African Scientist Vol. 21, No. 3 September 30, 2020 1595-6881/2020 $10.00 + 0.00
Printed in Nigeria

http://www.niseb.org/afs

AFS2020010/21302

Selected Biochemical Parameters and Oxidative Stress Status of Rats Administered Antimalaria Herbal Extract – 'Agbo'

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(Received July 16, 2020; Accepted in revised form September 23, 2020)

ABSTRACT: Herbs are increasingly used across the globe; In fact, the World Health Organization reported that most African countries, including Nigeria, depend on herbal medicines for primary health care, without much documented evidence of adverse effects. Agbo polyhebral extract is popular in Nigeria, especially among (but not limited to) the local populace of the south-western areas. The aim of this study is to determine the effects of malaria alcoholic herbal extract (Agbo) on renal, liver, oxidative stress markers and hematological parameters on wistar rats. Twenty rats weighing between 150g to 200g were divided into 4 groups of 5 each. Control A and experimental groups B, C D. The extract was administered for 8 weeks after at the end of which weights of the rats were taken and the rats sacrificed. Blood samples were collected into plain tubes and EDTA bottles, urea, creatinine, electrolytes, full blood count, liver enzymes (ALP, AST, ALT), oxidative stress markers (glutathione peroxidase, catalyze, malondialdehyde, SOD) were assayed in blood spectrophotometrically. Results were analyzed using ANOVA and expressed in mean± sem. Liver enzymes were not statistically significant, When catalase values of control (236.2 ± 5.89) was compared with the different doses (146.0 ± 0.70, 140.3 ± 0.47, 132.7 ± 0.65) and glutathione peroxidase control values (142.9 ± 1.03) was compared with the various doses (127.8 ± 0.52, 122.8 ± 0.79, 122.2 ± 0.73), there was a reduction in the experimental groups. Malondialdehyde (31.55 ± 0.43, 39.65 ± 0.60, 66.95 ± 0.99) were significantly increased ($p<0.05$) when compared with control (26.91 ± 0.59). SOD values (12.41 ± 0.38, 14.45 ± 13.47) significantly reduced compared with control (236.2 ± 5.89). Serum urea, creatinine, electrolytes and hematological parameters were insignificant. In conclusion, administration of malaria alcoholic herbal extract "Agbo" induced oxidative stress in the rats.

Keywords: Herbal, *Agbo*, Liver, Renal, Oxidative stress

Introduction

The use of herbal medicines in developing nations has risen in recent times. It is estimated that over three-quarters of the population in Africa depends on traditional herbal remedies for primary health care (Oreagba *et al.*, 2011). Herbal medicines have gained popularity in general because patients believe these products are natural in origin, and hence safe for consumption. In contrast to this belief, it has been proven to be untrue, with multiple reports of hepatotoxicity (Stikcel *et al.*, 2005). Interestingly, some plants produce toxic compounds as secondary metabolites which may not easily be distinguishable from the active pharmacological constituents. Some of these herbs are produced in very unhygienic conditions using potentially toxic ingredients, subsequently exposing the consumers to

multiple hepatotoxins (Nwokediuko *et al.*, 2013). It is also worthy of note that some marketed herbal products are composed of complex mixtures, hence the exact component that is responsible for injury is difficult to discern. Despite the widespread belief that herbs and herbal products are of natural origin unlike orthodox medicines, are to be considered safe without many side effects, there have been many reports of adverse effects linked with herbal remedies (Koh and Woo, 2000).

Agbo is a Yoruba word that describes a concoction of plant parts – bark, root, trunk, leaves – steeped or boiled in alcohol or water. Five brews of this agbo exist; they include agbo iba for malaria, agbo iba ponto for typhoid fever, agbo jedi jedi for dysentery, agbo ara riro for body aches and agbo atosi touted to cure sexually transmitted infections (Judd-Leonard *et al.*, 2018).

