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# Metabolic Alterations in Male Rats after Oral Exposure to Alcoholic Bitters

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## Abstract
*Alcoholic bitters have been widely consumed in Nigeria for the management of several medical conditions such as hypertension, menstrual cramps, hormonal imbalance and sexual inadequacies, without adequate information on the consequent biochemical changes. This study was therefore carried out to assess the effects of selected alcoholic bitters on some biochemical parameters of the liver and kidney of male rats.*
*Twenty- five male Wistar rats were assigned into five groups (A-E) consisting of five animals each and administered 0.4, 0.2, 0.2, 0.16, and 0.3 ml/kg of water, ethanol, Alomo, Strikers and Orijin alcoholic bitters respectively for 28 days. The animals were sacrificed 24 hours after the last treatment and the liver and kidney were used for the determination of the concentrations of total protein, total cholesterol, triglyceride, malondialdehyde and the activities of β-Hydroxy β-methylglutaryl-CoA reductase, superoxide dismutase and catalase using standard methods.*
*The alcoholic bitters significantly reduced ($p<0.05$) the protein concentration in both the liver (26%) and kidney (23%) when compared with control. There were significant reductions ($p<0.05$) in the levels of total cholesterol and β-Hydroxy β-methylglutaryl-CoA reductase activity in both the liver and the kidney whereas the level of triglycerides was not significantly ($p>0.05$) altered. Malondialdehyde levels reduced significantly whereas activities of catalase and superoxide dismutase significantly increased ($p<0.05$) especially in the liver after the administration of the alcoholic bitters when compared with control. This study lends credence to the beneficial effects of alcoholic bitter as it may reduce hypercholesterolemia and enhance antioxidant status.*

**Keywords**: Alcoholic bitters, Oxidative stress, Cholesterol, β-Hydroxy β-methylglutaryl-CoA reductase

## Introduction
In recent years, the request for herbal solution in Nigeria has been on the rise. Still, there are little or no scientific data to support medicinal claims of these herbal formulations. The scenario contributes to the increasing safety concerns of herbal preparation consumption in Nigeria (1). Herbal medicines have received greater attention as alternative to clinical therapy in recent times leading to subsequent increase in their demand. The damaging effect of herbal preparations to the human body is generally considered to be minimal compared to their synthetic counterpart (drugs), and as such, herbal medicines are generally regarded as safe. Herbals are usually sought after for their health benefits and these have become common medicines in many Nigerian homes (2).
Bitters are sharp flavouring extracts and regular cures that are ordinarily utilized in growing nations as a remedy for indigestion and other stomach afflictions, and even infections. Its utilization has been on the expansion currently without researching of the conceivable dangerous impact or metabolic adjustment. Studies and findings over the last two decades have continually show expanding utilization of home grown cures. It has been utilized as the most widely recognized type of common medication by around 60-80% of the world populace (3).
Herbal bitters are most often 'polyherbal formulations prepared from mixtures of many plant parts, obtained from various plant species and families. Thus, they contain multiple bioactive constituents that could have interacted with one another in solution, thereby posing some difficulties in their characterization. Herbal supplements are administered in most clinical conditions over a long period of time, without the consideration of toxic effects that might result from such prolonged usage and also, the proper dosage monitoring. However, in 2007 it has been described that the inability to bear the cost of present day restorative medicinal services in growing nations, has constrained patients to look for customary therapeutic considerations such as the bitters (4), (5).
Local survey carried out within Omu-Aran community concludes that a large percentage of young adults and artisans take daily doses (morning and/or night) of alcoholic bitters (especially Alomo Bitters, Striker Bitters and Origin Bitters) due to their claim of general wellness immediately after such consumption. The aim of this research therefore, is to determine the effects of these bitters on some biochemical parameters of the liver and kidney and to ascertain its safety and medicinal benefits in experimental animal model.

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## Materials and Methods

***Alcoholic Bitters***

The alcoholic bitters (Alomo, Striker and Orijin bitters) used were obtained from a local market in Omu-Aran, Kwara State, Nigeria.

