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INFLUENCE OF AFRICA STAR APPLE (*CHRYSOPHYLLUM ALBIDUM*) WINE EXTRACT ON SOME BIOCHEMICAL AND OXIDATIVE STRESS PARAMETERS IN EXPERIMENTAL RATS

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Abstract

The polyphenol content in wines have been documented to exhibit many health benefits in mammals. In this study, the effect of African Star Apple (ASA) wine extract on some biochemical and oxidative stress parameters were investigated in experimental rats. Animals were administered with the ASA wine extract and Baron Wine at 5% and 9% concentration *ad libitum* for 21 days. Oxidative stress markers and antioxidant enzymes activities were determined in the erythrocytes and brain tissue homogenates of rats. The ASA wine extract and the standard red wine had no significant difference ($p < 0.05$) on the activities of ALT, AST, ALP and acetylcholineesterase when compared with the control group. Both extracts at 9% concentration produced a significant increase ($p < 0.05$) in catalase, superoxide dismutase and gluthathione activities in the brain when compared with the control of the group. The ASA wine extract and the standard red wine caused a reduction in the malodialdehyde and NO levels in the treated rats as compared to the control. These findings suggest that ASA wine may have similar biochemical and cholinergic effect on biological system. These results suggest that red wine may have a neuro–protective effect against oxidative stress possibly via antioxidant activities.

Keywords: *Chrysophyllum albidum; Oxidative Stress; Red wine; Acetylcholinesterase; Antioxidant*

Introduction

Plants are a rich source of bioactive compounds with antioxidant activity. Examples of such bioactive compounds are phenolic acids, flavonoids, vitamins and carotenoids from which pharmacologically active products may be derived [1]. There is a surge of interest on dietary polyphenols because of their potential health benefits on humans with keen interest on their ability to protect against diseases connected with oxidative stress like neurodegenerative disease, cancers, diabetes, cardiovascular diseases etc. [2, 3]

Acetylcholinesterase enzyme (AChE, EC 3.1.1.7) performs a crucial role in the activity of the nervous systems (central and peripheral), because it catalyzes the hydrolysis of the acetylcholine neurotransmitter, thus producing choline and acetate (ACh) [4]. The most common neurodegenerative disorder is Alzheimer's disease (AD) which its pathogenesis still not fully understood. One of the most recognised theories has been "cholinergic hypothesis", hence inhibition of acetylcholinesterase (AChE) preserves the levels of acetylcholine and improves the cholinergic function and consequently has become the standard guideline in the symptomatic treatment of AD [5-7].

A plethora of plants has been documented to exhibit acetylcholinesterase inhibitory activity. In traditional system of medicine, several plants have been used to treat cognitive disorders, including neurodegenerative diseases, such as AD and other memory related disorders. According to different cultural traditions, the use of complementary medicines such as plant extracts in dementia therapy varies [8-10].

African Star Apple (*Chrysophyllum albidum*) belongs to the family *Sapotaceae* is an indigenous wild fruit tree which is vastly distributed in Nigeria, Uganda, Niger Republic, Cameroon and Cote d'Ivoire [11]. In folklore medicine, the bark is used for the treatment of malaria and yellow fever, while the leaf is useful in preparing an emollient for the treatment of skin eruption, stomachache and diarrhea [12]. The cotyledons from the seeds of *C. albidum* are used as ointments in the treatment of vaginal and dermatological infections in Western Nigeria. Some of the reported pharmacological activities of *C. albidum* includes: antioxidant [13, 14] anticholinesterase [14], hypolipidemic [15], antimicrobial [16] activities.

The fruits are also suitable for the production of fruit jams and jellies [17]. It has been documented that the plant is an excellent source of vitamins, irons, flavours to diets and raw materials to some manufacturing industries [18-20]. The fleshy and juicy fruits of *Chrysophyllum albidum*, which is commomly eaten, have the potentials as an ingredient of soft drinks and can be fermented into wine or other alcohol production [21]. In view of this, the aim of this study is to investigate the effect of wine extracted from *Chrysophyllum albidum* fruits on the some biochemical and oxidative stress parameters in the brain of Wistar albino rats.

