



## **Evaluation of the Toxicological Profile of *Yagari***

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Author UMMA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors UMMA and OFCN managed the literature searches, analyses of the study performed the spectroscopy analysis and author UMMA managed the experimental process. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background and Objective:** Data from researches have shown a rise in disease, ill health and death linked with the utilization of herbal products, thereby raising global awareness in the last few years. On that account, the safety and toxicity evaluations of herbal products and preparations was essential. This study evaluated the toxicological profile of *Yagari* – a herbal mixture.

**Materials and Methods:** Acute oral toxicity (LD<sub>50</sub>) was carried out in Swiss mice according to Lorke's method while sub-chronic toxicity study was carried out with 20 adult albino rats which were divided into 4 groups of 5 animals each. Group one served as control and received normal saline while Groups 2 to 4 received 250, 500 and 1000 mg/kg *yagari* respectively for 28 days. The body weights of the rats were monitored while on day 29, the rats were sacrificed and blood samples and organs were collected for biochemical/hematological analysis and histopathological examination respectively.

**Results:** Results showed that *Yagari* is not noxious up to 5000 mg/kg following acute oral toxicity study. The sub-chronic toxicity test divulged that *Yagari* had no serious end results on the biochemical, hematological and histopathological parameters, although the body weight of the animals significantly increased.

**Conclusion:** It was concluded that *Yagari* is not toxic, still further investigations on a large number of animals are essentially needed to denote safety and efficacy of the herbal formulation.

**Keywords:** Toxicological; *yagari*; herbal mixture; sub-chronic.

## 1. INTRODUCTION

Herbal medicine is a key component of traditional medicinal practices such as Ayurvedic, Homeopathic, Naturopathic, Traditional oriental and native American-Indian medicine [1]. It is coming to the fore as another treatment to synthetic drugs for the treatment of disease [2] possibly due to lower cost, availability, fewer adverse effects and perceived effectiveness [3]. Studies have documented lower incidences of cardiovascular, neurological and malignant lesions amidst vegetarians [4-6]. Herbal medicines are thought to be safe due to their 'natural source' [6]. Nevertheless, this hypothesis is not correct as herbal concocting could be inherently virulent, infected with contagious such as aflatoxins and contaminations such as heavy metals, pesticide residues [6]. These contaminants if present in herbal medicine pose health risk to all that rely on herbal medicine for their health care [6,7]. Clinical toxicity can vary from mild to severe and even life threatening making the toxicological evaluations of these preparations necessary [6]. In most countries, herbal products are launched into the market without proper scientific evaluation, and without any mandatory safety and toxicological studies [8]. Polyherbal formulation is the use of many plant parts in the treatment of disease and may have multiple bioactive compounds [6]. It is an assented truth among many people that polyherbal formulations are as effective as the orthodox drugs [6]. It was suggested that herbs provide ingredients that act in synergy or counterbalance undesirable effects. Hence, the body's healing process can utilize a balance of ingredients provided by nature [8] through herbs or natural plants products in their complete form. Characterization of herbal medicine is arduous due the presence of multiple bioactive constituents, which can induce pharmacokinetic drug interactions. Therefore, it is necessary to monitor or consider the toxic effects of herbal medicines while administering it over a long period of time to avert health related adverse effects [9].

*Yagari*, is a local herbal mixture specifically prepared for management and treatment of prostate disorder in the Eastern parts of Nigeria. It consists of *Nauclea latifolia*, and *Erythrophileum suaveolens*. Prostate disorder describes any medical problem that affects the prostate gland. It is major health problems

characterized by inflammation, enlargement and abnormal cell division of the prostate gland [10]. To the best of our knowledge, the toxicological study of *Yagari* is yet to be experimentally demonstrated. Here, we evaluated the toxicological profile of the herbal mixture using animal models. We investigated the sub-chronic and acute toxicity of *yagari* as well as analysis of the phytochemical composition of *yagari*. Our results underscore the potential safety of the herbal mixture, *yagari*.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The herbal mixture, *Yagari* was obtained from a traditional herbal shop in Nsukka, Enugu State, Nigeria and was filtered with muslin cloth via Whatman No 4 filter paper. The filtrate was concentrated in rotary evaporator and stored in the refrigerator for further analysis.

### 2.2 Animal

Eighteen albino mice of Swiss strain (22–28 g) were used for index of acute toxicity study while twenty Wistar rats weighing 180–250g were procured from the animal house facility of the Department of Pharmacology, University of Nigeria, Nsukka and handled according to the guide for the Care and Use of Laboratory Animals, published by the National Institute of Health (NIH), USA. The rats were maintained at  $25.0 \pm 2^{\circ}\text{C}$  on a 12 h light/dark cycle with access to standard animal feed and water *ad libitum* for 7 days before the commencement of the experiment.

### 2.3 Chemical

All the chemicals and reagents were of analytical grade, and were used as obtained from the suppliers.

### 2.4 Methods

#### 2.4.1 Phytochemical analyses of *yagari*

The qualitative phytochemical analyses of *Yagari* were carried out according to the methods of Harborne [11], methods as described by AOAC [12] and Trease and Evans [13].