***Experimental Animals***

Twenty-five healthy male Wistar rats were obtained from the animal house, University of Ilorin, Ilorin, Nigeria. The rats were housed in a clean well-ventilated room with a photo period of 12-hour light and 12-hour dark cycle with free access to feed and water.

***Chemicals and Reagents***

Assay kits for the cholesterol, triglyceride, were products of Randox Laboratories, Co-Atrim, (UK). All other reagents used were of analytical grade.

***Animal Treatment***

Twenty-five male rats were acclimatized for a week and thereafter, divided into five groups (A-E) of five animals each. Group A (Control group) were orally administered 0.4 ml/kg body weight of distilled water, once daily with the aid of a metal oropharyngeal canula while those in groups B, C, D, and E were treated with 0.2, 0.2, 0.16 and 0.3 ml/kg body weight of ethanol, Alomo bitters, Striker bitters and Orijin bitters respectively. The treatment lasted for 28 days.

***Preparation of Tissue homogenate***

After the twenty-eight days of treatment the rats were anaesthetized by placing them in a jar containing fumes of diethyl ether and sacrificed. The liver and kidney were excised from the rats and immersed in ice-cold 0.25 M sucrose solution to maintain the integrity of the harvested organs. The organs were blotted with filter paper, weighed and homogenized in ice-cold 0.25 M sucrose solution (1:4 w/v). The homogenates were then centrifuged at 5000 × g for 10 minutes and the supernatant stored in the freezer until when needed for biochemical analysis.

***Determination of Biochemical Parameters***

Total Cholesterol concentration was determined by the methods of (6), Total Protein concentration (7), Triglycerides (8), Antioxidant enzymes; Catalase (CAT) was assessed according to the methods of (9), Superoxide Dismutase (SOD) (10), Malondialdehyde (MDA)-(11) and HMG CoA reductase (12).

***Statistical Analysis***

All results were expressed as Mean ± SEM (n = 5). One ANOVA analysis was done using Statistical Package for Social Sciences (SPSS Inc.; Chicargo USA, 1989, 2013). Statistical significance was set at $P<0.05$.

## Results

The oral administration of ethanol and the alcoholic bitters caused a significant ($P<0.05$) decrease in protein concentration in both the liver and kidney when compared to the control as depicted in Figure 1. Similarly, as shown in Figure 2, there was a significant ($P<0.05$) decrease in concentration of total cholesterol in the liver and kidney in the groups treated with ethanol and alcoholic bitters when compared to the control group.

Meanwhile, triglyceride level was not significantly ($P>0.05$) altered across the groups as illustrated in Figure 3. The four weeks oral exposure of the animals to ethanol and the alcoholic bitters resulted in significant ($P<0.05$) increase in the enzyme HMGCo-A reductase level in all the groups when compared to the control in both the liver and kidney (Figure 4).

