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Depletion of Hepatic Antioxidant Enzymes in Experimental Albino Rats Due to Polyherbal Medicines Administration

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Abstract

Objective: Plants and their derived products have served as veritable sources of foods and medicines for humans and animals from the outset, and the emergency of polyherbs (plants-derived products) in recent years has received the widest publicity and patronage by the Nigerian population as alternative medicines. It is not over statement to say many of them lack empirical data or validation to support the upsurge and prevalence in their usage as folk medicines, and little or no scientific data exist on their potential side effects. This study therefore investigates "the effects of some of these polyherbal drugs on hepatic antioxidants in experimental rats".

Methods: Eight of the nine groups containing five animals each used for this study were administered eight different polyherbal drugs following different manufacturer's recommended dosage, while the ninth group served as control with no polyherb treatment. The study lasted for seven weeks i.e. forty-nine days, and on the 50th day, the animals were sacrificed after 12 h of previous overnight fasting, and their livers were excised for antioxidant assays.

Results: Fidson bitters and Asheitu Adams blood purifier (ABP) significantly decreased superoxide oxidase (SOD) and glutathione-S-transferase (GST), while Yoyo bitter and Asheitu Adams formula for diabetes (AD) mostly decreased reduced glutathione (GSH) in a non-significant manner at ($p<0.05$).

Conclusion: All polyherbal medicines caused depletion of hepatic antioxidant enzymes (SOD and GST), which is an indication of oxidative stress condition, but some of them improved non-enzymatic antioxidants like malondialdehyde (MDA) and vitamin C.

Keywords: Alternative medicines; Antioxidants enzymes; Hepatic; Non-Enzymatic antioxidants; Oxidative stress; Polyherbal medicines

Introduction

The discovery since prehistoric era that plants products, in addition to their nutritive values, could serve as therapeutic weapons against various human, animal and even plant diseases has made plants a *sine qua non* to human and animal lives [1], and has led to the formulations of many herbal and/or polyherbal medicines. Polyherbs are plants-derived herbal medicines popularly known as "polyherbal drugs or phytomedicines", and are currently renowned and recognized as the most common form of alternative medicines in recent years [2,3]. According to Pieme *et al.* [4], they are herbal recipes in the form of teas or extracts often prepared from the combinations of two or more plants and/or plant-products that contain active constituents with multiple physiological activities.

They could be used in the treatment of many diseases, and their use as herbal remedies in the treatment of various diseases is gaining increasing popularity; and thus making them the main stay of health care system, especially among the rural populace in the developing countries. In fact, according to Ogbonnia *et al.* [2], about 60% of the world population both in developed and developing countries where modern medicines are being practiced predominantly used herbal or polyherbal medicines for therapeutic purposes.

Some of these polyherbal formulations are; Evans healthy bitter, Yoyo bitter, Fidson bitter, Swedish bitter, Oroki herbal mixture, Pax herbal mixture, Asheitu Adams blood purifier, Asheitu Adams formula for diabetes, Goko Cleanser, Living bitter etc. and their various manufacturers claimed they could heal and/or prevent all manners of diseases when used at their recommended dosages.

For instance, the manufacturer of Oroki herbal mixture claimed it is formulated for pile, dysentery, constipation, diarrhea, waist and stomach pain etc. [5], while Evans healthy bitter manufacturer claimed; it could stimulate and maintain the production and flow of bile, improve digestion and appetite, give feeling of well-being etc. [6], and the manufacturer of Fidson bitter recommends it for poor digestion, painful digestion, loss of energy, poor appetite, anemia, immune disorder, bacterial and viral infections, intestinal cramps etc. [7].

However, in spite of all these acclaimed therapeutic uses/medicinal benefits of these polyherbal medicines by the manufacturers, as well as their wide patronage by the populace, there are only little or no scientific information (empirical data) on the effects of these polyherbs on *in vivo* antioxidants, because according to Adeyemi *et al.* [8], scientific data on safety and toxicity profiles of these polyherbal medicines are in dearth.

Recent studies have demonstrated the need to subject some of these herbal mixtures (polyherbal drugs) to scientific scrutiny and systemic approach evaluations, so as to ascertain their efficacy, side effects, toxicity and safety limits through experimentations and clinical findings [8-11], and more importantly because, there is no stringent government regulation in Nigeria on herbal/polyherbal medicines like as it is with conventional drugs.

