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# Kargasok Tea Consumption and Liver Toxicity in Rats

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# ARTICLE INFO ABSTRACT

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**Keywords:** Kargasok tea, Kombucha, Liver, Toxicity, Rats. **Background** - Kargasok tea (kombucha) is a widely consumed tea (even in Nigeria) made from a symbiotic culture of bacteria and fungi. While many persons consume kargasok tea because of its potential benefits like anti-cancer effects, cure for chicken pox, anti-hypertensive, improvement of poor eye sight, treatment of insomnia etc, some studies have shown that some toxicity exist following acute and/or chronic consumption. The aim of this study is to determine the toxicity of kargasok tea on the liver of rats.

**Method** - The median lethal dose (LD $_{50}$ ) done earlier using this tea did not produce any mortality even with a dose as high as 5000mg/kg. The study was carried out using a total of 50 wister rats which was divided into 4 groups. Group 1 (3 rats) was used as control while group 2 to 4 (12 rats each) was the test group. Kargasok tea extracts was administered to the rats in groups 2 to 4 for a period of 28days with group 2 receiving 250mg/kg of the extract, group 3 received 500mg/kg while group 4 received high doses (1000mg/kg). The rat liver was harvested (after sacrifice) every week under the effect of chloroform anaesthesia for histopathological evaluation. Also, blood was collected for LFT. The mean values of the biochemical parameters were evaluated for significant differences using SPSS 23. ANOVA test was employed for calculation of means and standard error determinations.

**Results -** Results revealed that the rats that consumed kargasok tea had increased liver enzymes. Also, there was histologically distorted liver from the rats that consumed kargask tea. This showed that kargasok tea has the potential of causing damage to the liver and kidney especially upon chronic consumption. **Conclusion -** Consumers of kargasok tea need to be wary of its deleterious effects especially from chronic intake as it has been shown to possess the potential of causing damages to vital organs of the body especially the liver.

#### INTRODUCTION

Kargasok tea is a symbiotic culture of bacteria and yeast (SCOBY). Yeast/bacteria fungal symbiont, zoogleal mat, T'chai from the sea, Teekwass, Tschambucco, Zoogleal mat, and Champagne of life are other names for this beverage.

Kargasok tea is a fermented tea beverage produced by adding sugar to black tea and letting it to ferment. The Symbiotic Culture of Bacteria and Yeast (SCOBY) produces several metabolic products, such as acetic acid and other organic acids, that are antibacterial in nature and prevent harmful bacteria from infecting the beverage<sup>1</sup>.

The fermentation of Kargasok tea is facilitated by a mutualistic connection between bacteria and yeasts<sup>2</sup>. It's not as easy to pinpoint the exact bacterial makeup of kombucha because of how changeable the bacterial composition of kombucha is. The inoculum used to brew the tea might have come from a variety of other places. There are plenty of prokaryotes in the kombucha culture with the most abundant belonging to two genera – *Gluconobacter* and *Acetobacter*. *Acetobacter xylinum* is regarded as the basic bacterium<sup>3,4,5</sup>. The cellulose layer's microflora, as reported by Sievers et al. (1995), includes both Zygosaccharomyces and A. xylinum sp.<sup>5</sup> Acetobacter xylium, A. pasteurianus, A. aceti, and Gluconobacter oxydans are the most common acetic acid bacteria in the tea fungus, as described by Liu et al. (1996)<sup>6</sup>.