Previous study done by Akande *et al.*, 2010), investigated the phytochemical content of agbo iba and was found to contain 40-50% alcohol,_Allamanda cathartica (Golden trumpet), Bixa orellana (Orellana), Cymbopogon citratus (English lemon grass), Ficus exasperate (Sand paper tree), and Momordica charantia (Bitter lemon) used traditionally for the anti-malarial preparations of Agbo in Nigeria. The phytochemical screening of the plants showed the presence of flavonoids, alkaloids, phenolic compound, tannis and reducing sugars. Phenolic compounds such as flavonoids (Pietta 1998), phenolic acids and phenolic diterpenes (Shadidi *et al*., 1992), have received increased attention as useful pharmaceuticals in managing diseases like malaria. Free radicals, reactive oxygen species and reactive nitrogen species are associated with many pathological conditions such as inflammation, metabolic disorders, cell aging and carcinogenesis. A study by Yildirim *et al.* (2001), implicated reactive oxygen species in several diseases including malaria. These antioxidants play a role in delaying, intercepting or preventing oxidative damages catalyzed by free radicals (Veliogllu *et al.*, 1995).

The liver is one of the vital organs in the human body, and is highly susceptible to a wide array of metabolic, toxic, microbial, circulatory, and neoplastic injury. Common liver diseases include: viral hepatitis, inflammatory diseases, alcoholic liver disease, non-alcoholic fatty liver disease and hepatocellular carcinoma (Abdulkareem *et al.*, 2006; Crawford *et al.*, 2010). In most cases, liver diseases start as a gradual and subtle process in which clinical detection and manifestation could occur weeks, months or even many years following onset of injury. Therefore, at the point at which most patients with hepatic dysfunction are referred to hepatologists, they already have chronic liver disease (Ugiagbe and Udoh, 2013). Liver injury associated with the consumption of herbal medicines is referred to as "herb-induced liver injury", which occurs rarely in only a few susceptible individuals (Pantano *et al.*, 2017). The clinical manifestations of herb-induced liver injury are identical to those of drug-induced liver injury (Ugiagbe and Udoh, 2013). Furthermore, herb-induced liver injury and drug-induced liver injury share common features, as both cases are caused by chemical components that can be produced either by natural or synthetic processes. These natural and synthetic chemicals are foreign to the body and require metabolic breakdown to be eliminated. However, in the course of metabolism, substances that are toxic to the kidney could be produced, resulting in kidney injury in susceptible individuals (Frenzel and Techke, 2016). This study aims determining the effects of commonly consumed anti malaria alcoholic herbal extract "Agbo" on renal and liver parameters as well as its effects on oxidative stress markers.

Materials and methods

Experimental design: Twenty adult wistar rats (*Rattus norvegicus)* weighing between 150-200g were used for the study. The animals were housed in well ventilated plastic cages and hygienic animal house under room temperature. They were given water and feed adlibitum after acclimization for two weeks into four groups with five animals per cage. Group A - Control, while the groups B, C, and D were administered different doses of the alcoholic agbo iba extract for a period of eight weeks. The control group was administered water and rat feds in pellets while the test groups were administered doses of 5.0ml/kg body weight, 7.5ml/kg body weight, and 10.0ml/kg body weight, of alcoholic agbo iba extract once daily using a gavage feeding needle for a period of eight weeks. At the end of the eight weeks, the animals were sacrificed. Blood was collected via cardiac puncture into plain tubes and EDTA bottles for analysis. Full blood count (FBC), kidney, liver, function tests and oxidative stress markers were analyzed in the samples.

Biochemical assays

Serum antioxidant status

Superoxide dismutase (SOD): The estimation of the superoxide dismutase in the blood was carried out using modified epinephrine assay method (Misra and Fridovich, 1970).

*Catalase (CAT)***:** The estimation of catalase in the blood was carried out using spectrophotometric method (Cohen *et al*., 1970). Absorbance was read at 480nm between 30–60seconds.
Glutathione peroxidase (GPx): The estimation of glutathione peroxidase was done using assay kits (Flohe and Gunzler, 1984). The biochemical function of glutathione peroxidase is to reduce lipid hydro peroxides to their corresponding alcohols and reduce free hydrogen peroxide to water.
Malondialdehyde (MDA) estimation: The MDA estimation was done using the thiobarbituric acid assay (TBARS) (Botsoglou *et al*, 1994). Malondialdehyde which is formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for the determination of the extent of the peroxidation reaction. Malondialdehyde has been identified in the product of lipid peroxidation that results with thiobarbituric acid to give a red species absorbing at 535nm.

Liver function status

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP) were estimated in the serum of experimental animals using Randox reagent kits laboratories Ltd. United Kingdom BT294QY) spectrophotometric method (Reithman, and Frankel, 1957).