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Therefore, the aim of this research study is to "investigate the effects of some of these polyherbal medicines on *in-vivo* antioxidants' concentrations in albino rats", because any negative effect on these antioxidants could lead to oxidative stress condition and ultimately death if condition persists thus, the reason for embarking on this study.

Materials and Methods

Chemicals and reagents

Epinephrine- E1635-5G, ascorbic acid- A92902-100G, iodine solution- 053-001-00-3, Ellmans reagent (5, 5-dithiobis-2-nitro benzoic acid (DTNB))- D218200-5G, hydrogen peroxide- 18312 used are products of Sigma-Aldrich, USA and Germany, while sodium hydrogen carbonate-102474V, hydrochloric acid- 10125-4H, dichromate-acetic acid reagent (potassium dichromate-10202 and acetic acid glacial-27013) are products of BDH Laboratory Supplies, Poole England. All other chemicals and reagents used are of analytical grade.

Polyherbal medicines

Evans healthy bitter was purchased from Evans Medical PLC, KM 32, Lagos-Badagry Expressway, Lagos; Yoyo bitter from Ablatt Pharmaceutical Limited, Lagos; Fidson bitter from Fidson Healthcare PLC, 268 Ikorodu Road, Lagos; Swedish bitter from Starling Nigeria Limited, Surulere, Lagos; Oroki herbal mixture from Nure Ind. & Comm. Company, Ijaye, Lagos; Pax herbal mixture from a pharmaceutical store, Ajegunle, Lagos; while Asheitu Adams blood purifier (ABP) and Asheitu Adams formula for diabetes (AD) were gotten from the company distributor at Ikorodu market, Lagos.

Laboratory animals

Forty-five growing male albino rats with the average weight of 212 ± 22.18 g were purchased from Animal Facility Centre of University of Lagos, College of Medicine, Idi-Araba. They were randomly distributed into nine groups of five animals each after two weeks of acclimatization, and were orally administered the different polyherbal medicines according to the recommended dosages of their manufacturers once a day for seven weeks using oral canula. The treatments were as follow:

Group 1: Control 0.25 ml of distilled water

Group 2: Fidson bitters 0.000290 ml/g body weight (61.5 µl per 212 g)

Group 3: Asheitu Adams Blood Purifier 0.000290 ml/g body weight (61.5 µl per 212 g)

Group 4: Swedish bitters 0.000290 ml/g body weight (61.5 µl per 212 g)

Group 5: Yoyo bitters 0.000429 ml/g body weight (91.0 µl per 212 g)

Group 6: Asheitu Adams formula for diabetes 0.000290 ml/g body weight (61.5 µl per 212 g)

Group 7: Pax herbal mixtures 0.000290 ml/g body weight (61.5 µl/212 g)

Group 8: Oroki herbal mixtures 0.001143 ml/g body weight (242.3 µl/212 g)

Group 9: Evans healthy bitters 0.000143 ml/g body weight (30.3 µl/212 g)

The animals were maintained in line with the regulations guiding the use of animals for experimental research stated by Animal Welfare Act (Laboratory Animal Welfare Act) as amended in 2013, which is also in line with National Institutes of Health guide for the care and use of Laboratory animals. They were fed *ad libitum* with certified feed (Grow Fast Mash; Animal Care Feeds), NAFDAC Reg. NO: A9-0025 and clean tap water in iron gauze cages lined with wooden shaves, at room temperature with adequate ventilation under natural 12 h of illumination/light and 12 h of darkness in the College.

At the end of the study period, the animals were sacrificed by cervical dislocation after 12 h overnight fasting, and livers were quickly removed, washed in ice cold 1.15% KCl solution, weighed and kept in sterile plain bottles containing 10% formaldehyde for storage before been processed for antioxidant assays.

Enzymatic antioxidants assay

Assay of superoxide dismutase (SOD) activity: SOD activity was determined following the protocols of Imaga and Valentine [12], Samarghandian *et al.* [13] and Samarghandian *et al.* [14] with slight modifications using its ability to inhibit auto-oxidation of epinephrine.