Methods

Sample collection

Fresh samples of *Chrysophyllum albidum* were purchased from Iworoko local market, in Ekiti State, Nigeria. Fresh fruits were handpicked and sorted for the best grade and fruits were washed under running tap water to remove the dirt. The plant identification was done by Mr. Omotayo F. with herbarium number UHAE2019104 at the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti Nigeria.

Chemicals and Reagents

The standard wine used Baron Romero was bought at NAO supermarket in Ado-Ekiti. Trichloroacetic acid (TCA), 1-chloro- 2,4-dinitrobenzene (CDNB), thiobarbituric acid (TBA), 5′,5′-dithiobis(2-nitrobenzoic acid) (DTNB), hydrogen peroxide (H_2O_2), reduced glutathione were procured from Sigma (St Louis, MO, USA). All other chemicals used were of available analytical grade.

Standard Wine Sample

The red wine (Baron Romero) used as standard in this present work was purchased at NAO Supermarket (7.6373° N, 5.2181° E), Ado-Ekiti, Ekiti State Nigeria.

Wine Extraction from African star apple fruit

Wine was extracted from the sorted African star apple fruit (ASA) fruits according to the procedure described by Awe et al. [22]. The wine extraction process was done in stages which includes: preparation of Must, aerobic fermentation, anaerobic fermentation, addition of yeast (*Saccharomyces cerevisiae*). Briefly, the fruit samples of African star apple were washed and skinned, weighed and surface sterilized with sodium metabisulphite solution to remove any microbial contamination. Warm water was added to fruit pulp to give the 'must', Camden tablet was added for juice sterilization. Standardized amount of yeast (*Saccharomyces cerevisiae*) strain (K1V-1116) was added to must after 24hrs by sprinkling. The 'must' was stirred daily for 6days (primary fermentation). The filtrate was racked into the secondary fermenter and the pomace discarded. Air-lock of the secondary fermenter was fixed and the filtrate fermented for six weeks. The wine was racked, the lee discarded, and store for 6months to mature (Ageing). Mature wine was then filtered and bottled.

Experimental Animals

Healthy male Wistar rats weighing (150 - 180g) were purchased from the animal house of the Department of Medical Biochemistry, College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria. The rats were kept under a natural condition of 12h light/12h dark cycle. The rats had access to standard pellets and water ad libitum. In this study, all the animals received humane care as described in the established protocol in the Guide for the Care and the Use of Laboratory Animals. The animal experiments were carried out in line with our institutional supervision of the ethics committee and standards on animal care.

Experimental Design

The animals were divided into five groups. Group 1 animals received distilled water and served as control, groups 2 and 3 were administered with 5% and 9% of standard red wine (Baron) while groups 4 and 5 were administered with 5% and 9% *Chrysophylum albidum* wine extract respectively for 21 days. At the end of the treatments, all the animals were sacrificed after 12 h fast.

Blood Sample and Tissue Preparations

Blood samples was by cardiac puncture into heparinized tubes, and left for 1 h at room temperature and later centrifuged at 3000g at room temperature for 10min to separate the plasma while the brain was excised from the rats. After which, it was placed in an iced cold phosphate buffer (pH 7.4) and then homogenized. The resultant brain homogenate was subjected to centrifugation at 12,000 × g for 15 min at 4°C to obtain the post mitochondrial fractions which was kept at 4°C and used for further biochemical assays.

Biochemical Assays

Biomarkers of oxidative damage

Lipid peroxidation was determined by estimating the thiobarbituric acid reactive substances (TBARS) formed (expressed as MDA equivalents) following the method of Ohkawa *et al.* [23]. The level of malondialdehyde (MDA) was deduced from the absorbance as described by Adam-Vizi and Seregi [24] (1982) and the unit given as nmol MDA/mg protein. The reduced GSH estimation was carried out according to Jollow *et al.* [25].

The antioxidant enzyme activities

The activity of Superoxide dismutase (SOD) was evaluated using the method of Misra and Fridovich [26]. The catalase (CAT) activities was Catalase activities were assayed by the method described by Sinha [27].

Serum Marker Enzymes

The measurement of Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities in the plasma of the experimental animals were carried out using commercially available kits purchased from Sigma–Aldrich Co., St. Louis, MO, USA.