Measurement of Urea in Serum

Serum urea: Serum urea was measured spectrophotometrically using Randox reagent kit by Berthelot method (Fawcett *et al.*, 1960)
Measurement of Serum Creatinine: Serum creatinine was measured spectrophotometrically using Randox reagent kit by two point's kinetic, modified Jafee increasing reaction (Bartels and Bolumer 1972).
*Electrolytes Estimation***:** Serum samples were assayed for electrolytes using ISE Analyzer (Scholz, 2010).
Full blood count: Full blood count was determined in whole blood using kx-21n, sysmex (Dacie and Lewis, 2002)
Statistical analysis: Data were collected and analyzed using the statistical package for social sciences SPSS. Values obtained in this study were presented as mean ± standard error of the mean (SEM) for test and control groups. Analysis of variance (ANOVA), graph pad prism statistical software version 5.0 was used to compare the probability at 95% confidence intervals ($p<0.05$).

Results

The mean values of liver function test and oxidative stress markers following the administration of antimalarial alcoholic herbal extract "Agbo" in rats are presented in Table 1. There was a significant decrease in the medium dose compared with the control ($P < 0.05$), in Alkaline phosphatase. Malondialdehyde and Superoxide dismutase (SOD) significantly increased when compared with Control. Glutathione peroxidase and Catalase increased significantly when compared with Control. However, there were no significant differences in AST and ALT in the three doses when compared with control.

Table 1: Mean values of liver function test and oxidative stress markers following the administration of antimalarial alcoholic herbal extract "Agbo" in rats.

Parameters	**Control**	**Low Dose (5 ml/kg)**	**Moderate Dose (7.5 ml/kg)**	**High Dose (10 ml/kg)**
Alkaline phosphatase (IU/l)	109.8 ± 12.72	108.2 ± 20.97	61.50 ± 3.62*	81.50 ± 18.59
AST (IU/l)	183.6 ± 9.82	184.6 ± 8.04	206.8 ± 9.50	217.5 ± 25.50
ALT (IU/l)	41.80 ± 4.21	35.20 ± 3.61	37.50 ± 3.71	47.00 ± 8.96
Malondialdehyde (µmol/ml)	26.91 ± 0.59	31.55 ± 0.43*	39.65 ± 0.60*	66.95 ± 0.99*
SOD (U/ml)	8.268 ± 0.47	12.41 ± 0.38*	14.45 ± 0.62*	13.47 ± 0.90*
Catalase (U/ml)	236.2 ± 5.89	146.0 ± 0.70*	140.3 ± 0.47*	132.7 ± 0.65*
Glutathione Peroxidase (U/ml)	142.9 ± 1.03	127.8 ± 0.52*	122.8 ± 0.79*	122.2 ± 0.73*

*$P < 0.05$ indicates significant difference in the treated groups with respect to control

The mean values of hematological parameters following the administration of anti-malaria alcoholic herbal extract "Agbo" in rats are presented in Table 2. There were no significant changes in all the hematological parameters in the treated groups (low, medium and high doses) when compared with the control respectively ($P > 0.05$); also there were no significant changes within the groups ($P > 0.05$).

Table 2: Mean values of hematological parameters following the administration of anti-malaria alcoholic herbal extract "Agbo" in rats.

Parameters	Control	Low Dose (5 ml/kg)	Moderate Dose (7.5 ml/kg)	High Dose (10 ml/kg)
WBC Count ($x10^3$/mL)	4.700 ± 0.492	5.300 ± 0.466	5.450 ± 0.771	5.050 ± 0.333
RBC Count ($x10^6$/mL)	7.886 ± 0.163	7.568 ± 0.058	7.833 ± 0.166	7.543 ± 0.165
Haemoglobin (g/dl)	18.34 ± 0.463	17.64 ± 0.469	17.78 ± 0.512	18.03 ± 0.464
Haematocrit (%)	47.34 ± 1.090	45.32 ± 1.088	47.55 ± 0.659	47.58 ± 1.748
Lymphocytes count ($x10^3$/mL)	4.020 ± 0.482	4.600 ± 0.415	4.825 ± 0.664	3.950 ± 0.119
Granulocytes count ($x10^3$/mL)	0.260 ± 0.060	0.340 ± 0.125	0.275 ± 0.085	0.375 ± 0.048
Platelets count ($x10^3$/mL)	547.2 ± 83.20	520.8 ± 83.84	642.3 ± 93.46	729.5 ± 28.89
Urea concentration (mg/dl)	43.60 ± 4.946	37.20 ± 2.557	40.75 ± 3.172	41.00 ± 3.136

The mean values of renal parameters following the administration of anti-malaria alcoholic herbal extract "Agbo" in rats are presented in Table 3. There were no significant changes in all renal parameters in the treated groups (low, medium and high doses) when compared with the control respectively ($P > 0.05$), also there were no significant changes within the groups ($P > 0.05$).

