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Research Article

PROTECTIVE EFFECT OF COMBINATION OF ETHANOLIC EXTRACTS OF *XIMENIA AMERICANA* AND *TERMINALIA MACROPTERA* AGAINST ALCOHOL-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: The aim of this study was to evaluate the hepatoprotective effect of the combination.

Methods: Serum liver markers, tissue antioxidant activity, and histological changes in the livers of rats from the blank, negative (distilled water), positive (silymarin 100 mg/kg bw), and test (combination 500 mg/kg bw) groups were measured after 7 days of pretreatment and induction of hepatotoxicity by 10 g/kg bw alcohol every 12 h for 48 h.

Results: Rats in the negative control group showed a highly significant ($p<0.001$) increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (BT) levels by 281.13%, 221.7%, and 93.44%, respectively, compared to rats in the blank group. Pretreatment with the combination resulted in a highly significant ($p<0.001$) decrease in AST, ALT, and BT levels of 69.19%, 62.24%, and 41.52%, respectively. The study of tissue oxidative stress parameters revealed a very significant ($p<0.01$) increase in superoxide dismutase (123.08%), glutathione (131.66%), and catalase (49.01%) activities and a significant ($p<0.05$) decrease in malondialdehyde concentration (59.72%) in the group pretreated with the combination compared with the negative control group. Steatosis and necrosis estimated at 50% were observed in rats in the negative control group. In contrast, necrosis observed in the group pre-treated with the combination was <10%.

Conclusion: These data suggest that the combination is effective in preventing the elevation of biochemical markers and the imbalance of enzymatic and non-enzymatic antioxidant systems caused by alcohol.

Keywords: Antioxidant, Hepatoprotective, *Ximenia americana*, *Terminalia macroptera*, Combination.

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INTRODUCTION

Alcohol has been consumed by humans for thousands of years. It is a common cause of reactive oxygen species (ROS) damage in the liver. Although it has been shown that alcohol consumption at levels of <10 g/day has a protective effect on the risk of coronary heart disease and ischemic stroke [1,2]. It should also be noted that at levels of alcohol consumption in excess of 30 g/day, alcohol is likely to cause damage. Worldwide, more than 2.5 million deaths were attributed to alcohol consumption in 2019. Africa had the second highest level of alcohol-related deaths, with 52.2 deaths/100,000 people [3,4]. In Africa, in low-income countries, alcohol consumption is 41 g of pure alcohol per drinker per day, which is associated with very high mortality rates [5]. There is a dose-response relationship between alcohol consumption and the risk of cirrhosis of the liver, cancer of the oropharynx, larynx, esophagus, liver and breast, and stroke. An increased risk of arrhythmia, cardiomyopathy, and sudden coronary death has been associated with heavy alcohol consumption. However, liver disease in alcoholics is one of the most serious liver disorders and encompasses three major related entities: Steatosis, alcoholic hepatitis, and cirrhosis. Steatosis is the first histological manifestation of alcoholic liver disease and is found in 85% of patients who consume excessive quantities of alcohol [6-8]. This damage is the consequence of alcohol metabolism in the liver. It generates an excess of free radicals and an increase in the peroxisomal oxidation of fatty acids, which ultimately affects the functionality of antioxidant systems to eliminate ROS in the organism. Consequently, the mechanism for restoring liver damage caused by alcoholic oxidative stress is closely regulated by the antioxidant status of a living system [9].

Many plants with antioxidant activity are also associated with liver protection potential. These include *Ximenia americana* and *Terminalia macroptera*, medicinal plants used in Mali in West Africa to treat a variety of illnesses, the most common being infectious and inflammatory conditions. Ethnopharmacological studies of *X. americana* and *T. macroptera* in Mali have shown that the roots and leaves, respectively, are the most commonly used. Pharmacological studies on these two plants have shown anti-inflammatory [10-12], antiradical [10,13,14], antioxidant [15], anticancer [16], antimicrobial [17,18], analgesic [10,19], and immunostimulant [20,21]. Previous *in vivo* studies on the combination have shown anti-inflammatory properties in models of acute (synergistic activity), sub-acute, and chronic inflammation [22,23]. This study is the first to assess the hepatoprotective effect of the above-mentioned combination on alcohol-induced liver damage in rats.