Neuronal Enzyme Assay

Inhibition of Acetylcholinesterase (AChE) was assessed by a modified method of Ellman *et al* [28] while the scavenging effect of the extracts on Nitric Oxide (NO) was assayed colorimetrically in brain tissue homogenate by using method of Berkels et al. [29].

Data Analysis

The results of three (3) experiments were pooled and expressed as mean ± standard deviation (SD). Mean values were appropriately analyzed and compared using one-way ANOVA followed by Duncan post hoc test; significance was accepted at p<.05. All statistical analyses were carried out using Graph Pad Prism version 6.00 for windows

Results

Effect of ASA and Baron Red wine on antioxidant enzymes

There was significant increase in the GSH content of the brain tissue of rats administered 9% ASA and Baron Red wine in the treated groups as compared to the control (Figure 1). However no significant difference was observed in the blood of rats administered 5% ASA and Baron Red wine. Also, there was no significant difference in SOD and CAT activities in brain tissue of rats treated with 5% ASA and 5% Baron red wine in all the groups when compared with the control group. However, rats administered 9% ASA and Baron red wine produced a significant increase in SOD and CAT activities of brain tissues (Figures 2 and 3). However, no significant difference was observed in the blood of rats administered ASA and Baron Red wine respectively (Figures 4-6).

Effect of ASA and Baron Red wine on marker enzymes

There was no significant difference for marker enzymes ALP, AST and AST observed in all the treated groups when compared to the control group (Figures 7- 9)

Effect of ASA and Baron Red wine on Biomarkers of oxidative damage

There was significant decrease in the MDA levels of the brain and blood in the rats administered ASA and Baron Red wine in all the groups when compared with the control group (figure 10 and 11).

Effect of ASA and Baron Red wine on Nitric Oxide

There was significant decrease for scavenging activities of NO in the blood of rats with treated with ASA and baron red wine groups when compared to the control group (figure 12).

Effect of ASA and Baron Red wine on Acetylcholine esterase

As shown in Figures 13, there was no significant difference for inhibitory activities of acetylcholine esterase in the blood of rats with treated with ASA and baron red wine groups when compared to the control group.

Discussion

The red wine is specifically rich in polyphenolic compounds. These bioactive substances have been implicated in boosting the antioxidant defense systems, modulatory enzymes and metal chelators [30-32]. In this study, our group shows the effect of standard red wine and wine extracted from *Chrysophylum albidum* fruit on biochemical and oxidative parameters in the brain and erythrocytes of experimental rats.

As shown in figures 1-3, the consumption of both standard red wine (baron) and wine extracted from *Chrysophylum albidum* increases the level of antioxidants enzymes in the brain of the experimental rats. The wine extract *C. albidum* of and that of standard red wine at 9% concentration produced a significant increase (P< 0.05) in the enzyme activities of SOD, CAT, and non-ezymic GSH activities in the brain when compared with the control group. However, there exists no significant difference in the activities of plasma CAT, SOD and GSH in all the groups (figures 4-6). These findings may imply that both red wine and wine from ASA contains considerable same amount of antioxidants boosting abilities in them which helps them to scavenge free radicals generated in the body. This result is in line with the findings of Van der et al. [33] that moderate consumption of red wine does not affect plasma antioxidants capacity but they contain good antioxidants such as polyphenols and flavonoids which can help fight cancerous cells in the body.

The results of serum liver biomarker enzymes are presented in Figures 7, 8, and 9. There was no significant difference in the activities of ALT, AST and ALP in the treated groups when compared with their corresponding control group. These marker enzymes are regularly used in the clinical application for the diagnostic screening of liver diseases, investigating the progression of known diseases and

monitoring the potential effects of xenobiotic drugs in the liver [34-35]. An elevation levels of these marker enzymes in the blood are an indicator of liver injury. In view of this, the results obtained from this study portends that both red wine and ASA wine does not elevate the level of these liver biomarker enzymes the blood of the experimental rats.

