

1 **Effect of *Xylopi*a *aethi*opica, *Ficus mucuso* and *Anthocleista vogelli* extracts on some**  
2 **Biochemical Parameters following ethanol-Induced Toxicity.**

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## ABSTRACT

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

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

Comment [I1]: aspartate

Comment [I2]: alanine

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

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

44 **Key Words:** Ethanol, *Xylopiya aethiopica*, *Fiscus mucuso*, *Anthocleista vogelli*

## INTRODUCTION

45  
46 Natural compounds have been adopted as protective and therapeutic agents against various  
47 toxicities caused by necrotizing agents such as ethanol. Some of the extracts of plants are very  
48 beneficial due to the antioxidant properties; others have cytotoxic effects [1]. Although there is  
49 gradual decline in the use of medicinal plants due to the introduction of modern synthetic  
50 medicine, information has it that traditional medicine still accounts for about 80% of the health  
51 needs of the rural populace in most regions of Africa. Despite the huge benefits attached to  
52 medicinal herbs, it is not recommended to use it without adequate knowledge of its toxicity,  
53 dosage and purity.

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

61 The genus *Ficus* is made up of about 1000 species across tropical and warm temperate regions  
62 with greatest diversity in Asia, Malaysia and tropical South America. The tree is large and up to  
63 21m in height. In Latin, ficus means fig, which is derived from the Persian ‘fica’. The common  
64 name for *Ficus mucuso* is fig. It is a semi-deciduous spreading savannah tree with greenish  
65 flowers. The seeds are very tiny and numerous [6]. Because of the high nutritive value, apes [7,  
66 8] and indeed humans [9] depends so much on *Ficus* as part of their diet.

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

Comment [I3]: genus

Comment [I4]: 21 m

68 *Anthocleista vogelli* is predominantly found in swampy areas, river banks and Raphia grooves  
69 [11, 12]. It is about 20m in height. It is a medicinal plant that is widely used in West Africa [11,  
70 12]. It is used to manage constipation and also regulate menstruation. It acts as a strong purgative  
71 and diuretic. In some countries such as Sierra Leone, it is used in the treatment of jaundice and  
72 hepatitis [11]. In Nigeria and Congo, the bark and seed of this promising plant is used in the  
73 treatment of ovarian problem, bronchitis, hernia and fever. *Anthocleista vogelli* contains  
74 compound such as 1,7-dihydroxy-3,8-dimethoxy-xanthone and 1,8-dihydroxy-3,7-dimethoxy-  
75 xanthone. These compounds are responsible for its anti-malaria and anti-ulcer potential.  
76 Antioxidants occur naturally in some plants which constitute part of human daily diet [6]. The  
77 intake of such nutritious plants display antioxidant properties which mop up free radicals thus  
78 preventing oxidative stress and maintaining good health. The most common antioxidants present  
79 in diets are vitamin E, vitamin C and carotenoids. Other non-nutrient food substances, including,  
80 phenolic and polyphenolic compounds also exhibit antioxidant properties [6, 13]. Based on the  
81 documented antioxidant evidences and nutritive value of these three plants, it is convenient to  
82 evaluate their basic role against ethanol toxicity.

83 The present study was thus initiated to comparatively evaluate the effects of three aqueous  
84 extracts of *Xylopiya aethiopica*, *Ficus mucoso* and *Anthocleista vogelli* on some biochemical  
85 parameters following ethanol administration with a view to ascertain their effect in ameliorating  
86 ethanol toxicity.

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90 **MATERIALS AND METHODS**

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92 **Plant Materials**

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

97 **Preparation of Extract**

98 The fresh fruit of *Xylopia aethiopica*, leaves of *Fiscus mucuso* and *Anthocleista vogelli* were air  
99 dried and powdered using a grinding and crushing machine (Daiki Rita Kogyo Co Ltd, Japan).

100 The powders were extracted in cold water with intermittent shaking for 48 hours. The aqueous  
101 filtrate was concentrated in vacuum rotary evaporator (Buchi Ratavapour R110, Schweiz). The  
102 fruit of *Xylopia aethiopica* and leaves of *Fiscus mucuso* and *Anthocleista vogelli* yielded 18.39g  
103 (5.93%), 19.75g (5.34%) and 51.39g (3.06%) respectively. Extracts were dissolved in normal  
104 saline solution and administered orally at a dose of 200mg/kg to animals in groups C-H for  
105 twenty one days. The extract was administered 24hrs after ethanol toxicity was established for  
106 animals in groups C-E. Ethanol toxicity characterized by gastric heamorrhagic patches was  
107 established one hour after administration in line with previous study [14]

108 **Animals**

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

**Comment [15]:** Lines 103 to 107 should be moved to appear below line 123. See the first review

114 **Experimental Design**

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

116 **GROUP A:** Control (administered with normal saline)

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

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

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

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

121 **GROUP F:** *Xylopi*

122 **GROUP G:** *Fiscus mucuso*

123 **GROUP H:** *Anthocleista vogelli*

124 At the end of the experiment, the animals were sacrificed. Before the sacrifice, blood samples

125 were collected via cardiac puncture after the animals were placed under slight anaesthesia. Serums