#### 2.4.2 Acute toxicity study of *yagari*

The acute toxicity study of the herbal mixture (*Yagari*) was carried out by the method of Lorke

[14] to define the range of lethal dose and safe dose for the crude extract. Eighteen (18) albino mice were utilized in the study. They were starved of food for 18 hr but allowed access to water prior to the study. The study involved two stages. In stage one, the animal were grouped into three (3) groups of three mice each and were administered 10, 100 and 1000 mg/kg body weight of the herbal mixture, after which they were observed for 24 hours for signs of toxicity and/ or mortality. Based on the results of the first phase, nine mice were again divided into 3 groups of 3 mice each and were also treated with the herbal mixture at doses of 1600, 2900 and 5000 mg/kg body weight respectively in the second phase. The animals were observed for 24 hr for nervousness, dullness, in-coordination and/or death. The extract was dispersed in normal saline and administered orally. The median lethal dose (LD50) was estimated as the geometric mean of the maximum dose that caused 0% death and the maximum dose that caused 100% death.

$$\text{Mathematically, LD50} = \sqrt{(D_0 \times D_{100})}$$

$D_0$  = Highest dose that gave no mortality

$D_{100}$  = Lowest dose that produced mortality

#### 2.4.3 Sub-chronic toxicity studies of *yagari*

Chronic toxicity study was conducted according to the guidelines of Organization for Economic Cooperation and Development [15]. Twenty (20) adult male albino rats were randomly divided into four groups of five rats each. Rats in Groups 2, 3 and 4 were administered 250, 500 and 1000 mg/kg of the herbal mixture while Group 1 served as control and administered normal saline for 28 days. The rats were maintained on diet and tap water ad libitum throughout the study. All groups were observed for signs of toxicity and mortality for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> hours and thereafter, daily for 28 days. On day 29, all rats were fasted overnight and then euthanized using chloroform, organs were harvested for histopathological examination and blood samples were collected via cardiac puncture into EDTA/ plain bottles for the following analyses.

#### 2.5 Weekly Body Weight Changes

Rats in all groups were weighed weekly during the period of treatment and on the day of sacrifice for changes in body weight.

#### 2.6 Determination of Blood Glucose Concentration

The determination of blood glucose in whole blood was done according to the method of Trinder [16] using accu-check glucometer by Roche diagnostic.

#### 2.7 Determination of Hematological Parameters

The haematological parameters were done according to the method of Ochei and Kolhatkar [17] using hematological auto-analyzer (Mindray BC-2800 Auto Hematological Analyzer, England). The hematological parameters analyzed included: Packed cell volume (PCV), Red Blood Cell (RBC) count, White Blood Cell (WBC) count and Hemoglobin (Hb) concentration.

#### 2.8 Assay of Liver Function Biomarkers

Aspartate aminotransferase (AST) was evaluated using the method of Reitman and Frankel [18] as described by Randox laboratories, United Kingdom using Randox kits; Alanine aminotransferase (ALT) was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine using the method of Reitman and Frankel [18] as described in Randox kits; alkaline phosphatase (ALP) was assayed based on the methods of Babson et al. [19] while total bilirubin was determined according to the method of Jendrasik and Grof [20].

#### 2.9 Lipid Profile Determination

Total cholesterol was evaluated using enzymatic colorimetric CHOD- POD test method described by Abell et al. [21] with Randox test kit; Triglycerides was also determined spectrophotometrically using the method of Tietz [22]; High density lipoproteins (HDL) was evaluated by the method of Kamaswera et al. [23] as described in Quimica Clinica Applicada test kit.

#### 2.10 Oxidative Stress Biomarkers

The degree of lipid peroxidation was determined spectrophotometrically by measuring the concentration of malondialdehyde (MDA) as described by Wallin et al. [24]; Superoxide

dismutase was assayed using enzymochrom superoxide dismutase assay kit produced by Bioassay system, USA according to the method of Ukeda et al. [25]; and the activity of catalase was assayed by method of Sinha [26].

### 2.11 Histopathology

The organs (liver and kidney) were collected for histopathological studies. Tissue samples collected were fixed in 10% formal saline for 24 hrs. They were washed in ascending grades of ethanol, cleared with xylene, embedded in paraffin wax, sectioned with a microtome and stained with haematoxylin and eosin (H and E) and mounted on Canada balsam [27]. All the sections were examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

### 2.12 Data and Statistical Analysis

All data were expressed as Mean  $\pm$  SEM and statistical differences between means were determined by one-way ANOVA followed by Duncan's *post-hoc* test for multiple comparison tests using Statistical Package and Service Solution. Values were considered significant at  $P \leq 0.05$ .

## 3. RESULTS

### 3.1 Phytochemical Composition of *Yagari*

Table 1 shows the quantitative phytochemical composition of *Yagari*. Bioactive compounds such as alkaloids were found to be in high amount ( $2333.339 \pm 0.003$  mg/g). Terpenoid, phenol, tannin and flavonoid were moderately present.

### 3.2 Acute Toxicity Study of *Yagari* in Mice

The acute toxicity test of *Yagari* showed that the herbal mixture does not cause death with doses

up to 5000 mg/kg body weight. Table 2 shows the observations in phase I and II of the acute toxicity test ( $LD_{50}$ ) of the *yagari*. These observations indicated that no mortality or uncoordinated movement was recorded in the rats treated with different doses of the herbal mixture in both phases of the study.

### 3.3 Body Weight Changes in Wistar Rats Treated with Graded Doses of *Yagari*

Table 3 showed the effect of *yagari* on the animals' body weight. When different doses of *yagari* were administered to rats, there was a non-significant ( $p > 0.05$ ) increase in the mean body weight when compared to control.