Table 3: Mean values of renal parameters following the administration of anti-malaria alcoholic herbal extract "Agbo" in rats.

Parameters	Control	Low Dose (5 ml/kg)	Moderate Dose (7.5 ml/kg)	High Dose (10 ml/kg)
Sodium concentration (Mmol/L)	144.2 ± 1.068	144.4 ± 1.122	145.8 ± 1.377	145.5 ± 1.555
Potassium concentration (Mmol/L)	5.900 ± 0.332	6.360 ± 0.337	6.700 ± 0.252	5.900 ± 0.332
Bicarbonate concentration (Mmol/L)	25.40 ± 2.400	28.00 ± 1.304	28.00 ± 1.472	23.25 ± 1.652
Chloride concentration (Mmol/L)	105.4 ± 0.748	107.2 ± 0.374	107.0 ± 0.913	108.0 ± 0.707
Creatinine concentration (mg/dl)	0.66 ± 0.04	0.59 ± 0.01	0.650 ± 0.029	0.60± 0.02

The mean weights following the administration of anti-malaria alcoholic herbal extract "Agbo" in rats are presented in Table 4. There was no significant difference in the weights of the rats when weights prior to administration of extracts were compared to weights 8 weeks after administration.

Table 4: Comparing the mean values of weight following the administration of anti-malaria alcoholic herbal malaria "Agbo" extract on rats.

Parameters	Control	Low Dose (5 ml/kg)	Moderate Dose (7.5 ml/kg)	High Dose (10 ml/kg)
Weight before (g)	149.6 ± 3.64	166.0 ± 1.30	173.8 ± 0.63	182.8 ± 2.39
Weight after (g)	166.0 ± 3.63	168.6 ± 7.41	197.5 ± 10.09	205.3 ± 11.95

Discussion

Findings from this study showed a significant difference in all the oxidative stress markers evaluated in this study. MDA is an end product of lipid peroxidation and represents a credible marker of oxidative stress. The formation of MDA can be extensive in ROS-induced degradation of polyunsaturated lipids (Zang *et al;* 2018). Plasma MDA levels usually increase in proportion to the severity of oxidative damage. There were significant ($p<0.05$) increases observed in the different test groups compared with the control, indicating that lipid peroxidation increased as extract doses increased. A previous study has reported similar findings with other herbal mixtures Adeyemi and Suliaman, 2014). This could also be attributed to the alcoholic solvent used in the preparation of the extract, alcohol which is primarily metabolized in the liver leads to the production of dangerous by-products such as acetaldehyde

and highly ROS which cause oxidative stress and are capable of causing damage to macromolecules such as DNA, proteins and lipids (Bartsch and Nair, 2000). This result is in line with previous study carried out by Ighodaro *et al.,* (2010). Endogenous antioxidants activity such as SOD, CAT, and GPX which are the first line of defense against oxidative stress in the body were compromised in the rats based on the result obtained from this study. SOD catalyzes the conversion of highly reactive superoxide (O_2^-) radical into molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). H_2O_2 is also harmful to the cells; hence it is degraded into water by other enzymes such as catalase. Superoxide is produced as a by-product of oxygen metabolism and if not regulated may cause cell damage. The results obtained from this study shows a significant ($p<0.05$) increase in low, medium and high doses compared with control. This may be as a result of the increased production of MDA during lipid peroxidation. The enzyme catalase (CAT) catalyzes the decomposition of H_2O_2 into water and molecular oxygen, hence a very important enzyme that protect the cell from oxidative damage. Catalase values decreased ($p<0.05$) significantly in all test groups compared with control. GPX enzyme, catalyze the production of oxidized glutathione (GS-SG) to the reduced form of glutathione (GSH), hence the reduction of hydrogen peroxides to water and molecular oxygen was compromised. This reaction is important in removing low levels of H_2O_2 that might damage the cell. The result from this study shows a significant ($p<0.05$) decrease in all test groups when compared with control. Agbo extract caused the generation of free radicals (ROS and RNS), which in turn triggered continuous mobilization of SOD, CAT, and GPx *in vivo,* thus leading to the reduction of the enzymes in the course of scavenging the overproduced free radicals. Previous studies have reported the potential of herbal mixtures to reduce SOD, CAT and GPx (Emaleku, 2018).

