0.66 ± 0.033[a]	35.61 ± 2.08[ab]	1.68 ± 0.33[a]	37.46 ± 0.66[bc]	37.25 ± 12.95[ab]	10.80 ± 0.80[a]
Group B	0.62 ± 0.035 [a]	31.82 ± 5.60 [ab]	1.94 ± 0.13[a]	37.50 ± 0.28[bc]	53.00 ± 11.93[b]	12.33 ± 2.02[a]
Group C	0.65 ± 0.012 [a]	58.15 ± 1.16[c]	1.79 ± 0.12[a]	36.20 ± 1.15[bc]	19.00 ±1.15[a]	11.00 ± 1.16 [a]
Group D	0.66 ± 0.015 [a]	28.76 ± 1.32[a]	1.50 ± 0.01[a]	35.55 ± 0.02[b]	25.00 ± 3.46 [a]	16.00 ± 2.30[ab]
Group E	0.78 ± 0.011 [a]	68.28 ± 1.19[c]	1.70 ± 0.12 [a]	32.80 ± 1.16[a]	36.00 ± 1.16[ab]	13.00 ± 1.15[a]
Group F	0.66 ± 0.043 [a]	62.18 ±11.99[c]	1.82 ±0.09 [a]	36.22 ± 0.69[bc]	30.50 ± 3.27[ab]	14.75 ± 1.31[ab]
Group G	0.71 ± 0.022 [ab]	29.35 ± 4.16[a]	1.70 ± 0.04 [a]	36.06 ± 0.62[bc]	24.60 ± 5.60 [a]	13.20 ± 2.65[a]
Group H	0.78 ± 0.025[b]	51.95 ± 11.01[bc]	2.07 ± 0.09 [a]	39.05 ± 1.58[c]	36.66 ± 7.75[ab]	19.50 ± 1.04 [ab]

Values are given as Mean ± SEM. Letters a, b, c, ab and bc within a column signifies that means with different letters differs significantly at $p < 0.05$ while means with the same letters does not differ significantly at $p < 0.05$ (using one way ANOVA with Duncan multiple range test)

DISCUSSION

Ethanol toxicity has been a point of reference in biomedical researches due to its basic role in eliciting oxidative stress which is capable of causing serious harm if left unchecked. Oxidative stress occurs in cells as result of cascade of reactions such as lipid peroxidation produced by oxidants [16]. Lipid peroxidation is elicited by many environmental factors such as infections, toxins and ethanol. Even though biochemical determination of lipid peroxidation status was not performed in this study, evidence from previous researches has linked ethanol toxicity to lipid peroxidation [17, 18].

In this study, uric acid levels were decreased in the serum of ethanol fed rats as compared to the control. However, this decrease was not significant. This may either be due to the inhibition of nucleotide (adenine nucleotide) turnover or alteration in the catabolism of purines. It is also possible that the uric acid may have been utilized in scavenging free radicals produced as a result of ethanol intoxication. Free radicals such as superoxide anion and hydroxyl radical are unstable [6, 19]. For instance, superoxide anion interacts with nitric oxide to form reactive peroxynitrite while hydroxyl radical react rapidly with most biological molecules [19]. The increase in the uric acid concentration of the group treated with *Xylopia aethiopia* and *Anthocleista vogelli* after ethanol toxicity (Group C and E) and also in the group administered with *Xylopia aethiopia* and *Anthocleista vogelli* only (group F and G) is likely due to the antioxidant properties of these plants. Investigations have shown that *Xylopia aethiopia* possesses antioxidant properties [5]. A study of Adefegha and Oboh [20] about the effects of diets supplemented with *Xylopia aethiopica* and *Piper guineense* on some biochemical parameters in normal rats revealed that the flavonoid content of *Xylopia aethiopica* was significantly higher than *Piper guineense*.

There was no change in the uric acid concentration of *Fiscus mucuso* fed groups (group D and G), an indication of its poor protective role. In this study, there was no significant effect of ethanol administration on ALT even though AST was significantly increased. AST and ALT are liver enzymes that are expected to increase in response to liver damage. There is a possibility that the body adjusted itself to the systemic presence of ethanol by producing endogenous antioxidants to mop up the elicited free radical thus protecting the liver from excessive damage. Recent studies have shown that in the event of toxicity, the body is capable of adjusting itself to cope so long the threshold of intoxication is not exceeded [21]. The failure to attain the threshold of intoxication in the ethanol fed group may be due to the duration of ethanol administration which may not be long enough to result in excessive liver damage. Vasconcelos *et al.,* [22] in a related study reported that daily administration of ethanol for 7 days produced no effects on ALT and AST levels which later increased significantly with a prolonged treatment for 14 days. This may probably be responsible for the non significant difference in the total protein, urea and creatinine concentrations in all the groups.

It is therefore suggestive that body adaptability due to short duration of ethanol administration rather than antioxidants properties of *Anthocleista vogelli* and *Fiscus mucuso* was responsible for the non significant changes in the AST, ALT, total protein, urea and creatinine concentrations in the ethanol fed group. The fact that creatinine concentration was not affected may be an indication that the kidney function was not hindered.

It can therefore be concluded that *Xylopia aethiopica* had a better reno-protective and hepatoprotective effect than *Anthocleista vogelli* and *Fiscus mucuso* extracts as evidenced in its role in normalizing the negative influence of ethanol toxicity.