### 3.4 Effect of *Yagari* on Fasting Blood Glucose Level of Wistar Rats

The effect of *yagari* on fasting blood glucose is shown in Table 4. The blood glucose concentration decreased in dose dependent manner. The herbal mixture at the concentration of 1000 mg/kg b.w. decreases blood glucose level significantly ( $p < 0.05$ ) when compared to normal control.

### 3.5 Effect of *Yagari* on Some Haematological Parameters of Wistar Rats

Table 5 shows the result of the hematology of rats exposed to sub-chronic medicament with different doses (250, 500 and 1000 mg/kg) of *yagari*. *Yagari* at all the dose levels produced no significant changes ( $p > 0.05$ ) in the haematological parameters (PCV and Hb concentration) when compared to control. However, at 1000 mg/kg, *yagari* produced a significant ( $p < 0.05$ ) increase and decrease in the levels of RBC and WBC when compared to the control respectively.

Table 1. Phytochemical composition of *yagari*

Phytochemical	Relative Abundance (mg/g)
Reducing Sugars	72.870 $\pm$ 0.003
Tannins	788.420 $\pm$ 001
Flavonoids	883.949 $\pm$ 0.020
Phenols	1740.645 $\pm$ 0.004
Steroids	41.580 $\pm$ 0.005
Terpenoids	2177.030 $\pm$ 0.006
Saponins	63.2 $\pm$ 0.002
Glycosides	38.157 $\pm$ 0.005
Alkaloids	2333.339 $\pm$ 0.006

Results are expressed in Means  $\pm$  SD ( $n = 3$ )

**Table 2. Acute toxicity study of yagari in mice**

<b>Groups</b>	<b>Dosage (mg/kg b.w)</b>	<b>Mortality (Phase I)</b>
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3

<b>Groups</b>	<b>Dosage (mg/kg b.w)</b>	<b>Mortality (Phase II)</b>
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

**Table 3. Body weight (g) changes in wistar rats treated with graded doses of yagari**

<b>Treatment group</b>	<b>Body weight (g)</b>				
	<b>Day 0</b>	<b>Day 7</b>	<b>Day 14</b>	<b>Day 21</b>	<b>Day 28</b>
1	209.68 ± 6.42	212.24 ± 7.43	217.26 ± 8.51	223.63 ± 10.70	230.71 ± 15.70
2	188.62 ± 11.28	191.66 ± 12.27	195.77 ± 8.40	200.15 ± 3.51	201.77 ± 1.70
3	212.30 ± 13.77	211.85 ± 18.99	216.81 ± 16.15	220.99 ± 19.43	231.51 ± 27.58
4	199.29 ± 19.08	201.15 ± 20.16	207.65 ± 17.76	210.65 ± 25.03	213.80 ± 22.37

*Data represented as mean ± SD (n=5).*  
*Group 1: Normal control, 5 mg/ml of normal saline*  
*Group 2: 250 mg/kg b.w of Yagari*  
*Group 3: 500 mg/kg b.w of Yagari*  
*Group 4: 1000 mg/kg b.w of Yagari*

**Table 4. Effect of *yagari* on fasting blood glucose level of wistar rats**

Groups	Glucose Conc. (mg/dl)
1	96.67 ± 4.16
2	88.33 ± 8.96
3	87.33 ± 2.08
4	84.33 ± 3.06*

Data represented as mean ± SD (n=5) \*p < 0.05 compared to control.

Group 1: Normal control, 5 mg/ml of normal saline

Group 2: 250 mg/kg b.w of *Yagari*

Group 3: 500 mg/kg b.w of *Yagari*

Group 4: 1000 mg/kg b.w of *Yagari*

**Table 5. Effect of *Yagari* on Some Haematological Parameters of Wistar Rats**

Groups	PCV (%)	Hb (g/dl)	RBC (×10 <sup>12</sup> /L)	WBC (×10 <sup>9</sup> /L)
1	46.33 ± 1.53	24.34 ± 2.36	303.33 ± 49.33	5600.00 ± 400.00
2	46.33 ± 3.21	25.31 ± 1.77	321.33 ± 26.03	5233.33 ± 351.19
3	47.00 ± 3.46	26.15 ± 1.18	334.67 ± 27.30	5133.33 ± 152.73
4	49.67 ± 2.08	27.17 ± 1.34	418.00 ± 19.08*	4933.33 ± 152.75*

Data represented as mean ± SD (n=5) \*p < 0.05 compared to control.

Group 1: Normal control, 5 mg/ml of normal saline

Group 2: 250 mg/kg b.w of *Yagari*

Group 3: 500 mg/kg b.w of *Yagari*

Group 4: 1000 mg/kg b.w of *Yagari*

### 3.6 Effect of *Yagari* on Liver Function Biomarkers of Wistar Rats

Table 6 shows the results of the effect of *Yagari* on liver function biomarkers of rats treated with varied doses (250, 500 and 1000 mg/kg) of *yagari*. The herbal mixture has no significant (p > 0.05) changes in the parameters (AST and T. Bil). In contrast, *yagari* at 1000 mg/kg effected a significant (p < 0.05) decreases in ALT and ALP compared to the control.