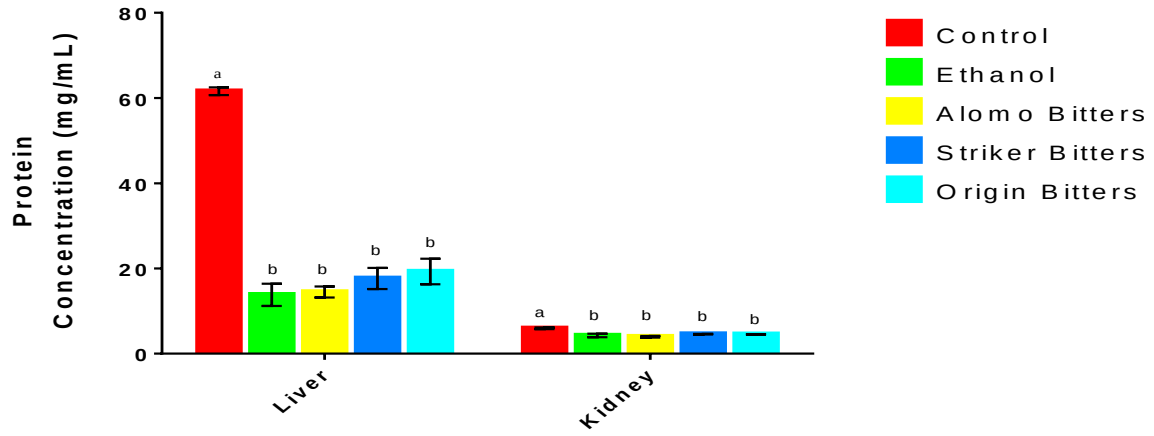


**Figure 1:** Effects of alcoholic bitters on protein concentration in the liver and kidney of male wistar rats.

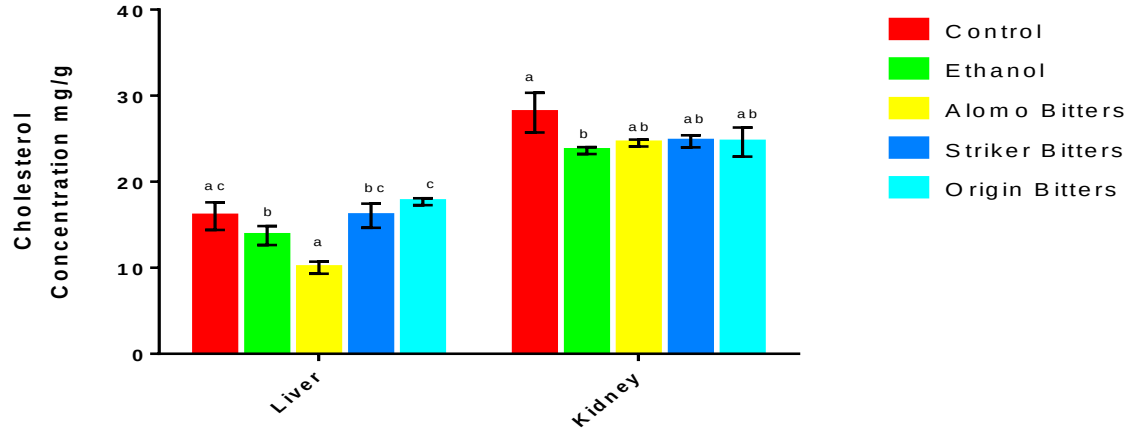


**Figure 2:** Effect of alcoholic bitters on total cholesterol concentration in the kidney and liver of male Wistar rats.