REFERENCES

1. Ju EM, Lee SE, Hwang HJ, Kim JH. Antioxidant and anticancer activity of extract from Betula platyphylla var. japonica. Life Sci. 2004; 74:1013. doi: 10.1016/j.lfs.2003.07.025.

2. Nnodim, J.,Emejulu, A.,Amaechi, a., and NwosuNjoku, E.C. Influence of Xylopia aethiopica fruits on Some haematological and biochemical Profile. Al Ameen J Med Sci. 2011; 4 (2): 191-196.

3. Nwangwa EK. Antifertility Effects of Ethanolic Extract of Xylopia aethiopica on Male Reproductive Organ of Wistar Rats American Journal of Medicine and Medical Sciences 2012; 2(1): 12-15

4. Burkhills HM. Useful plants of West Tropical Africa, 2nd edi Royal botanical garden. 1985; 1: 130-132.

5. Amayaw Y, Owusu-Ansah E. Morphohistological studies of two plants species used in Ethnomedicine J.Herds, spices med plants. 1998; 5 (4):60-85.

6. Ahoua ARC, Kone MW, Konan AG, Tra BFH and Bonfoh B. Antioxidant activity of eight plants consumed by great apes in Côte d'Ivoire. African Journal of Biotechnology. 2012; 11(54): 11732-11740.

7. Boesch C. Hunting behaviour of wild chimpanzees in Taï national Park. Am. J. Phys. Anthropo, 1989; 78: 547-573.

8. Goné Bi ZB. Phénologie et distribution des plantes dont divers organes (principalement les fruits) sont consommés par les chimpanzés dans le Parc National de Taï. DEA

252 Ecologie tropicale option végétale, Laboratoire de Botanique, UFR Biosciences,
253 Université de Cocody-Abidjan (Côte d'Ivoire), 1999; 95.

254 9. Kamanzi AK. Plantes médicinales de Côte d'Ivoire : Investigations phytochimiques
255 guidées par des essais biologiques. Thèse Biologie Végétale option Phytochimie, U.F.R.
256 Biosciences. Université de Cocody-Abidjan (Côte d'Ivoire). 2002; 176.

257 10. Abdel-Hameed ESS. Total phenolic contents and free radical scavenging activity of
258 certain Egyptian *Ficus* speces leaf samples. Food Chem. 2009; 114: 1271-1277.

259 11. Burkill HM. The useful Plants of West Tropical Africa, 2nd Ed., Vol. 3, Royal Kew
260 Botanical Gardens. Kew. London. 1995; 522-527.
261 12. Sonibare MA, Soladoye MO, Ekine-Ogunlana Y. A chemotaxonomic approach to the
262 alkane content of three species of *Anthocleista* Afzel. (Loganiaceae). African Journal of
263 Biotechnology. 2007; Vol. 6 (13): 1516-1520.
264 13. Bagchi K, Puri S. Free radicals and antioxidants in health and disease. East Mediterr.
265 Health J.1998; 4: 350-360.
266 14. Al-Qaraghuli AMS, Abdel Wahab EMN, Al-Ani IM, Faisal GG. Effects of Xiang Sha
267 Yang Wei Wan on Ethanol-Induced Gastric Ulcer in Sprague Dawley Rats: a
268 Histological Study. IMJM. 2013; 12(2): 3-10.
269 15. National Institute of Health Guide for the Care and Use of Laboratory Animals. DHEW
270 Publication (NIH). revised, Office of Science and Health Reports, DRR/NIH, Bethesda
271 1985; USA.

16. Pan M, Cederbaum AI, Zhang YL, Ginsberg HN, Williams KJ, Fisher EA. Lipid peroxidation and oxidant stress regulate hepatic apolipoprotein B degradation and VLDL production. J. Clin Invest. 2004; 113(9):1277-1287.

17. Montoliu C, Vallés S, Renau-Piqueras J, Guerri C. Ethanol-induced oxygen radical formation and lipid peroxidation in rat brain: effect of chronic alcohol consumption. J Neurochem 1994; 63(5):1855-62.

18. Ostrowska J, Łuczaj W, Kasacka I, Rózański A, Skrzydlewska E. Green tea protects against ethanol-induced lipid peroxidation in rat organs. Alcohol. 2004; 32 (1):25-32.

19. Ofusori D. A and Adejuwon A. O. Histopathological studies of acute and chronic effects of Calliandra portoricensis leaf extract on the stomach and pancreas of adult Swiss albino mice. *Asian Pacific Journal of Tropical Biomedicine,* 2011; 11: 182-185

20. Adefegha SA, Oboh G. Effect of diets supplemented with Ethiopian pepper [Xylopia aethiopica (Dun.) A. Rich (Annonaceae)] and Ashanti pepper [Piper guineense Schumach. et Thonn (Piperaceae)] on some biochemical parameters in normal rats. Asian Pacific Journal of Tropical Biomedicine 2011; 12:558-566.

21. WHO (2010). *Childhood lead poisining:* (http://www.who.int/ceh/publications/leadguidance.pdf, accessed 31 October 2013).

22. Vasconcelos SMM, Soares PM., Lima NM., Pereira RF, Alves RS,. Queiroz MGR, Macedo DS, Freitas RM, SOUSA FCF, Fonteles MMF, Viana GSB. Effects of Ethanol or Naltrexone after Ethanol Exposure on Plasma Levels of Hepatic Enzymes, Lipid Profile and Apolipoprotein in Rats Sci Pharm. 2008; 76: 305–320