### 3.7 Effect of *Yagari* on Lipid Profile of Wistar Rats

The result of the Lipid profile of Wistar rats that were sub-chronically exposed to *yagari* is presented in Table 7. There was no significant (p > 0.05) changes in the levels of Total cholesterol (CHOL) and high density lipoprotein (HDL) compared to the control. However, at 1000 mg/kg, *yagari* produced a significant (p < 0.05) decrease in the concentration of triacylglycerol when compared to the control.

### 3.8 Effect of the *Yagari* on Oxidative Stress Biomarkers of Wistar Rats

The results of the effect of the herbal mixture on superoxide dismutase (SOD) activities in the

Wistar rats are presented in Table 8. SOD activities at 250 mg/kg b.w. in *yagari*-treated rats show no significant change (p > 0.05) compared to the control. The result of the mean malondialdehyde (MDA) concentration in *yagari*-treated rats in Table 8, depicts that there was no significant (p > 0.05) changes in the MDA concentration of *yagari*- treated rats compared to the control. The catalase (CAT) activities in *yagari*- treated rats as shown in Table 8 reveal no significant difference (p > 0.05) between the treated and control rats at 250 mg/kg body weight

### 3.9 Histopathology of the Liver and Kidney Organs of Wistar Rats Treated with *Yagari* after Day 28

The effect of *yagari* at 250, 500 and 1000 mg/kg on the histological architecture of the liver and kidney organs was microscopically evaluated after Haematoxylin and Eosin stain following 28 days of treatment with the herbal mixture. Plates 1 - 8 showed the photomicrographs of the liver and kidney of the control and treated rats. There was no abnormal in the anatomical architecture of the organs.

**Table 6. Effect of yagari on liver function biomarkers of wistar rats**

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TBIL (mg/dl)
1	159.26 ± 23.13	59.00 ± 1.73	17.44 ± 0.35	1.05 ± 0.78
2	160.87 ± 6.77	57.33 ± 0.58	17.21 ± 0.95	0.77 ± 0.46
3	146.53 ± 13.46	53.33 ± 3.06	17.27 ± 0.32	0.65 ± 0.33
4	142.68 ± 9.21	45.67 ± 7.37*	15.47 ± 1.34*	0.29 ± 0.06

Data represented as mean ± SD (n=5) \*p < 0.05 compared to control.

Group 1: Normal control, 5mg/ml of normal saline

Group 2: 250mg/kg b.w of Yagari

Group 3: 500mg/kg b.w of Yagari

Group 4: 1000mg/kg b.w of Yagari

**Table 7. Effect of yagari on lipid profile of wistar rats**

Groups	CHOL (mmol/L)	TAG (mmol/L)	HDL (mmol/L)
1	90.44 ± 15.66	121.26 ± 36.43	45.42 ± 10.96
2	87.42 ± 13.81	97.68 ± 21.03	57.38 ± 7.17
3	87.42 ± 18.82	97.68 ± 32.48	59.77 ± 18.05
4	63.30 ± 18.09	67.37 ± 21.04*	64.55 ± 18.98

Data represented as mean ± SD (n=5) \*p < 0.05 compared to control.

Group 1: Normal control, 5mg/ml of normal saline

Group 2: 250 mg/kg b.w of Yagari

Group 3: 500 mg/kg b.w of Yagari

Group 4: 1000 mg/kg b.w of Yagari

**Table 8. Effect of yagari on oxidative stress biomarkers of wistar rats**

Groups	MDA (mg/dl)	CAT (IU/L)	SOD (IU/L)
1	2.77 ± 0.09	1.33 ± 0.09	65.90 ± 2.45
2	2.69 ± 0.19	1.34 ± 0.03	68.21 ± 3.39
3	2.65 ± 0.09	1.61 ± 0.13*	73.83 ± 6.47*
4	2.62 ± 0.13	1.84 ± 0.06*	86.26 ± 2.14*

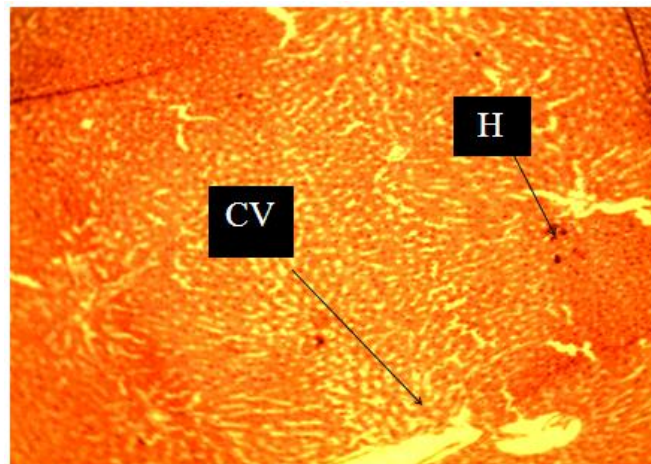
Data represented as mean ± SD (n=5) \*p < 0.05 compared to control.