Assay of glutathione–S-transferase (GST) activity: GST activity was determined using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate, following the method described by Samarghandian et al. [13] with slight modifications.

Assay of catalase (CAT) activity: CAT activity was determined colorimetrically following the methods of Imaga and Valentine [12], Samarghandian *et al.* [13] and Samarghandian *et al.* [14] with slight modifications, using the amount of H_2O_2 consumed as yardstick.

Non-enzymatic antioxidants assay

Reduced glutathione determination: The reduced glutathione (GSH) content of liver tissue as non-protein sulfhydryl was estimated using 5-dithiobisnitro benzoic acid (DTNB) enzymatic colorimetric reaction, according to the method described by Imaga and Valentine [12] and Baker *et al.* [15].

Lipid peroxidation: Malondialdehyde (MDA), an index of lipid peroxidation was determined using the methods of Imaga and Valentine [12], and Samarghandian *et al.* [13] with slight modifications.

Vitamin C concentration: Vitamin C content was determined using titration method described by College of Science, University of Canterbury [16].

Statistical Analysis

All data collected were statistically evaluated with Graph Pad Prism version 5.0 software using One-Way Analysis of Variance (ANOVA) with Turkey's Multiple Comparisons Tests. The results were presented as mean ± standard error of mean (SEM) in tables and were also used to construct bar charts. F-test at 95% (i.e. 0.05) level of significance was used to assess significance difference and p-value less than 0.05 i.e. ($p<0.05$) was considered statistically significant.

Results

Table 1 results showed that Fidson and ABP significantly reduced or decreased GST and SOD concentrations in comparison with the control, but these concentrations did not differ significantly from the concentrations of other polyherbal drugs' treatments. The results also revealed that there was no statistically significance difference in CAT concentrations between the control and the various polyherbal medicines' treatments however; polyherbal medicines caused decrease in CAT levels of the experimental animals.

More also, Table 2 showed that reduced glutathione (GSH) concentrations did not differ significantly between treatments although; GSH concentrations of Swedish, Oroki, and Evans were non-significantly higher than that of the control group, while Yoyo B. and AD mostly but non-significantly decreased GSH concentrations. More also, there was no statistical difference at all in MDA and vitamin C concentrations between polyherbal medicines nevertheless; vitamin C concentrations of all treatments except Swedish and Evans were higher than that of the control group, while all the polyherbal medicines except Pax brought about decrease or reduction in MDA concentrations in comparison with the control.

Furthermore, Figures 1- 3 showed that all the polyherbal medicines decreased hepatic concentrations of GST, SOD and CAT respectively in the experimental animals. While Figure 4 showed that few of the polyherbal medicines (Swedish, Evans and Oroki) brought about increase in reduced GSH concentrations in experimental animals, while the rest polyherbs (Fidson, ABP, Yoyo B., AD and Pax) caused reductions GSH levels in comparison with the control group.

Additionally, Figure 5 revealed that; all polyherbal medicines except Pax decreased MDA concentrations in comparison with the control, and Figure 6 showed that all polyherbal medicines except Swedish and Evans conversely increased endogenous vitamin C.

Treatments / Parameters	Control	Fidson	Abp	Swedish	Yoyo B.	Ad	Pax	Oroki	Evans
GST (µmol/ml/min)	34.22 ± 27.71^{a}	21.02 ± 2.51^{b}	18.40 ± 2.69^{b}	24.35 ± 8.16^{ab}	28.76 ± 3.06^{ab}	31.76 ± 2.94^{ab}	24.87 ± 2.22^{ab}	27.17 ± 2.35^{ab}	28.73 ± 0.53^{ab}
SOD (µmol/ml/min)	1097 ± 86.78^{a}	690 ± 84.13^{b}	608 ± 86.86^{b}	1028 ± 317.80^{ab}	841 ± 28.56^{ab}	967 ± 31.04^{ab}	797 ± 49.29^{ab}	897 ± 70.91^{ab}	925 ± 16.41^{ab}
CAT(µmol/ml/min)	734 ± 15.45	659 ± 43.57	678 ± 19.18	684 ± 27.22	728 ± 27.56	702 ± 31.92	680 ± 29.25	714 ± 12.29	717 ± 31.77

Note: comparison is strictly within parameter. All values expressed as Mean ± SEM. Values with the same alphabet superscript notation (s) do not differ significantly, while those with different superscript notation (s) do at ($p<0.05$) and those without
Keys: ABP-Ashetu Bitters blood Purifying tonic; AD- Ashetu Adams formula for Diabetes; B-Bitters.