Due to the high concentration of polyunsaturated fatty acids in brain with regards to other organs, lipid peroxidation is one of the major outcomes of free radical-mediated injury to brain which directly damages neuronal membranes and yields a number of products responsible for extensive cellular damage [36]. As shown in figures 10 and 11, the response of ASA wine extract and standard wine to malondialdehyde levels in both the plasma and brain homogenate is concentration dependent and is able to reduce lipid peroxidation. At higher concentration of 9%, both ASA wine extract and standard wine were statistically effective in lowering MDA content than that of the 5% concentration of both ASA wine extract and standard wine, in the brain homogenate and plasma of the experimental rats when compared to the control groups. It is interesting to note that the ASA wine extract was more effective in reducing this MDA content levels than the standard wine (Baron) at both concentrations used. This observation is similar to the work reported by Montilla et al [37] 2006. The reduction of this lipid peroxidation observed in the brain and brain homogenate might be as a result of the presence of polyphenolic compounds present in ASA fruit extracts as previous reported by Oboh et al [14] (2018). These phenolic compounds present in ASA extracts play a crucial function in scavenging and neutralizing free radicals, quenching singlet and triplet oxygen.

Nitric oxides (NO) plays an important role as a neurotransmitter in the brain and crosses cell membranes freely. The function of NO in the hypothalamus has largely been implicated in learning process and in memory formation [38]. Studies in experimental animals have well documented the synthesis of NO in the brain, and its role in a variety of neuronal functions including learning and memory processes, cortical arousal, nociception, food intake, penile erection, yawning, blood vessel dilatation and immune response [38, 39]. From the present study, it is shown that the level of nitric oxide is lowered in the groups administered with red wine and the ASA wine extracts when compared with the control group (Figure 12). Nitric oxide can be conceived as a double-edged sword. On one hand, in the low, constitutive mode, it has beneficial effects, mediating and protecting neuronal activity. On the other hand, in the high, unregulated mode, it is an indiscriminately damaging molecule. The possibility that NO can exist in distinct oxidation/reduction states with different biological actions provides further elucidation of mechanisms underlying the neuroprotective and neurotoxic effects of NO [40]. It can be suggested from this study that ASA wine extracts promotes the positive effects of NO activities in the brain of albino rats.

Acetylcholine (ACh) is critical for communication between neurons and muscle at the neuromuscular junction. It is involved in direct neurotransmission in autonomic ganglia, and has been implicated in cognitive processing, arousal, and attention in the brain [41]. The principal biological role of acetylcholinesterase is the termination of impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine to acetate and choline. In certain neurological disorders such as Alzheimer's disease (AD), acetylcholinesterase is over activated in the synapses so that levels of acetylcholine in the brains is significantly diminished, which leads to weakened neurotransmission and thereby memory loss and other adverse effects. This study showed no significant difference in the level of the enzyme in the brain of all the experimental rats (Figure 13). At 5% and 9%, ASA wine extract has no induced effect on acetylcholine esterase enzymes/

Conclusion

In conclusion, this present study showed that *Chrysophyllum albidum* fruits (ASA) wine extract has the same induced effect as the standard wine (baron). The wine stimulates the activities of glutathione, catalase and superoxide dismutase in brain homogenate but has no effect on the plasma catalase, glutathione and superoxide dismutase activities. Also, there is no observable effect on liver

enzyme biomarkers and acetylcholinesterase. It was also observed that the ASA wine extract decreased lipid peroxidation and nitric oxide activities on concentration dependent manner. Further prospective studies on effect of higher *Chrysophyllum albidum* fruits wine concentrations on neuronal activities are required.