Group 1: Normal control, 5 mg/ml of normal saline

Group 2: 250 mg/kg b.w of Yagari

Group 3: 500 mg/kg b.w of Yagari

Group 4: 1000 mg/kg b.w of Yagari



**Plate 1. Photomicrograph of sections of liver administered with 5 ml/kg b.w. normal saline. H and E, x100. CV = Central vein, H =Hepatocytes**



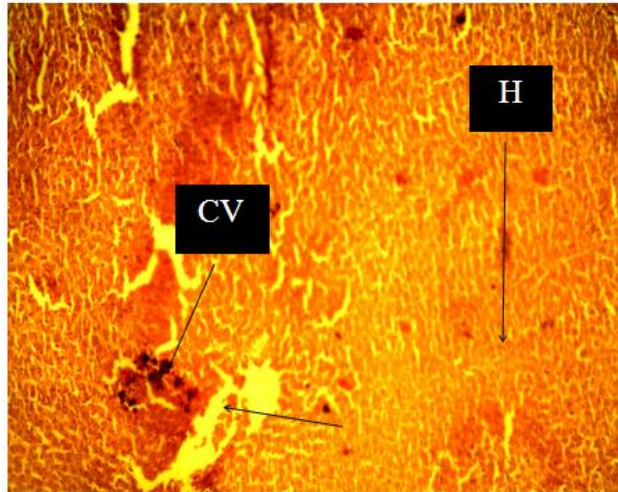


Plate 2. Photomicrograph of sections of liver administered with 250 mg/kg b.w. herbal mixture. H and E, x100. CV = Central vein, H =Hepatocytes

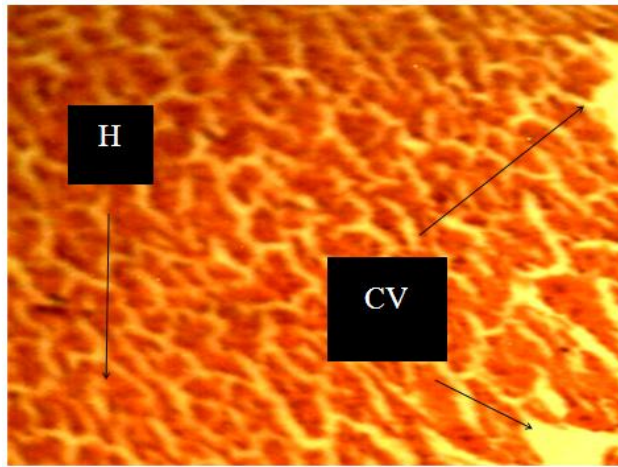


Plate 3. Photomicrograph of sections of liver administered with 500 mg/kg b.w. herbal mixture. H and E, x100. CV = Central vein, H =Hepatocytes

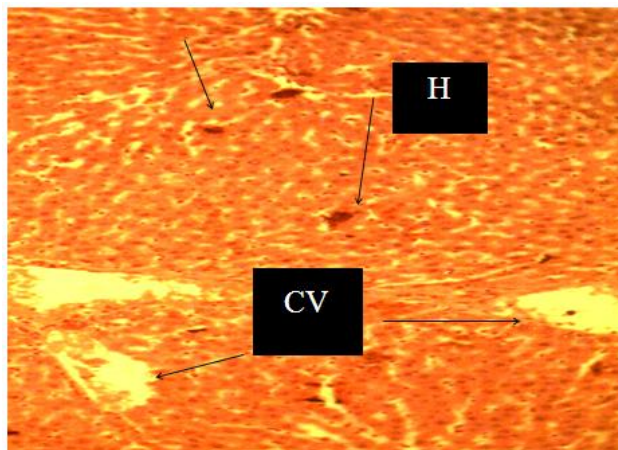
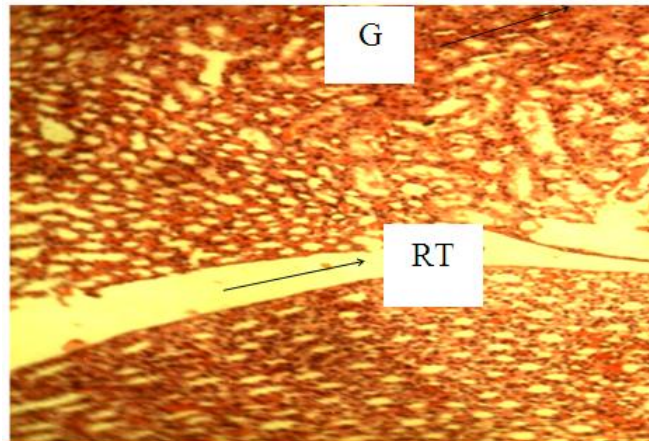
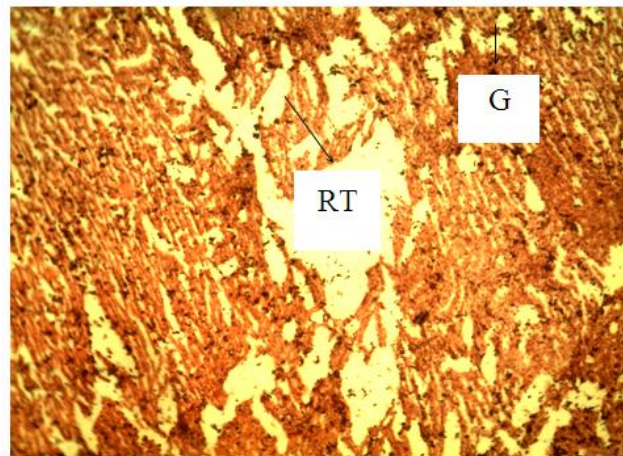