Table 1: Hepatic concentration of antioxidant enzymes of experimental animals

Treatments / Parameters	Control	Fidson	Abp	Swedish	Yoyo B.	Ad	Pax	Oroki	Evans
GSH (µmol/ml)	5.73 ± 0.95^{ab}	4.02 ± 0.62^{ab}	3.79 ± 0.67^{ab}	7.86 ± 1.79^{a}	1.60 ± 0.68^{b}	2.51 ± 0.46^{b}	4.36 ± 0.93^{ab}	7.47 ± 0.64^{a}	7.66 ± 1.46^{a}
MDA (µmol/ml)	4.40 ± 0.65	3.78 ± 0.36	3.71 ± 0.58	4.21 ± 0.82	3.57 ± 0.54	3.54 ± 0.43	4.50 ± 0.76	4.03 ± 0.23	3.35 ± 0.78
VIT. C (mg/100 g)	16.89 ± 2.26	17.90 ± 2.48	21.32 ± 2.00	16.45 ± 0.61	19.28 ± 1.54	17.27 ± 2.25	17.76 ± 1.40	17.68 ± 1.34	15.66 ± 2.19

Note: comparison is strictly within parameter. All values expressed as Mean ± SEM. Values with the same alphabet superscript notation (s) do not differ significantly, while those with different superscript notation (s) do at ($p<0.05$) and those without superscript implies there is no statistical difference at all.
Keys: ABP-Ashetu Bitters blood Purifying tonic; AD- Ashetu Adams formula for Diabetes; B-Bitters.

Table 2: Hepatic non-enzymatic antioxidant concentration of experimental animals.

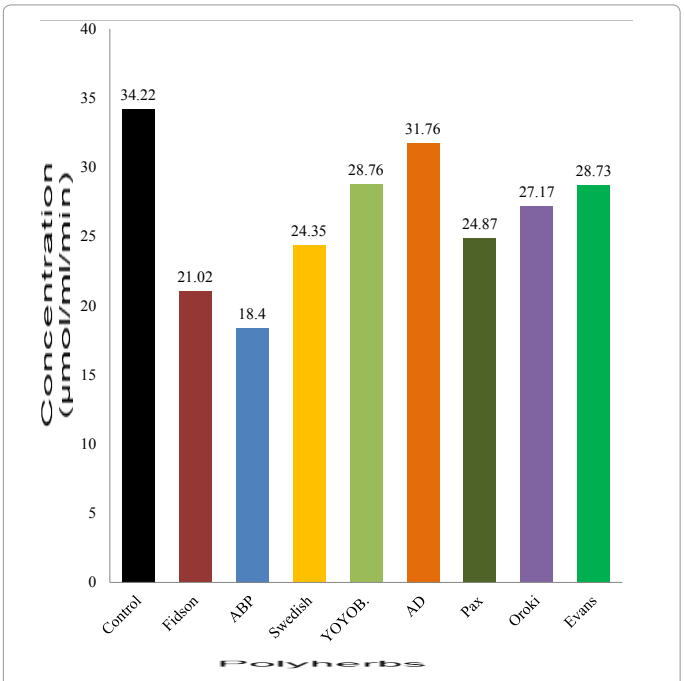

Figure 1: Effect of polyherbal medicines on glutathione-s-transferase (GST) concentrations in experimental animals.