METHODS

Collection and authentication of the plants

The leaves and root barks of *T. macroptera* and *X. Americana,* respectively, were collected in Same (Bamako, Mali). They were identified by Dr. Mamadou Lamine Diarra, a botanist. A herbarium of each sample was deposited with identification numbers 2468 and 0027, respectively, in the department of medicine.

Extraction methods

After drying and pulverizing the samples, hydroethanolic maceration (30: 70 v/v) was carried out on each sample powder as described previously [24].

Experimental animals
Twenty male Wistar rats weighing between 150 and 200 g were placed in cages to adapt to laboratory conditions, temperature: (22±3)°C, humidity: 50–55%, and light/dark cycle: 12 h. They were adapted to laboratory conditions within 2 weeks. Water and food were permanently available. The experiment was carried out in accordance with the authorization of the ethics committee of the University of Science, Techniques and Technology of Bamako #Reg. No. 2021/234/USTTB.

Reagents and drugs
Anhydrous absolute ethanol (Carlo Erba); Ellman's reagents; trichloroacetic acid; thiobarbituric acid; dichromate; acetic acid; adrenaline; silymarin (Cefasilymarin® Cefak, Kempten), formalin (Cooper).

Groups and treatment schedule
After 12 h of fasting, the rats were randomly divided into 4 groups of 5. Groups 1 and 2, the blank and negative controls, respectively, were given distilled water. Rats in Group 3, the positive control, received 100 mg/kg bw of silymarin. Rats in Group 4, the test group, received 500 mg/kg bw of the combination (1:1 w/w). The various substances were administered by gavage as a single daily dose for 7 days.

Induction of hepatic injury and sample collection
The method previously used by Feng was slightly modified for this experiment [25]. A solution of pure (99.9%) absolute ethyl alcohol was then diluted to 50° according to Gay Luisac's table (100 mL of pure alcohol were added to 107.44 mL of distilled water). Rats in Groups 2, 3, and 4 were given 50° ethyl alcohol orally at a dose of 10 g/kg or 12.67 mL/kg bw every 12 h for 48 h. 12 h after the last dose, the rats were sacrificed by decapitation.

Blood was collected in a dry tube. The liver was harvested and washed immediately with cold 0.9% NaCl. Part of the liver was used to assess oxidative stress parameters and the remainder was fixed in 10% dilute formalin for histological assessment.

Determination of the hepatic index
The body weight and liver weight of each rat were measured before and immediately after sacrifice. The liver index was determined according to this formula [26].

$$\text{Liver index} = 100 \times \frac{liver\ weight}{body\ weight}$$

Serum preparation
Blood collected in dry tubes was left to stand for 30 min. The blood samples were centrifuged at 3000 rpm for 10 min at 4°C to afford the serum, which was stored at 2–8°C for biochemical analysis.

Biochemical tests
To assess liver injury, serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (BT) were measured at 37°C according to the manufacturer's instructions [27].

Preparation of liver homogenates
The liver was weighed and a mass of 0.20 g was transferred to a graduated cylinder into which 1 mL of 50 mM Tris-HCl was added. After grinding in a mortar on an ice tray, the homogenate obtained was centrifuged at 3000 rpm for 20 min at 4°C. The supernatant collected was aliquoted and stored in the freezer at −20°C for assay of oxidative stress parameters.

Determination of antioxidant activity in liver tissue
The supernatant was used to measure oxidative stress parameters. Catalase (CAT) and superoxide dismutase (SOD) activity were determined as previously described [28,29]. Reduced glutathione (GSH) and malondialdehyde (MDA) levels were measured using the method described by Ellman [30] and Ohkawa *et al.* [31], respectively.