Kombucha tea has a plethora of different yeast species including Koleckera, Pichia, Mycoderma, Saccharomyces, Saccharomyces, Schizosaccharomyces, Zygosaccharomyces, Brettanomyces/Dekkera, Candida, Torulospora.<sup>3</sup>

Following the chemical analysis of Kargasok tea, the presence of the following have being identified – sugars, organic acids such as gluconic acid, citric acid, acetic acid, L-lactic acid, malic acid, tartaric acid, Vitamins  $B_1$ ,  $B_2$ ,  $B_6$ ,  $B_{12}$ , and C, amino acids, lipids, proteins, polyphenols, as well as yeast and bacterial metabolites.  $^{3.7,8.9}$ 

For the preparation of kargasok tea, about 5 grams of tea leaves are added to 1 litre of water (5g/L), followed by a similar amount of sugar (50g for 1 L of water), which functions as a substrate for the tea fermenting yeasts and bacteria throughout the kargasok tea making process. Before the SCOBY or a tiny amount of ready-to-drink kargasok tea (starter culture) may be added, the beverage has to be at a temperature near to 20°C. To avoid unjustified contamination by undesirable microorganisms, it is crucial to always operate in a clean atmosphere and only use sterilised tools throughout the process.<sup>1</sup>

It is also vital to control the levels of pH during the fermentation process. Preferably, the process should be stopped when the pH gets to 4.2 so as to avoid excessive production of acetic acid which may prove counterproductive. Kargasok tea fermentation might vary from culture to culture, kombucha can be fermented in as little as three days and as long as sixty days.<sup>1</sup>

Numerous positive benefits of drinking Kargasok tea have been observed. According to Dufresne and Farnworth (2000), the health benefits of drinking Kargasok tea include: removing toxins from the blood, lowering cholesterol levels, slowing the progression of atherosclerosis, reducing the risk of stroke in hypertensive patients, amongst others. 10

Antimicrobial<sup>11</sup>, <sup>12</sup>, Antioxidant<sup>13</sup> and Hepatoprotective<sup>14, 15</sup> benefits of kargasok tea have been observed in different studies by researchers.

It is worth noting that concerns about the safety of herbal medicines and supplements like kargasok tea have increased in recent years along with their popularity. The risks associated with kargasok use are nothing new. The increasing consumption of kargasok in the 1950s coincided with the proliferation of cancercausing rumours regarding the Russian tea Kwass, which is quite similar to kargasok. Sunghee et al. (2009) demonstrated lactic acidosis in an HIV patient who consumed kargasok tea within 12 hours of presentation.<sup>16</sup> Srinivasan et al. (1997) reported that four people who drank kargasok tea suffered hepatic and allergic issues. 17 After consuming kargasok tea for a month, a 58-year-old woman exhibited signs of cholestatic liver injury, as evidenced by a high alkaline phosphatase to aminotransferase ratio and increased bilirubin level. Biopsy results confirmed druginduced hepatotoxicity.18

#### Aim

To determine the toxicity of kargasok tea on the liver of rats

#### **MATERIALS AND METHODS**

#### **Animals**

Fifty (50) 200g to 360g of male (30) and female (20) wistar rats were used. They were sourced from the University of Port Harcourt animal house and allowed to acclimatize for two weeks at the Department of Pharmacology Laboratory of the University of Port-Harcourt, Rivers State, Nigeria.

#### Kargasok tea

Kargasok tea was prepared by fermenting sugared black tea with a SCOBY (Symbiotic Culture of Bacteria and Yeast). This was in turn orally administered to the animals.

#### **Ethical clearance**

Ethical commendation was obtained from the ethics committee of the University of Port Harcourt. The animals were handled according to the guidelines of National Institute of Health guide for care and use of Laboratory Animals.

#### **Experimental Design**

This study was carried out using a total of 50 rats. They were divided into four (4) groups (group 1 to group 4). Group 1 was used as the control while group 2 to 4 as test group. Group 2 to 4 had 12 rats each while group 1 had 3 rats only. Tea extracts was administered to the animals in groups 2 to 4 for a period of 28days with group 2 receiving low doses, that is 250mg/kg, group 3 receiving moderate doses, that is 500mg/kg while group 4 received high doses, that is, 1000mg/kg. The tea was administered once daily.

The liver was collected from the animals every week under the effect of diethyl ether anesthesia for histopathological evaluation. Also, blood samples were collected during this period of sacrifice for the evaluation of liver function tests (LFT).