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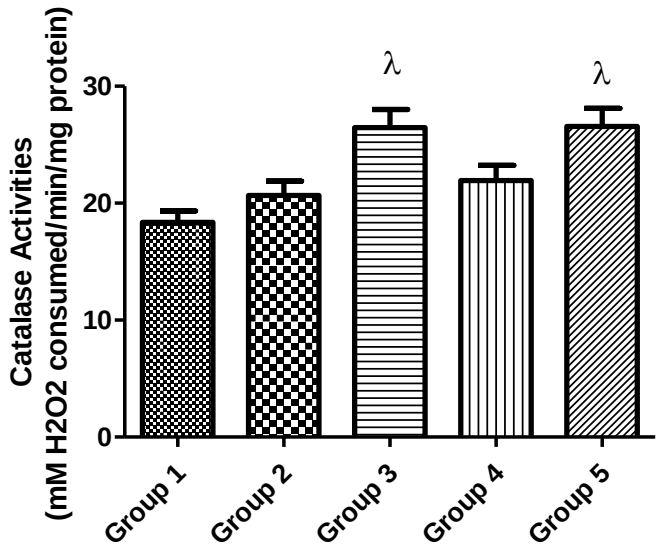
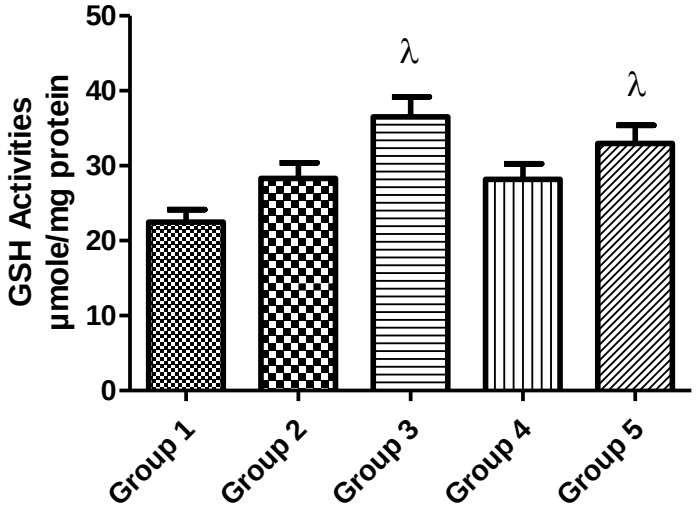
Figure 1: The activities of catalase in the brain of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with symbol(s) show significant (P<0.05) difference.



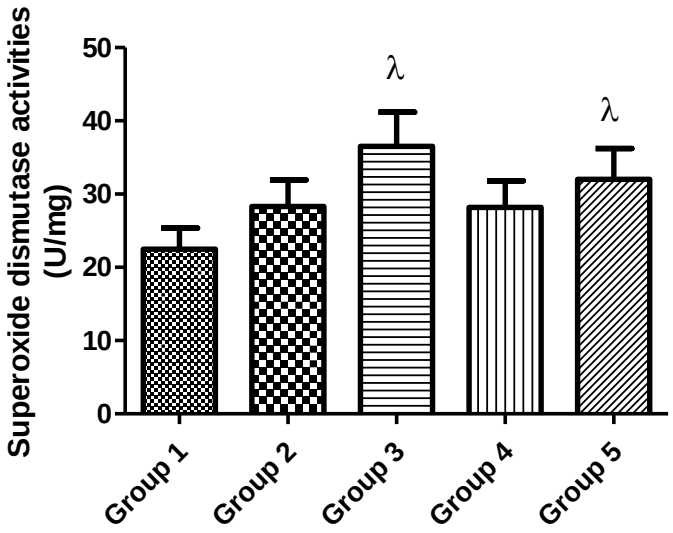
λ means significant difference from the control (group 1)

Figure 2: The glutathione activities in the brain of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with symbol(s) show significant (P<0.05) difference.



λ means significant difference from the control (group 1)

Figure 3: The superoxide dismutase activities in the brain of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with symbol(s) show significant (P<0.05) difference.



λ means significant difference from the control (group 1)

Figure 4: Catalase activities in the plasma of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with no symbol shows no significant difference (P>0.05).