Histological examination
Sections were prepared from formalin-fixed tissue and stained with hematoxylin and eosin. The stained areas were observed under a light microscope. Liver histology was assessed by an experienced pathologist without disclosing treatment or grouping information. Pathological changes associated with liver injury were assessed as the percentage of cells affected [32].

RESULTS AND DISCUSSION

Effect of the combination on the liver index
Induction of hepatic injury by massive doses of alcohol resulted in a highly significant increase ($p<0.001$) in the hepatic index of rats in the negative control group compared with rats in the blank control group (Fig. 1). Authors have described that the increase in the hepatic index may be due to severe damage to liver tissue, resulting in hepatomegaly [33,34]. Thus, the hepatic index effectively reflects the severity of liver damage. Rats pre-treated with the combination before alcohol induction showed a highly significant reduction in liver index ($p<0.001$) (Fig. 1). Studies have shown that exposure to ethanol inhibits the capacity of adipose tissue to absorb fatty acids on which lipid homeostasis depends, and which are the main cause of variation in body mass [33,35]. This is consistent with our results. Our results also showed that pre-treatment with the combination could prevent this inhibition.

Effect of combination on biochemical parameters
Several serum liver substances such as ALT, AST, and TB are used as biochemical markers of liver damage. Increased serum levels of AST and ALT have been attributed to deterioration in the structural integrity of the liver, as these enzymes are cytosolic and are released into the bloodstream following cell damage [36]. In the present study, the administration of alcohol to rats in the negative control group highly significantly ($p<0.001$) increased ALT and AST levels by 281.13% and 221.7%, respectively, compared with rats in the blank control group (Table 1). This observation indicates that the hepatotoxicity model has been successfully established in rats [37]. Furthermore, 93.44% increase in serum BT concentration was observed in the negative control group compared with the blank control group (Table 1). This could be due to damage to the liver parenchyma [38]. Pre-treatment with the combination resulted in a 69.19%, 62.24%, and 41.52% reduction in AST, ALT, and TB, respectively (Table 1). The reductions were highly significant ($p<0.001$) for transaminases and significant ($p<0.05$) for BT. Furthermore, the combination (500 mg/kg) produced reductions that were similar to those of silymarin (100 mg/kg bw) (Table 1). These results could indicate that the combination has the potential either to stabilize the plasma membrane, thereby preserving the structural integrity of hepatocytes, or to combat the free radicals produced by ethanol metabolism. These results corroborate those of other authors who have worked on *X. americana* or *T. macroptera* [10,39].

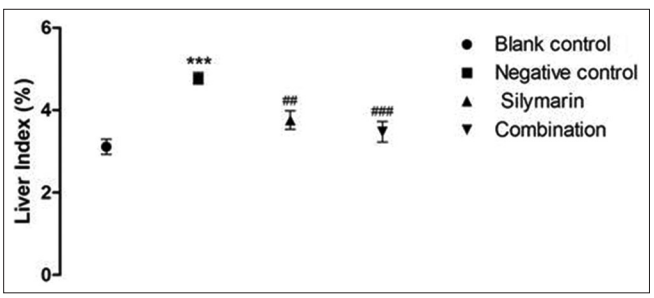

Fig. 1: Effect of combination on liver index in alcohol-induced rats. Data are represented as mean±SEM, n=5. *p<0.05, **p<0.01, ***p<0.001 significant difference when compared to blank control group and #p<0.05, ##p<0.01, ###p<0.001 significant difference when compared to negative control group