The increase in MDA and SOD and decrease in GSH, and catalase in the experimental groups when compared with control is a sign of oxidative stress in the rats was also observed in similar studies of Srilaxmi *et al.,* (2010) and Kalu *et al.,* (2011). However their studies were based on extracts from single plant, while "Agbo" herbal mixture is a combination of different plants. ALP showed a significant increase only in moderate doses, while AST and ALT did not show any significant increase when compared with control. This simply implies that the herbal extract is not hepato toxic. The liver function tests are most useful to make distinction between hepatocellular and cholestatic liver disease (Murali and Carey, 2017). Elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), higher than alkaline phosphatase (ALP) is typical in patients with hepatocellular disease, while elevated levels of ALP are typical in patients with cholestatic liver injury (Arvind and Murali, 2017). AST and ALT are important biomarkers of the liver hepatocytes, under pathological conditions of the liver including liver cirrhosis; there is a leak of these enzymes into plasma thus raising their activity (Nyblom *et al*., 2004). Our finding is similar to works of Momoh (Momo *et al.,* 2012; Olufunsho *et al.,* 2015), where their extracts were observed to have resulted in increase in ALP and ALT while AST did not increase significantly when compared with control, however their study was based on the extract of Bridelia ferruginea, while our study is based on "Agbo" extract which is a combination of different herbs. Creatinine and urea are used to access the functioning capacity of the kidney; hence serum increase in these parameters is an indication of diminished glomerular filtration rate (Panda, 1999). In this current study, urea and creatinine levels did not show any significant difference between the treated groups and control, this is suggestive of normal functional kidney which agrees with the work of Wurochekke *et al.* (2008) after administration of *Xemenia and Americana* to evaluate the effects on the kidney as well as findings of Kolawle and Sunmonu (2010), after administration of Bridelia ferruginea to rodents. The electrolyte levels (sodium, potassium, bicarbonate and chloride) did not show any significant difference when the experimental groups were compared with control, Long-term electrolytes homeostasis is maintained by the kidney (Wright aand Giebish, 1985), the normal electrolytes levels observed is also an indication that the "Agbo" extract did not compromise renal function.

Hematological parameters can be used as indices to access the effect of plant extracts in animals and man (Sunmonu and Oloyede, 1985). Red blood cells and hemoglobin are very important in the transport of respiratory gases (Dogruchy, 1976), hence normal results observed in the hematological parameters when compared with control in this current study is a sign that the extract did not result in hemolysis of the red blood cells. This is also in agreement with findings of Olufunsho Olufonsho *et al*. (2015). The result is also an Indication that the extract lacks the potential to stimulate erythropoietin release in the kidney, which is the humoral regulator of RBC production (Polenakovic and Sikole, 1974; Sanchez-Elsner *et al*., 2004). Total white blood cells and lymphocytes counts were not significant when compared with control; this implies that the extract does not result in immunosuppression. Our result did not agree with findings of Olufunsho *et al.* (2015), who observed a reduction in WBC and Lymphocytes after administering of the extract of *Bridelia ferruginous.* lymphocytes are the main effector cells of the immune system and their increase may be ascribed to the extract triggering neutrophils to promote phagocytosis (cellular ingestion of offending agents) (McKnight *et al*., 1999).

Platelet levels were also not affected when the experimental groups were compared with control, according to McLellan and co-workers (McLellan *et al.,* 2003), a reduction in platelet count in experimental animals has been

reported to indicate an adverse effect on the oxygen-carrying capacity of the blood as well as on thrombopoietin. The extract did not result in any significant difference in the weights of the experimental groups when compared with control.

Conclusion

Administration of the malaria herbal concoction "Agbo" induced oxidative stress in the rats based on the increased MDA and reduced catalase and GPx levels observed in this study. The herbal concoction "Agbo" is not hepatotoxic as the liver enzymes ALT and AST were not affected, however the increase in ALP observed in rats given the moderate dose could be a pointer to cholestatic liver injury as elevated ALP levels have been associated with cholestatic liver injury. The kidney was not affected; all the renal parameters observed in this study were within normal range. The Agbo Concoction did not also affect the hematological parameters as both the red blood cells; white cell count and platelet count were not altered. There is however need for further long term studies and also the use of early renal markers is recommended as creatinine which was used in this study might not increase until a large percentage of the glomerulus is affected.

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