126 obtained were assayed for total protein, uric acid, creatinin, urea, Aspartate aminotrasferase

127 (AST) and Alanine aminotransferase (ALT) using respective diagnostic kits. All biochemical

128 analysis were carried out in the Department of Biochemistry, Obafemi Awolowo University,

129 Nigeria.

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Comment [16]: anesthesia

Comment [17]: aspartate

Comment [18]: alanine

134 **Statistical Analysis**

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

**Comment [19]:** compare the means of the groups

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## RESULTS

In this study, uric acid levels were decreased in the serum of ethanol administered rats. Following the treatment with *Xylopiya 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 *Xylopiya 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 *Xylopiya aethiopia*, *Fiscus mucuso* and *Anthocleista vogelli* while only *Xylopiya 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.



179 **TABLE 1:** Showing the Effects of *Xylopia aethiopica*, *Fiscus mucoso* and *Anthocleista vogelli* on  
 180 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 <sup>a</sup>	35.61 ± 2.08 <sup>ab</sup>	1.68 ± 0.33 <sup>a</sup>	37.46 ± 0.66 <sup>bc</sup>	37.25 ± 12.95 <sup>ab</sup>	10.80 ± 0.80 <sup>a</sup>
Group B	0.62 ± 0.035 <sup>a</sup>	31.82 ± 5.60 <sup>ab</sup>	1.94 ± 0.13 <sup>a</sup>	37.50 ± 0.28 <sup>bc</sup>	53.00 ± 11.93 <sup>b</sup>	12.33 ± 2.02 <sup>a</sup>
Group C	0.65 ± 0.012 <sup>a</sup>	58.15 ± 1.16 <sup>c</sup>	1.79 ± 0.12 <sup>a</sup>	36.20 ± 1.15 <sup>bc</sup>	19.00 ± 1.15 <sup>a</sup>	11.00 ± 1.16 <sup>a</sup>
Group D	0.66 ± 0.015 <sup>a</sup>	28.76 ± 1.32 <sup>a</sup>	1.50 ± 0.01 <sup>a</sup>	35.55 ± 0.02 <sup>b</sup>	25.00 ± 3.46 <sup>a</sup>	16.00 ± 2.30 <sup>ab</sup>
Group E	0.78 ± 0.011 <sup>a</sup>	68.28 ± 1.19 <sup>c</sup>	1.70 ± 0.12 <sup>a</sup>	32.80 ± 1.16 <sup>a</sup>	36.00 ± 1.16 <sup>ab</sup>	13.00 ± 1.15 <sup>a</sup>
Group F	0.66 ± 0.043 <sup>a</sup>	62.18 ± 11.99 <sup>c</sup>	1.82 ± 0.09 <sup>a</sup>	36.22 ± 0.69 <sup>bc</sup>	30.50 ± 3.27 <sup>ab</sup>	14.75 ± 1.31 <sup>ab</sup>
Group G	0.71 ± 0.022 <sup>ab</sup>	29.35 ± 4.16 <sup>a</sup>	1.70 ± 0.04 <sup>a</sup>	36.06 ± 0.62 <sup>bc</sup>	24.60 ± 5.60 <sup>a</sup>	13.20 ± 2.65 <sup>a</sup>
Group H	0.78 ± 0.025 <sup>b</sup>	51.95 ± 11.01 <sup>bc</sup>	2.07 ± 0.09 <sup>a</sup>	39.05 ± 1.58 <sup>c</sup>	36.66 ± 7.75 <sup>ab</sup>	19.50 ± 1.04 <sup>ab</sup>

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

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## DISCUSSION

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

194 In this study, uric acid levels were decreased in the serum of ethanol fed rats as compared to the  
195 control. However, this decrease was not significant. This may either be due to the inhibition of  
196 nucleotide (adenine nucleotide) turnover or alteration in the catabolism of purines. It is also  
197 possible that the uric acid may have been utilized in scavenging free radicals produced as a result  
198 of ethanol intoxication. Free radicals such as superoxide anion and hydroxyl radical are unstable  
199 [6, 19]. For instance, superoxide anion interacts with nitric oxide to form reactive peroxynitrite  
200 while hydroxyl radical react rapidly with most biological molecules [19]. The increase in the uric  
201 acid concentration of the group treated with *Xylopiya aethiopia* and *Anthocleista vogelli* after  
202 ethanol toxicity (Group C and E) and also in the group administered with *Xylopiya aethiopia* and  
203 *Anthocleista vogelli* only (group F and G) is likely due to the antioxidant properties of these  
204 plants. Investigations have shown that *Xylopiya aethiopia* possesses antioxidant properties [5]. A  
205 study of Adefegha and Oboh [20] about the effects of diets supplemented with *Xylopiya*  
206 *aethiopica* and *Piper guineense* on some biochemical parameters in normal rats revealed that the  
207 flavonoid content of *Xylopiya aethiopica* was significantly higher than *Piper guineense*.

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

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

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

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