#### Statistical analysis

The mean values of the biochemical parameters were evaluated for significant differences using SPSS version 23 (IBM, United States of America). ANOVA test was employed for calculation of means and standard error determination. P-values were accepted at ≤ 0.05 significance level.

#### **RESULTS**

Figure 1 shows a graph of mean values of Aspartate transaminase (AST). Compared with normal control, the

AST values of the rats that consumed low dose (250mg/kg) of kargasok tea were higher for week 3 and 4. The AST values of rats that consumed medium (500mg/kg) and high dose (1000mg/kg) of the tea were higher for week 2 through to week 4. Statistically significant increases were found in medium and high dose week 3, all doses for week 4 (p≤0.05). This showed that increasing the dose of the tea led to an earlier increase in the AST values. Also, the AST values increased proportionally to the number of weeks of consumption of the tea.

Figure 2 shows a graph of mean values of Alanine transaminase (ALT). When compared with normal control, the ALT values of the rats that consumed low dose (250mg/kg) of the tea were higher for week 3 and week 4; while that of rats that consumed medium (500mg/kg) and high dose (1000mg/kg) of the tea were higher from week 1 through to week 4. Statistically significant increases were seen in high dose week 2, all doses for week 3 and week 4 (p≤0.05). This showed that an increase in the dose of the tea also led to an increase in the AST level. Also, the more the number of weeks spent on consumption of the tea, the higher the AST value was.

Figure 3 shows the mean values of alkaline phosphatase (ALP).

When compared with the normal control, the ALP values of the rat that consumed low dose (250mg/kg), medium dose (500mg/kg) and high dose (1000mg/kg) of the tea were higher from week 2 through to week 4; although a statistically significant difference was only observed in high dose, week 4 ( $p \le 0.05$ ). It was also noticed that this increase was directly proportional to the number of weeks.

Figure 4 shows the mean values of total bilirubin. There was a slight increase in values among the rats that consumed medium dose of the tea in week 1, 3 and 4 and also high dose week 2 and 4; however these slight increases were statistically insignificant.