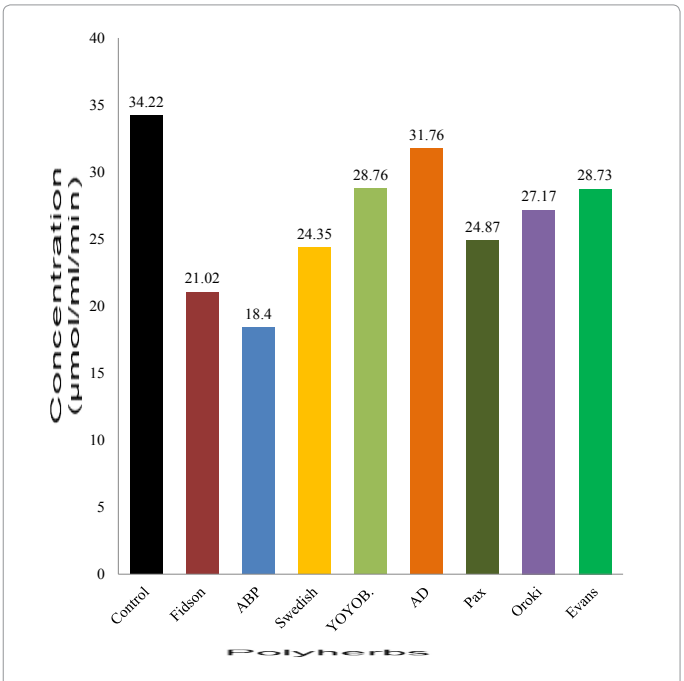

Figure 2: Effect of polyherbal medicines on superoxide dismutase (SOD) concentrations in experimental animals.

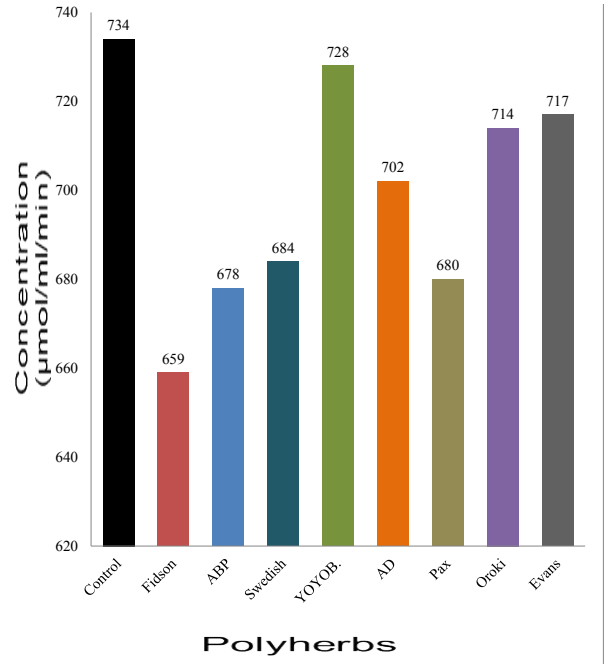

Figure 3: Effect of polyherbal medicines on catalase (CAT) concentrations in experimental animals.

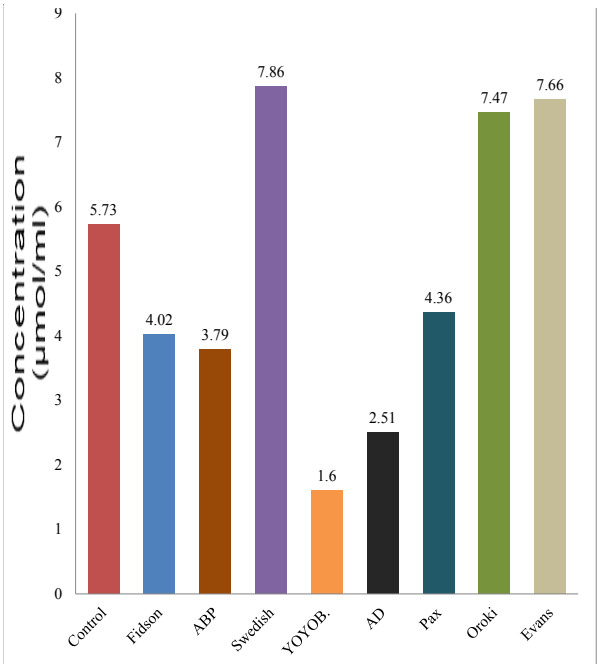

Figure 4: Effect of polyherbal medicines on glutathione (GSH) concentrations in experimental animals.