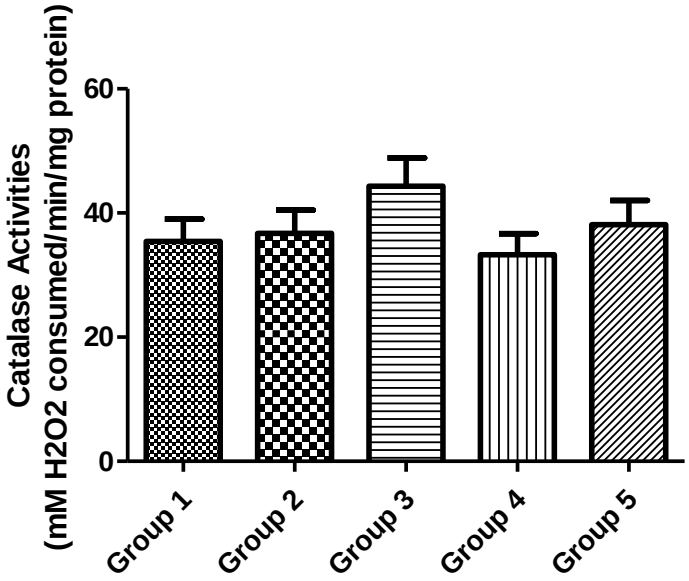


Figure 5: GSH activities in the plasma of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with symbol(s) show significant (P<0.05) difference.

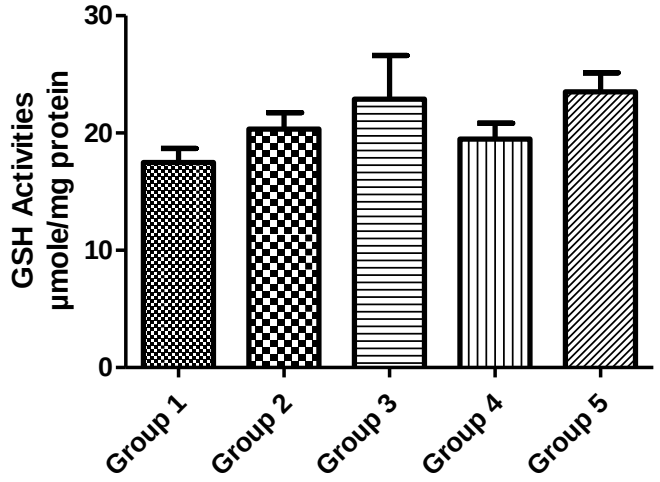


Figure 6: Superoxide dismutase activities in the plasma of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with no symbol shows no significant difference (P>0.05).

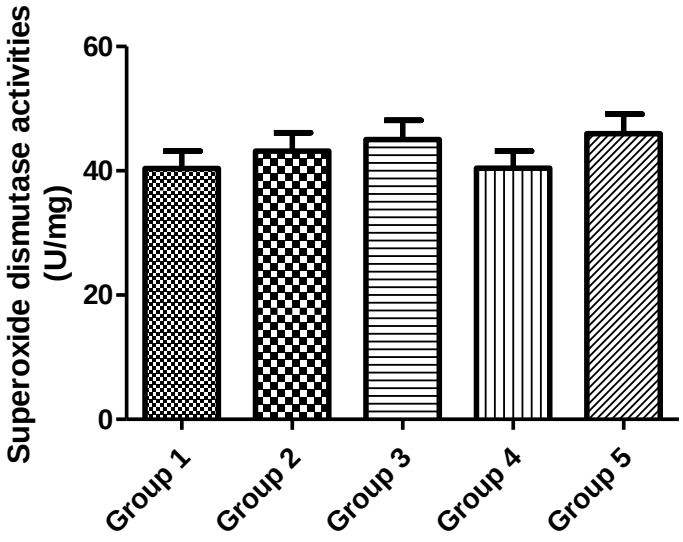


Figure 7: Aspartate transaminase (AST) activities in the plasma of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with no symbol shows no significant difference (P>0.05).

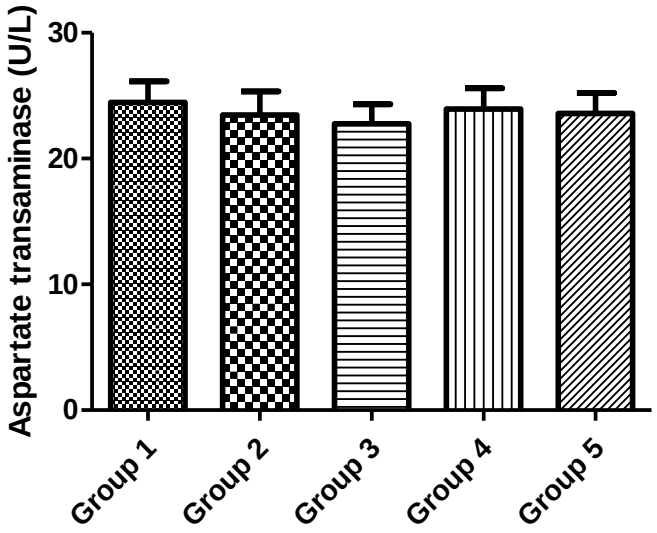


Figure 8: Alkaline phosphatase (ALP) activities in the plasma of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with no symbol shows no significant difference (P>0.05).