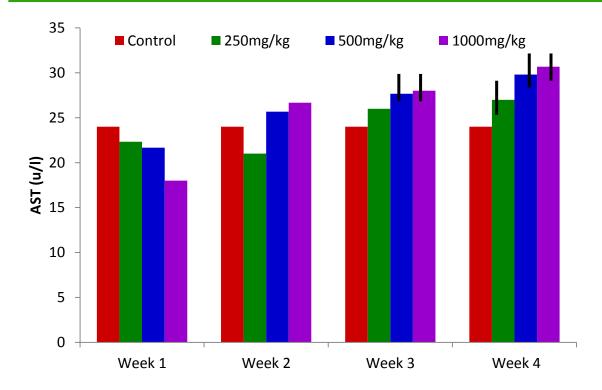


Figure 1: Effect of Kargasok Tea on AST in Wistar rats

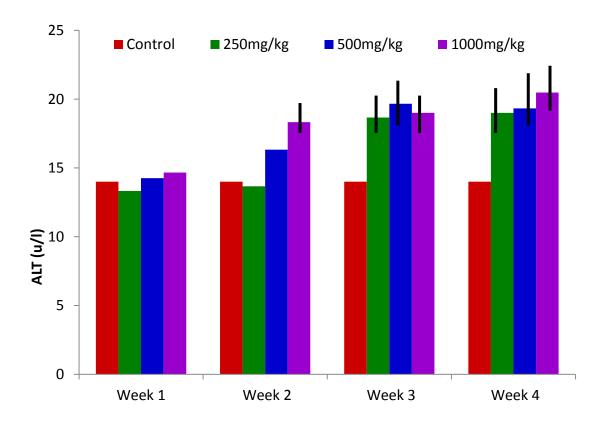


Figure 2: Effect of Kargasok Tea on ALT in Wistar rats

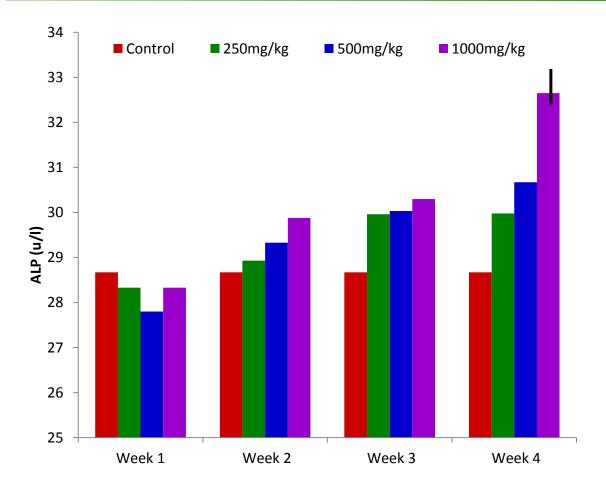


Figure 3: Effect of Kargasok Tea on ALP in Wistar rats

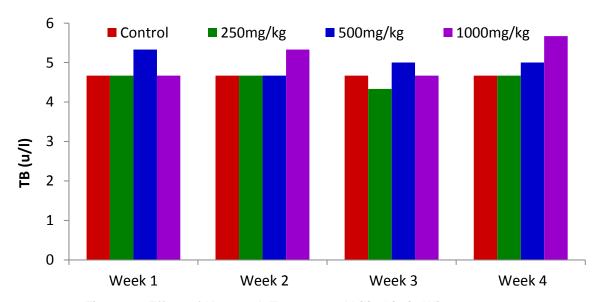
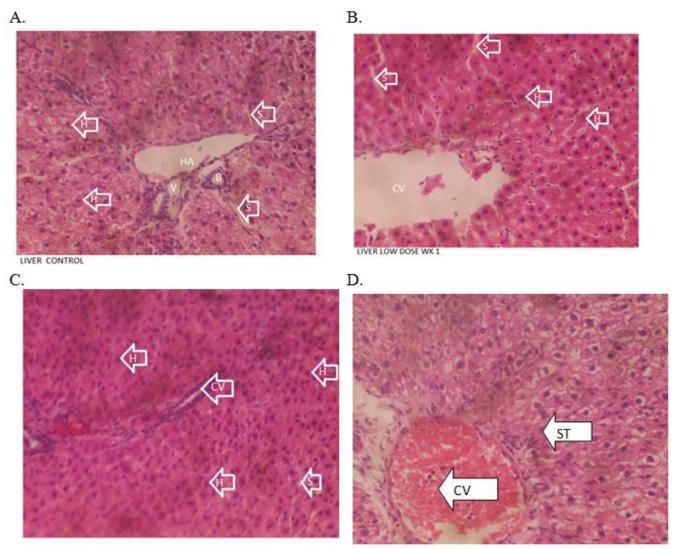
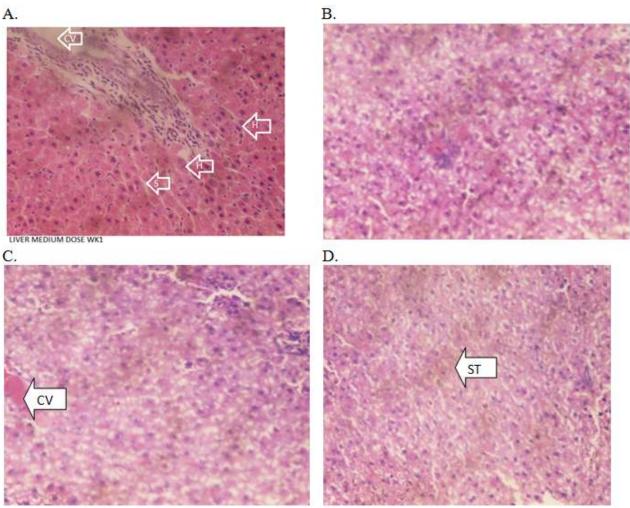


Figure 4: Effect of Kargasok Tea on total bilirubin in Wistar rats



**Figure 5:** Photomicrograph of the rat liver showing **(A)** control - histologically normal liver with intact hepatocytes (H), Sinusoids (S) **(B)** low dose wk 1 **(C)** low dose wk 2 **(D)** low dose wk 4- histologically distorted liver with congested central vein (CV), hepatocytes with different grades of steatosis.