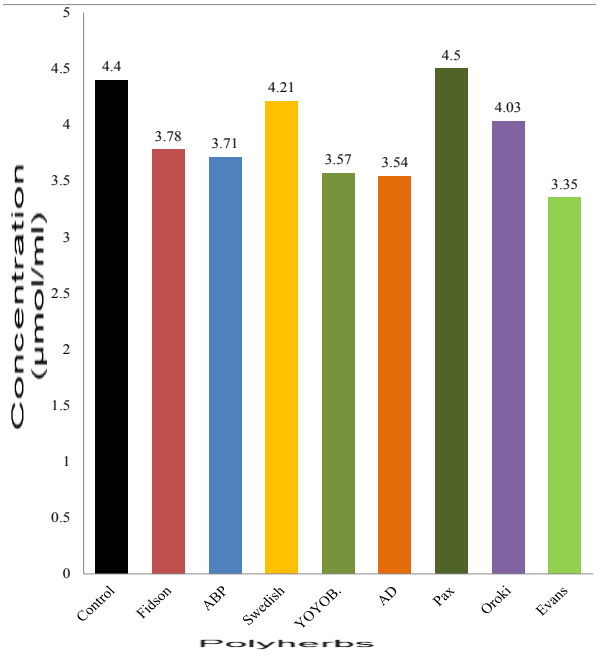

Figure 5: Effect of polyherbal medicines on malondialdehyde (MDA) concentrations in experimental animals.

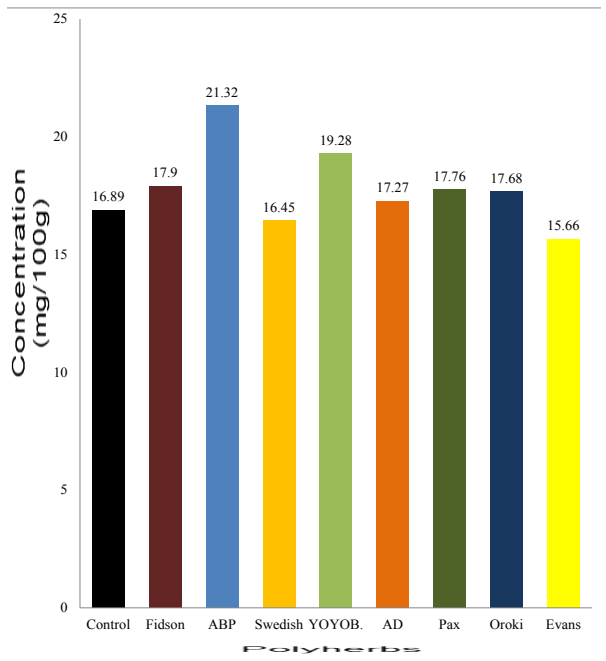

Figure 6: Effect of polyherbal medicines on vitamin C concentrations in experimental animals.

Discussion

Antioxidants are reducing agents that prevent oxidation of cellular components by molecular oxygen [17]. They terminate chain reactions that can result to the production of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) by removing free radical intermediates, and inhibit further or prevent other oxidative reactions, while they are being oxidized [18]. They are thus considered as important as nutraceuticals on account of many health benefits [17], and are used to label any substance whose availability, even in minute concentration can inhibit or delay the oxidation of a substrate or other molecules [19]. These antioxidants are known to diffuse free radicals leading to limited risk of oxidative stress [20], and are significant in oxidative stress reductions [21] i.e. they help combat oxidative stress [13].

Oxidative stress is the disproportion between oxidants and antioxidants in favor of oxidants, which potentially lead to oxidative damage of cells. In other words, it is made by an imbalance between the generations of ROS and detoxification of the reactive intermediates via biological system's ability [14].

Therefore, the significant decrease or depletion of hepatic antioxidant enzymes, most especially SOD and GST concentrations, and non-significant decrease in reduced GSH concentrations, most especially in Yoyo B. and AD treated groups are signal of oxidative stress condition. They are consistent with the findings of Adeyemi *et al.* [8]; Imaga and Valentine [12]; Adeyemi and Owoseni [22] on some of these polyherbal drugs (Swedish bitters, Yoyo bitters and Oroki herbal mixtures).