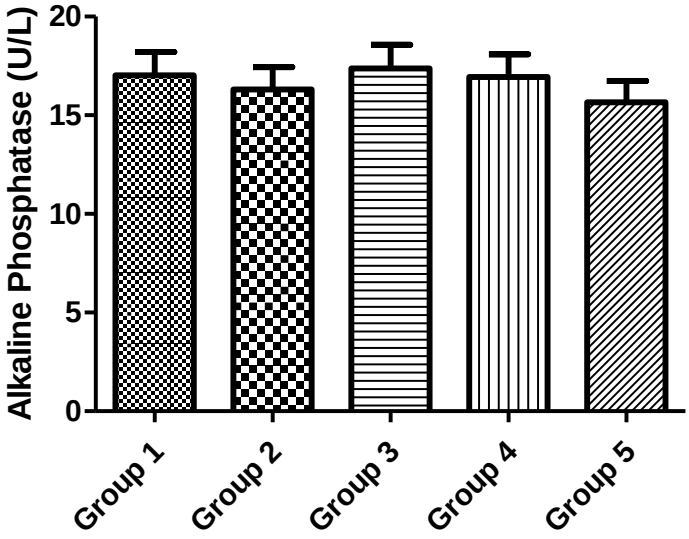


Figure 9: Alanine transaminase (ALT) activities in the plasma of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with no symbol shows no significant difference (P>0.05).

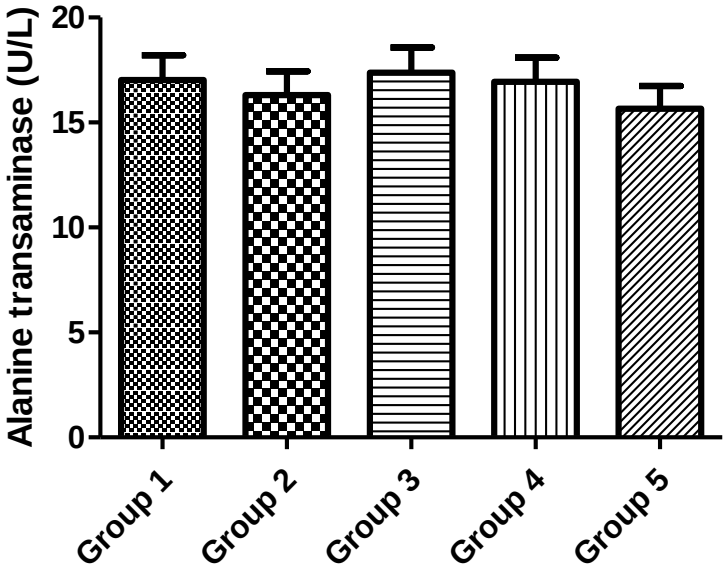
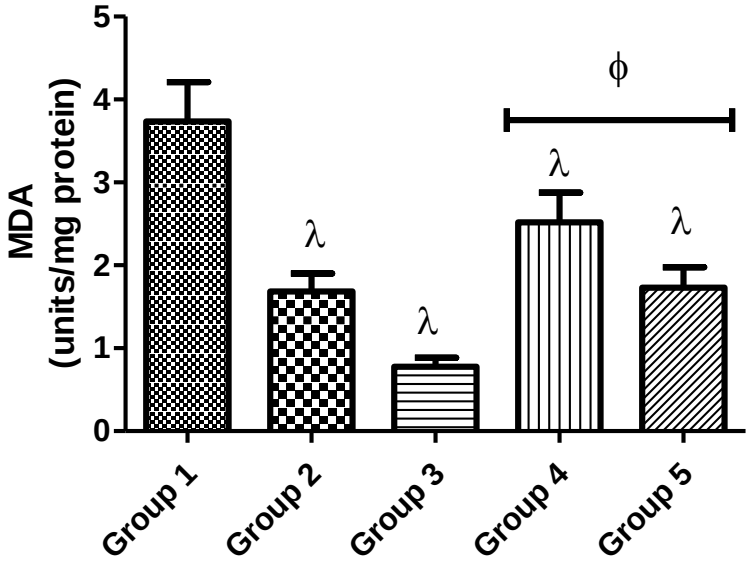
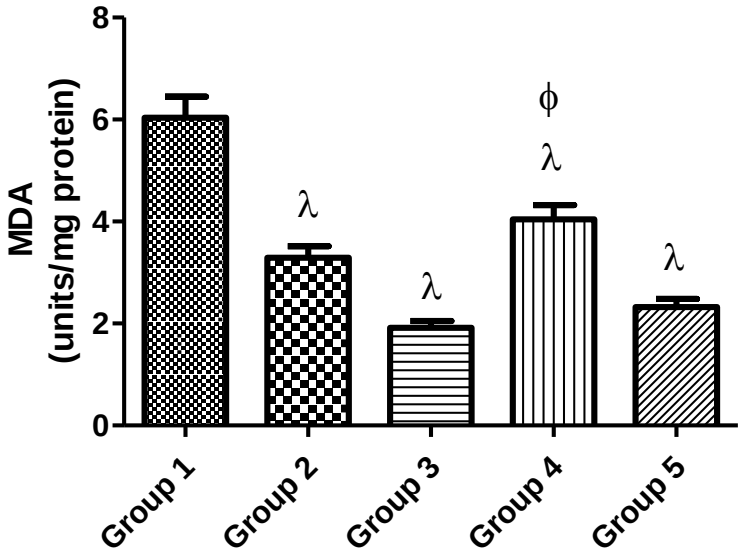