**Figure 6:** Photomicrograph of rat liver showing **(A)** Medium dose wk 1 – histologically normal rat liver with patent central vein (CV), intact hepatocytes and sinusoids (S). **(B), (C) and (D)** – medium dose wk 2, medium dose wk 3 and medium dose wk 4 respectively, all showing histologically distorted liver with congested central vein (CV), different grades of steatosis (ST).

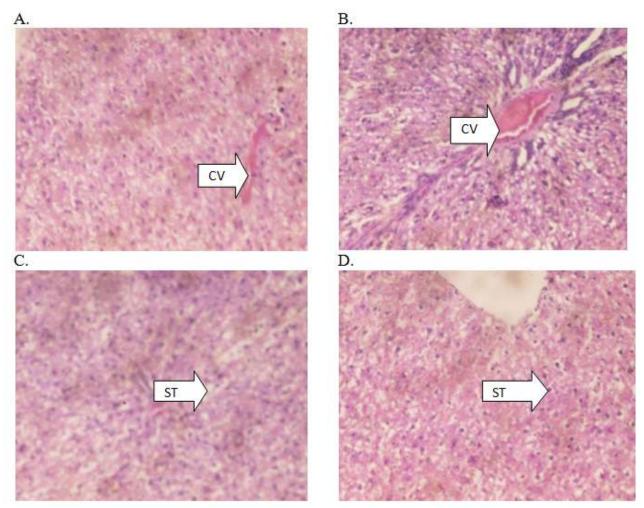


Figure 7: Photomicrograph of rat liver showing high dose wk 1 (A), high dose wk 2 (B), high dose wk 3 (C) and high dose wk 4 (D). These photographs show histologically distorted liver with congested central vein (CV) and different degrees of steatosis (ST).

### **DISCUSSION**

From the result of the liver function tests (LFT), it was observed that the liver enzymes (ALT, AST & ALP) were increased following consumption of kargasok tea with ALT and AST showing a greater increase when compared with ALP. These liver enzymes are usually increased in pathologic conditions of the liver, although there are non-hepatic causes of elevated ALT and AST like in cases of hemolysis, myopathy and even exercise; from the result, ALT and AST showed a greater increase relative to ALP. This is said to occur when there is a hepatocellular cause for the liver pathology as against a very elevated ALP relative to ALT and AST which would signify a cholestatic or hepatobilliary cause. 19 Therefore from this study, it can be inferred that the rats which consumed Kargasok tea for at least 2 weeks and beyond irrespective of the dose showed signs of hepatocellular injury. The laboratory results were also backed up by results of histology which showed distorted liver, hepatocytes with different grades of steatosis with a congested central vein. This is in keeping with an inflamed liver. This assertion is

supported by Srinivasan et al (1997) who reported onset of hepatic complications in four patients consuming kargasok tea and that these symptoms ceased when consumption was halted. Gedela et al (2016) has also observed hepato-toxicity from a liver biopsy following consumption of kargasok tea. Perron et al (1995) also observed elevated liver enzymes in a 53 yr old college professor who drank half cup of kombucha tea for two weeks of which symptoms and laboratory values returned back to normal after cessation of the drink.

#### CONCLUSION

Consumers of kargasok tea need to be wary of its deleterious effects especially from chronic intake as it has been shown from this study to possess the potential of causing damages to vital organs of the body especially the liver.

The authors share no conflict of interests.

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