Plate 4. Photomicrograph of sections of liver administered with 1000 mg/kg b.w. herbal mixture. H and E, x100. CV = Central vein, H =Hepatocytes

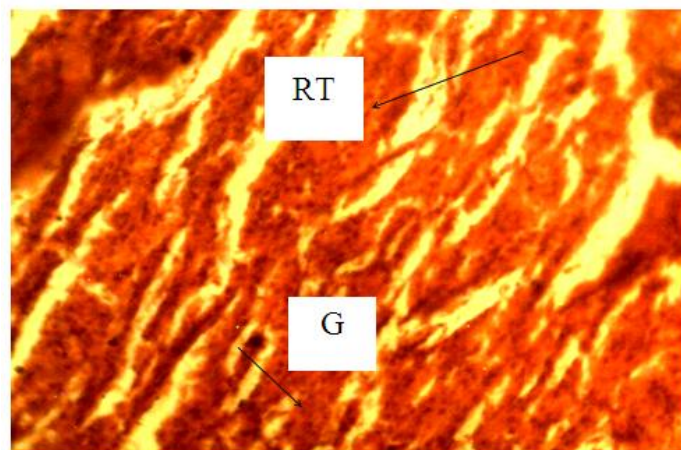




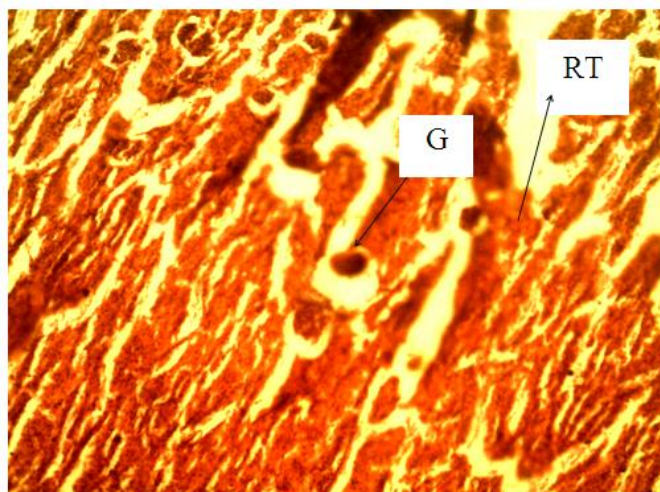
**Plate 5. Photomicrograph of sections of kidney administered with 5 ml/kg b.w. normal saline. H and E, x100. G = Glomerulus, RT = Renal tubule**



**Plate 6. Photomicrograph of sections of kidney administered with 250 mg/kg b.w. herbal mixture. H and E, x100. G = Glomerulus, RT = Renal tubule**



**Plate 7. Photomicrograph of sections of kidney administered with 500 mg/kg b.w. herbal mixture. H and E, x100. G = Glomerulus, RT = Renal tubule**



**Plate 8. Photomicrograph of sections of kidney administered with 1000 mg/kg b.w. herbal mixture. H and E, x100. G = Glomerulus, RT = Renal tubule**

#### 4. DISCUSSION

Polyherbal formulations are receiving greater attention as alternatives to clinical therapy resulting in an increase in their demands [28]. On preliminary phytochemical screening, *Yagari* divulged the presence of phenols, tannins, alkaloids, steroids, saponins, flavonoids, terpenoids, glycosides and reducing sugars. Their relative abundance was alkaloids ( $2333 \pm 0.006$  mg/g), flavonoids ( $883.949 \pm 0.020$  mg/g), phenols ( $1740.645 \pm 0.004$  mg/g), tannins ( $788.420 \pm 0.001$  mg/g), Terpenoids ( $2177.030 \pm 0.006$  mg/g) and Saponins ( $63.230 \pm 0.002$  mg/g).

Secondary metabolites are basic sources of enormous pharmaceutical agents [29]. Phenolics which include flavonoids, tannins, phenolic acids, stilbenes and coumarins [30], account for one of the major groups of compounds acting as anti-clotting agents, antioxidants, immune enhancers and hormone modulators [29,30]. They possess other biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [31-34]. Flavonoids in *Yagari* might be part of the active anti-inflammatory activities of the herbal mixture. Studies have shown that flavonoids have anti-inflammatory activities and are powerful antioxidants [35,36]. The anti-inflammatory activity of flavonoids is mediated by inhibition of arachidonic acid-metabolizing

enzymes (cyclooxygenase and lipoxygenase) as well as by anti-oxidative properties [37,38]. This is an indication that *Yagari* could be effective in treating inflammation and scavenging radicals.

In same vein, relatively high tannin content in *yagari* ( $788.420 \pm 0.010$  mg/g) could endow the mixture with medicinal properties such as anti-inflammatory, antimicrobial and astringent activities of the herbal mixture [39-41].

*Yagari* was also shown to hold in saponins which are known to produce suppressive effect on inflammation, free radical scavenging and cholesterol reducing activity [42]. Saponins also serve as anti-cancer agents and as antioxidants [43]. They stimulate antibody production and induce the production of lymphocytes [44]. Terpenoids as a group have anti-viral, anti-malaria, anti-inflammatory and anti-cancer [45]. They also inhibit cholesterol synthesis; reduce diastolic blood pressure and blood glucose level [46]. Steroids have been reported to have antibacterial properties [47] and they are very important compounds especially due to their relationship with compounds such as sex hormones [43]. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [48]. Several workers have reported the analgesic, antispasmodic, antibacterial, antifungal, anti-inflammatory, anti-fibrogenic effects of alkaloids [44,49]. Alkaloids also act as central nervous system stimulant [48]. Glycosides are known to lower the blood pressure according to reports [50].