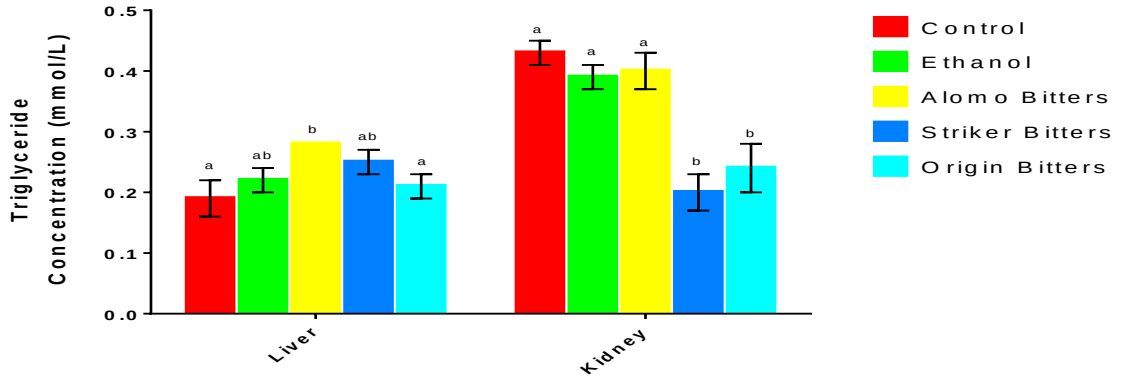


**Figure 3:** Effect of alcoholic biters on concentration of triglyceride in the liver and kidney of male Wistar rats.

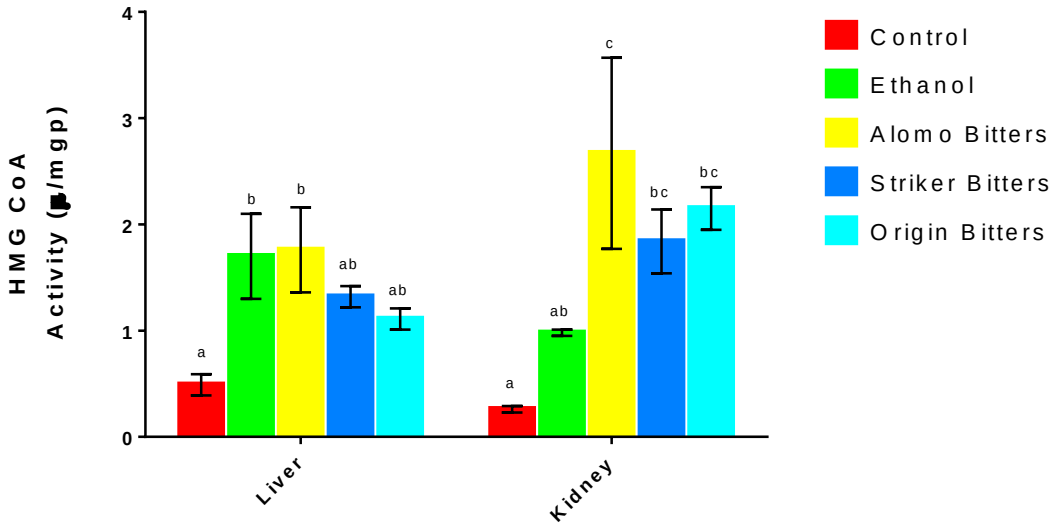


**Figure 4:** Effect of bitters on the activity of HMG CoA Reductase in the liver and kidney of male Wistar rats.

In the liver, the group administered Alomo bitters showed a significant ($P<0.05$) increase in MDA (marker for lipid peroxidation) when compared to the control. Conversely, there was a significant ($P<0.05$) reduction in the level of the MDA in the group treated with Alomo bitters in the kidney when compared to the control as shown in Figure 5.

The activities of the antioxidant enzyme superoxide dismutase and catalase were significantly increased in the alcoholic bitters groups when compared to the control group as depicted in figures 6 and 7 respectively.