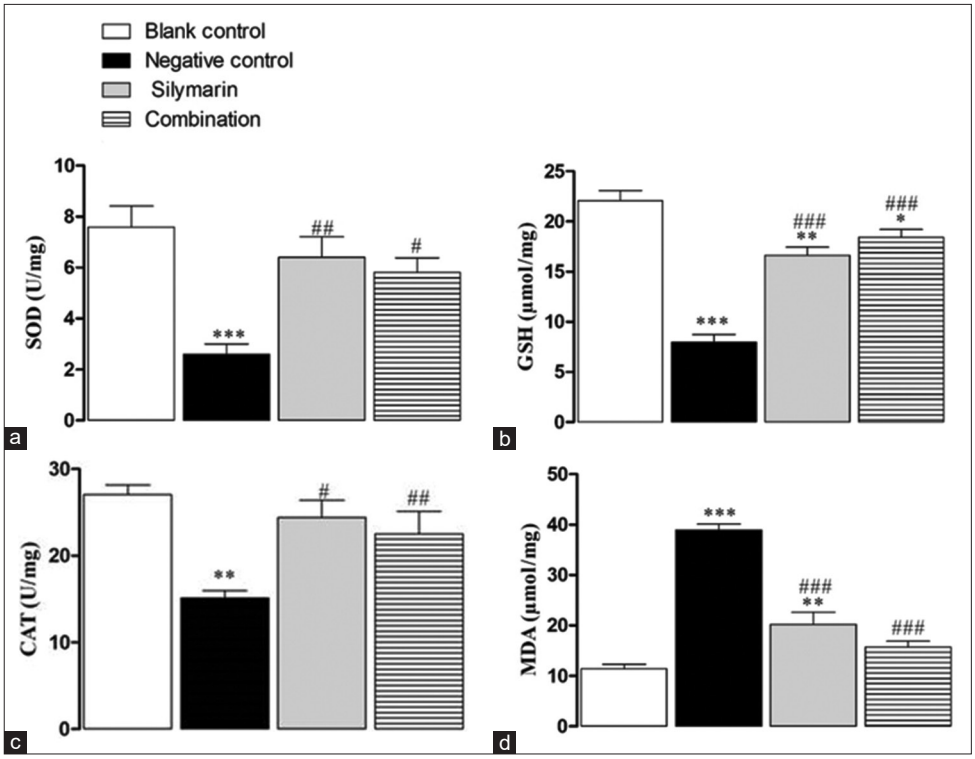

Fig. 2: Effects of the combination and silymarin on oxidative stress parameters in alcohol-induced rats. The values are expressed as mean±SEM, n=5. *p<0.05, **p<0.01, ***p<0.001 significant difference when compared to blank control group and #p<0.05, ##p<0.01, ###p<0.001 significant difference when compared to negative control group

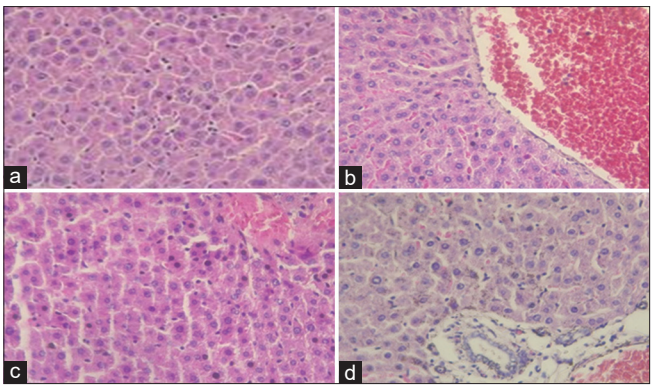

Fig. 3: Photomicrograph of liver cells of blank control rats (a), negative control rats (b), silymarin (c), and combination (d)

Table 1: Effects of the combination on the biochemical parameters in alcohol-induced rats

Treatment	AST (UI/L)	ALT (UI/L)	Bilirubin total (mg/dL)
Blank control	45.05±3.49	58.75±3.08	0.61±0.32
Negative control	171.7±8.49***	189.0±3.69***	1.18±0.29*
Silymarin	52.89±4.19*,###	71.36±3.63***,###	0.69±0.2#
Combination	58.04±2.87**,###	65.19±4.25*,###,ᵃ	0.72±0.31#