The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents of *Yagari*. Thus, *Yagari* is likely to be valuable reservoir of bioactive compounds of substantial medicinal merit.

Persistent nature of some diseases/disorders justifies lasting therapy [6]. Herbs and natural plants products consumption for treatment of long term disease conditions requires mindfulness of a possible harmful aftermath [6]. Reports of illnesses and fatalities over the use of medicinal plants have questioned the chemistry and pharmacology of herbal medicine [51]. It is therefore apt to evaluate scientifically the toxicological profiles of herbs and herbal products. In this study, we evaluated the acute and sub- chronic toxicity of *Yagari* using mice and Wistar albino rats respectively.

From the acute toxicity study, it was evinced that *Yagari* has no noticeable adverse effect throughout the duration of the experiment at all the doses used, which is an expression that *Yagari* was safe. Similarly, in the evaluations of sub- chronic toxicity, *Yagari* administered at doses of 250, 500 and 1000 mg/kg for twenty-eight (28) day did not reflect any observable alteration in the eating and drinking patterns of the rats.

Nevertheless, body weight which is one of the analytical indices for the assessment of primary signs of toxicity [52] increased in all the groups but was more elevated in the group treated with 500 mg/kg of *Yagari*. This may be ascribed to the normal growth of the rats with age or to enhanced feeding pattern by the rats. Reduction in body weight is considered a sensitive index of toxicity after exposure to toxic substance. In this study, the effect of *Yagari* showed an increase in body weight, thereby demonstrating that *Yagari* is not toxic when consumed sub-chronically.

*Yagari* decreased fasting blood sugar levels in dose-dependent manner, demonstrated the presence of hypoglycaemic agents [53]. The antihyperglycemic effect may be due to the phytochemical constituents like alkaloids, glycosides and flavonoids present in *Yagari* [54]. Flavonoids have been reported to possess hypoglycaemic agents [55-57]. Kaempferol, a flavonoid has been reported to decreased plasma glucose level and increase the insulin levels in streptozotocin- diabetic rats after 45 days of treatment [56]. They can also potentially

ameliorate glycoprotein abnormalities related to the risk of diabetes mellitus [56,57]. The antidiabetic activity of flavonoids depends on the chemical criterion (C-2-C-3 double bond and ketonic group at C-4 position on ring B) which is fundamental for the bioactivity of poly-phenol compounds [58]. Flavonoids suppress the blood glucose level by enhancing insulin release from pancreatic islets [57]. Saponins stimulate the release of insulin and block the formation of glucose in the bloodstream [59]. Alkaloids inhibit  $\alpha$ -glucosidase and decrease glucose transport through the intestinal epithelium [54,60]. These phytomedicines play significant role in maintaining blood glucose levels, glucose uptake, insulin secretory and immuno modulatory functions to prevent specific diabetes mellitus.

Study of the hematological variables like haemoglobin (Hb) concentration, Pack Cell Volume (PCV), Red Blood Cell (RBC) and White Blood Cell (WBC) count proffer useful information about the haematological toxicities [61]. *Yagari* at the dose levels of 250 and 500 mg/kg b.w produced no significant changes in the hematological parameters. However, at 1000 mg/kg body weight, *Yagari* produced a significant ( $p < 0.05$ ) increase in the levels of RBC and a decrease in WBC when compared to the controls. The marked increase observed in RBC, Hb and PCV demonstrated the hematinic potential of *Yagari*. The increase in RBC was an indication of changes in the rate of production of red blood cell. The significant increase in number erythrocytes and hematocrit counts after oral administration of *Yagari* suggests that *Yagari* may contain phytochemicals and compounds that stimulate the secretion or formation of erythropoietin in the stem cells of normal rats. Stimulation of stem cells in the bone marrow to produce red blood cells occurs due to the action of erythropoietin which is a glycoprotein hormone [62]. It affects the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and hemoglobin are very important in transferring respiratory gases [63]. It may also suggest that *Yagari* can cause polycythemia which is in agreement with previous studies that indicated that an increase in the count of erythrocytes and PCV is suggestive of polycythemia and positive erythropoiesis [64,65]. In addition previous studies by Esenowo et al. [66] showed that the leaves of *Peristrophe bicalculata* (Retz) are capable of improving erythrocyte counts in experimental animals. They confirmed the use of *Peristrophe bicalculata* (Retz) leaves in restoring

lost blood during excessive bleeding. In his studies, Okon et al. [67] worked on *Baphia nitida* (Lodd) also reported similar results. Therefore, *Yagari* can be used to restore lost blood during excessive bleeding.

Wambi et al. [68] suggested that the mechanism leading to the increase in erythrocyte count is probably mediated by the presence of antioxidant phytochemicals such as terpenoids and tannins. The presence of antioxidant phytochemicals may be responsible for the haemopoietic stimulating effects. This agrees with previous studies that showed that prophylactic and therapeutic oral administration of antioxidant supplements in plant extracts significantly increased cells of hemopoietic origin in animals exposed to potentially lethal doses of radiation. Erythrocytes have also been found to be protected from oxidative damage by flavonoids, tannins and terpenes [62].