Figure 10: MDA activities in the brain of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with symbol(s) show significant (P<0.05) difference.



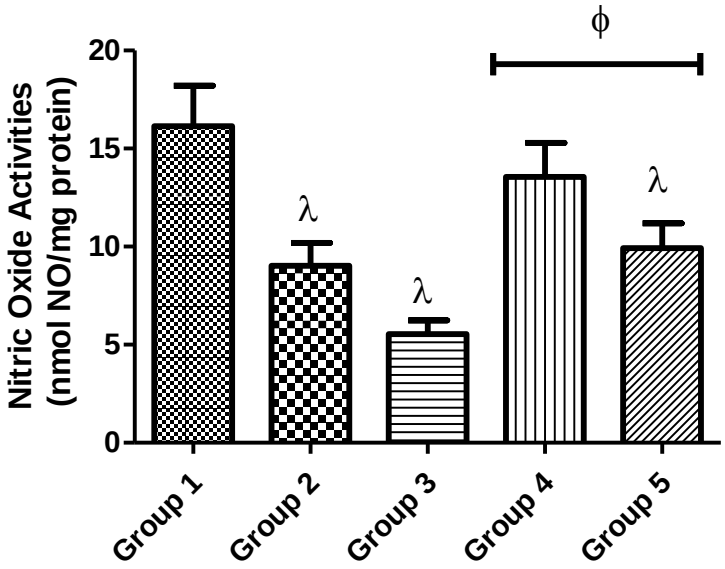
λ means significant difference from the control (group 1)
Φ means a significant difference of group 4 and 5 as compared to group 2 and 3

Figure 11: MDA activities in the plasma of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with symbol(s) show significant (P<0.05) difference



λ means significant difference from the control (group 1)
Φ means a significant difference of group 4 and 5 as compared to group 2 and 3

Figure 12: The nitric oxide activities in the brain of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with symbol(s) show significant (P<0.05) difference.



λ means significant difference from the control (group 1)
Φ means a significant difference of group 4 and 5 from as compared to group 2 and 3

Figure 13: Acetylcholinesterase activities in the brain of rats administered with 5% and 9% of standard red wine and wine extracted from African star apple. Values are expressed in mean ± SD (n=4). Bar with no symbol shows no significant difference (P>0.05).