The values are expressed as mean±SEM, n=5. *p<0.05, **p<0.01, ***p<0.001 significant difference when compared to blank control group and #p<0.05, ##p<0.01, ###p<0.001 significant difference when compared to negative control group and ᵃp<0.05: Silymarin versus combination. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Effect of the combination on oxidative stress parameters

Recent studies have shown that hydroxyethyl radicals are generated during ethanol metabolism. Ethanol also alters enzymatic and non- enzymatic antioxidant defenses. These variations are at the root of oxidative stress, resulting in functional and structural damage to cell membrane integrity. Oxidative stress contributes to the perpetuation of chronic hepatic inflammation [13,40]. Fig. 2 shows that compared to the blank control group, SOD, GSH, CAT activities were reduced in rats of the negative control group in a highly significant way (p<0.001) for SOD, GSH and very significant (p<0.01) for CAT, with reductions of 65.79%, 63.95%, and 44.06%, respectively. On the other hand, a highly significant increase (p<0.001) of 240% in the concentration of MDA was observed. These data could indicate an exaggeration of oxidative damage and lipid peroxidation in the liver cells of rats in the negative control group. These results are in accordance with the work of Bilanda *et al*. [41] and Singh *et al*., 2018 [42] who showed that chronic ethanol consumption leads to an increase in MDA levels and a decrease in SOD, GSH, and CAT levels in liver tissue. Pre-treatment with the combination induced a highly significant increase (p<0.01) in the activities of SOD (123.08%), GSH (131.66%), and CAT (49.01%) and a significant reduction (p<0.05) of the MDA concentration (59.72%) compared with the negative control group, indicating that both enzymatic (SOD and CAT) and non-enzymatic (GSH) antioxidant activities had been restored. These results suggest that the combination contains bioactive compounds able to activate or restore the activity of enzymatic and non-enzymatic antioxidant systems impaired by ethanol. Previous studies have found high levels of polyphenols, flavonoids, and tannins in the two hydroethanolic extracts forming the combination. Several studies have shown the ability of these secondary metabolites to scavenge free radicals and inhibit lipid peroxidation [22,24,43,44].

Effect of the combination on histological lesions

The above results are supported by observational histopathological data. As the liver is the main site of ethanol metabolism, it is particularly sensitive to ethanol-induced histological lesions, including pure steatosis, alcoholic hepatitis, fibrosis, and cirrhosis [45]. The livers of rats in the blank control group showed regular liver cell morphology. In contrast, rats in the negative control group showed inflammatory

infiltration, congestion, steatosis, and an estimated 50% necrosis (Fig. 3). This is indicative of alcoholic hepatitis. It combines steatosis with hepatic inflammation and death of hepatocytes by necrosis [46]. Pre-treatment with the combination and silymarin significantly improved the histopathological changes, as shown by a discrete inflammatory infiltrate and <10% necrosis (Fig. 3).

CONCLUSION

The results of this preventive study show that pre-treatment with the combination can effectively prevent ethanol-induced liver damage. It showed very good hepatoprotective activity on serum liver markers by reducing levels of serum markers (ALT, AST, and BT). The mechanisms by which the combination protects the liver may be linked to its ability to upregulate enzymatic (SOD and CAT) and non-enzymatic (GSH) antioxidant activity and prevent lipid peroxidation. These results could justify the use of *X. americana* and *T. macroptera* in traditional medicine and open up prospects for the development of improved traditional medicines.

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AUTHOR'S CONTRIBUTION

Conceptualization: Mahamadou Ballo, Sekou Bah, Estelle Youl: Methodology: Mahamadou Ballo, Sekou Bah, Estelle Youl, Formal analysis: Data collection, Writing - Preparation of the original version: Mahamadou Ballo, Final editing: Sekou Bah, Estelle Youl.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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