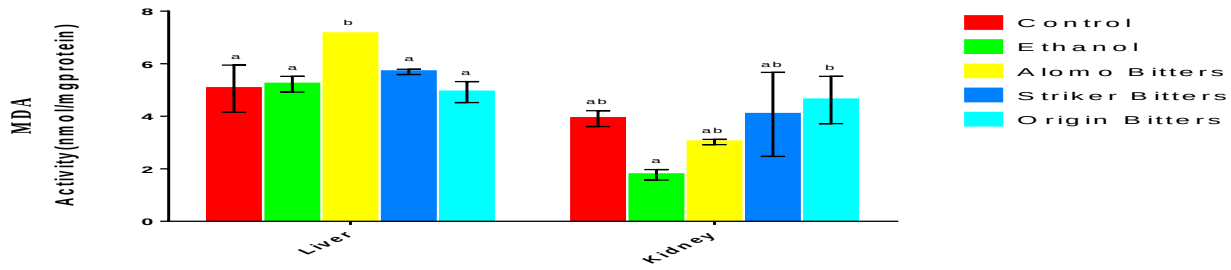


**Figure 5:** Effect of bitters on MDA level in the liver and kidney of male Wister rats.

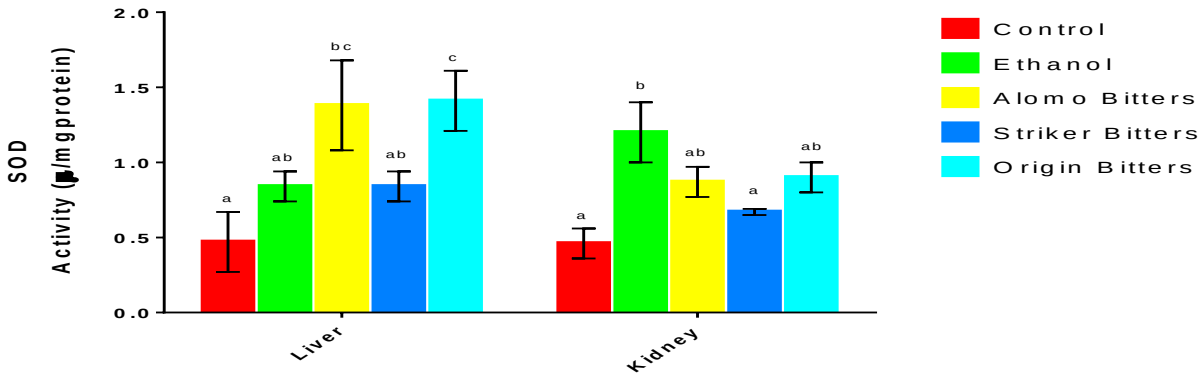


**Figure 6:** Effect of bitters on the activity of SOD in the liver and kidney of male Wistar rats.

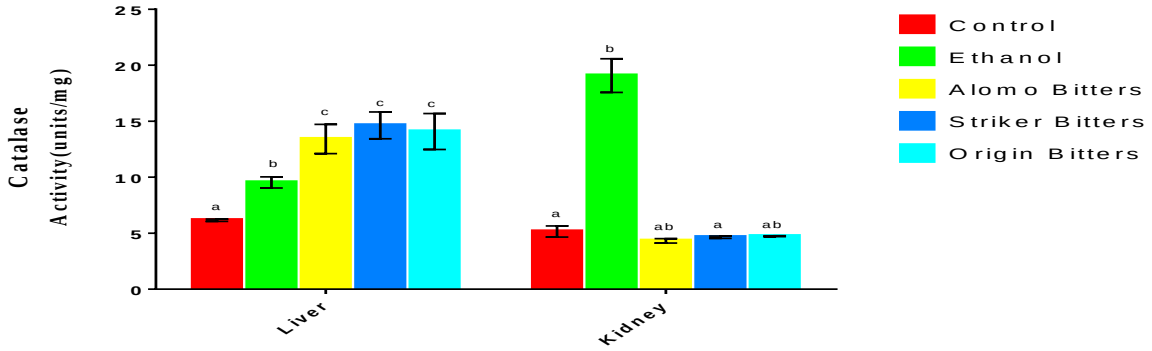


**Figure 7:** Effect of bitters on catalase activity in the liver and kidney of male Wister rat.

## Discussion

The oral administration of ethanol and alcoholic bitters caused a significant ($P<0.05$) decrease in total protein concentration in both the liver and kidney tissues of the animals, this may suggest an alteration in their protein synthesis ability and thus, indicating a non-desirable effect of the bitters on the tissue regeneration function. Since the ethanol group also showed a reduction in total protein concentration, it is likely that the alcoholic content of the bitters is responsible for the altered tissue protein synthesis function. (13)

The reduction of the total cholesterol in the tissues is probably as a result of the inhibition of the enzyme HMG-CoA reductase of the cholesterol biosynthetic pathway by the active components of the alcoholic bitters. This suggests that the alcoholic bitters might inhibit cholesterol biosynthesis in the liver. (14).

Oxidative stress which is measured by the biochemical marker, MDA, was observed in the ethanol groups. An increase in the level of hepatic MDA was observed in the Alomo bitters treated group, this was similar to the findings of Adeyemi and co-workers (1), where elevated levels of MDA was observed in rats following oral administration of yoyo bitters. Conversely, Strikers and Orijin bitters did not enhance production of reactive oxygen species. Increase in the activity of antioxidant enzymes induced by the bitters may also protect the cells of the liver and kidney from oxidative damage.

The study revealed that oral and repeated exposure to Striker, Alomo and Orijin alcoholic bitters seems to protect the liver and kidney tissues from oxidative stress and reduced lipid concentrations which may enhance cardiovascular health. The reduction in protein levels suggests that the long term oral consumption of these alcoholic bitters may be detrimental to tissue growth and maintenance.

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