This finding implies that the administration of the polyherbal drugs caused the generation of free radicals like ROS and RNS, which in turn triggered the mobilization of endogenous or *in vivo* biological antioxidant enzymes like GST, SOD and CAT in order to combat their detrimental effects. However, the continuous mobilization of these enzymes due to the daily introduction of free radical-generating polyherbs then led to the depletion of antioxidant enzymes as a result of exhaustion in the course of scavenging the overproduced free radicals. The scavenging activities of these enzymes are needed so as to prevent the deleterious effects of free radicals, and as well inhibit further oxidation of cellular components such as proteins, nucleic acids and lipids by molecular oxygen [23].

It should also be noted that; if oxidative stress condition is not properly managed or well handled by the body antioxidant defense system or mechanism, it could lead to oxidative damage of cellular components [23] and complication of chronic degenerative diseases such as diabetes, cardiovascular diseases, cancer etc. [24,25]. According to Dey and Cederbaum [26], oxidative stress from overproduction of free radicals could lead to the pathogenesis and progression of liver diseases if not properly controlled. They opined that, increased production and accumulation of free radicals in liver (in an oxidative stress condition) play an important role in the pathogenesis and progression of liver diseases e.g. cirrhosis. Adeyemi and Owoseni [22], Adeyemi *et al.* [8], Imaga and Valentine [12] and Gafrikova *et al.* [24] all stated oxidative damage of biomolecules to be one of the dangerous effects of depleted antioxidant enzymes as a result of overproduction of free radicals among other dangers.

Furthermore, the decrease in GSH concentrations; a naturally occurring antioxidant that prevents free radical damaged of cells and as well helps in detoxification process by conjugating with chemicals/oxidants [22], must have resulted from the free radical scavenging activity of GST. GST is an antioxidant enzyme that uses GSH as cofactor in its defense mechanism to prevent oxidative damage just like glutathione peroxidase (GPx) and glutathione reductase (GR). It is also possible that GR plays a role in the reduction of GSH concentration, because according to Samarghandian *et al.* [14], GSH can be changed to its oxidized form (GSSG) by GR when cells are exposed to overproduction of free radicals hence, this could also ultimately contribute to the decreased hepatic GSH concentrations observed.

In a nutshell; the depletion of antioxidant enzymes and GSH molecule observed in this study should be a reflection of the toxic effects of these polyherbal medicines on the liver as pro-oxidants, which may expose liver to oxidative damage. For instance, SODs are the major antioxidant enzymes that inactivate superoxides, thereby controlling oxidative stress [27]. It is an anti-oxidative enzyme that plays an important role against oxidative stress-generated complications such as diabetes and cardiovascular diseases by breaking down hydrogen peroxides [28]. Therefore, its depletion is a signal of its long time involvement in combating free radicals, which if not remedied could ultimately lead to cell death.

However, the slight decrease in hepatic MDA levels in all treatments except Pax and increase in vitamin C in all treatments except Evans and Swedish suggest that these polyherbal medicines are not altogether bad. This tends to be an attestation to the observed *in-vitro* antioxidant properties of these polyherbs by Emaleku *et al.* [29]. For instance, they found out that; these polyherbal medicines have high percentage MDA inhibition in a concentration dependent manner. MDA, the product of lipid peroxidation, is an index of the level of oxygen free radicals [30]. Therefore, this observed decrease in hepatic MDA levels in almost all treatments when compared with the control is an indication of prevention of lipid peroxidation.

It would therefore not be an over-statement, to say at this juncture, that the high percentage *in vitro* inhibition of MDA of these polyherbs reported by Emaleku *et al.* [29] might have helped in preventing lipid peroxidation in the polyherbs-treated animals. This positive non-enzymatic antioxidant property i.e. decrease in MDA levels in animals administered polyherbal medicines might be one of the likely reasons for the non-significant reductions in GSH and CAT concentrations observed in the polyherbs-treated animals. This is likely because, the higher the concentration of the free radicals released, the higher the concentration of the antioxidants that would be triggered by the body innate antioxidant defense system to counteract the free radicals' effects, and vice versa.

Conclusively, polyherbal medicines depleted antioxidant enzymes, most especially SOD and GST, while most of them, especially Yoyo B. and AD decreased GSH concentrations, but almost all prevent lipid peroxidation by decreasing MDA concentrations.

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