In this context, the possibility that *Yagari* does have the potential to stimulate erythropoietin release in the kidney was likely. This is similar to results obtained with some other plants [69]. The white blood cells (WBC) or leucocytes generally participate in body defence against invading bacteria, viral and parasitic organisms but each is kinetically and functionally independent. WBC shows increased concentration in response to toxic environment. In this study, WBC was not significantly altered. The findings from this investigation provided more information on the therapeutic safety of *Yagari*.

Levels of serum liver biomarker enzymes are biochemical parameters usually performed in order to evaluate toxic effects on the liver [70]. AST and ALT are common liver enzymes because of their higher concentrations in hepatocytes, but only ALT is remarkably specific for liver function [71]. Therefore an elevation in serum concentration of ALT is an indication of liver damage. AST is mostly present in the myocardium, skeletal muscle, brain and kidneys [72]. Thus, the liver and heart release AST and ALT and an elevation in plasma concentration are indicative of liver and heart damage [71]. ALP is present mostly in cells lining the biliary duct of the liver and is used to diagnose obstruction to the biliary system. Therefore, its elevation in the blood indicates cholestatic diseases such as gall stone or the presence of a tumor, blocking the bile duct [73]. In this study, chronic exposure of rats to *Yagari* at graded doses (250, 500 and 1000 mg/kg body weight)

did not cause significant increase in AST, ALT and ALP values when compared to the control group, rather there was a significant decrease of ALT and ALP activities at 1000 mg/kg b.w. This might be an indication that the herbal mixture is not toxic to the liver when consumed over a long term. Aside the liver, this indicates that the herbal mixture does not have nephrotoxic effects on the heart when consumed sub-chronically. This is further confirmed in the histopathological observations. Flavonoids are hepatoprotectives [74]. The observed hepatoprotective activity of *yagari* may be due to the presence of flavonoids.

The mechanism of action of flavonoids involves the suppression of the formation of a wide range of reactive oxygen, nitrogen and chlorine species by the inhibition of enzymes or by chelating trace elements involved in the production of free radical that is responsible for injury to the liver, whether acute or chronic [57,75]. The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years [76].

Bilirubin is a break down product of hemoglobin. An elevation in serum bilirubin level could be attributed to three major causes such as hemolysis, biliary obstruction and liver cell necrosis [77]. In this study there was no significant decrease in the levels of serum bilirubin in the treated group when compared to control rats. This suggests that *Yagari* may have no toxic effect on the erythropoietic system as was confirmed by the hematological parameters. *Yagari* perhaps protected the liver by enhancing bilirubin uptake and conjugation by the liver and subsequent secretion into the bile ducts.

*Yagari* administration of the different doses used did not produce significant changes in the serum total cholesterol and HDL after 28 days. *Yagari* at 1000 mg/kg, however, produced a significant decrease in serum TAG. The non-significant reduction in total serum cholesterol observed with the administration of the *Yagari* when compared to normal control could be attributed to the inhibition of endogenous synthesis of lipids probably by its antioxidant properties. The reduction in total cholesterol observed at the administration of *Yagari* may be attributed to the saponins content in *Yagari*. Saponins have been reported to reduce the uptake of cholesterol in the body by their interaction and binding with bile acids, thereby lowering the body's overall cholesterol levels [78]. The reduction in serum triacylglycerol levels may involve inhibition of the

lipolytic enzymes, hepatic triacylglycerol lipase and lipoprotein lipase. The reduction of the activity of these enzymes may lead to decreased removal of triacylglycerol from serum and the accumulation of triacylglycerol in the tissues. Observed reduction in the levels of triacylglycerol in serum of rats treated with *Yagari* demonstrates the beneficial effect of *Yagari*.

The observed increase in HDL concentration could be due to the fact that the antioxidant compounds present in *Yagari* increased the activity of lecithin cholesterol acyltransferase (LCAT) activity. The activity of LCAT may increase the transport of cholesterol esters from peripheral tissue to liver and stimulate the production and secretion of HDL for circulation [79]. According to Sani et al. [43] saponins also help in maintaining levels of high HDL.

Estimation of the activity of catalase and superoxide dismutase (SOD) and the concentration of malondialdehyde (MDA) were used as indices to study the effect of the herbal mixture on the oxidant- antioxidant balance of the rats. A decrease in the levels of catalase and SOD activities and increases in the concentration of MDA show an increased oxidative stress and reduced antioxidant activities in the system, which lessens the potentiality of the body to get rid of free radicals [6]. In this study, there was a remarkable increase in the activities of SOD and CAT at the doses of 500 and 1000 mg/kg b.w in *Yagari* treated rats. Nonetheless, *Yagari* administered did not cause consequential changes in the concentration of MDA when compared to the control. This suggests that the herbal mixture when consumed chronically might not induce oxidative stress.

This could be attributed to high phenolic content. The free radical-scavenging potential of phenol appears to depend on the OH groups found on the phenolic ring which serves as electron/hydrogen donor [80]. In addition, Anoop and Bindu [81] reported that flavonoids are capable of inhibiting the expression and activation of radical generating enzymes such as nitric oxide synthase and propensity to quench these free radicals as indicated.

## 5. CONCLUSION

The results underpin our hypothesis, indicating the absence of dose dependent adverse effects or harmful aftermath in the use of *Yagari*, following the acute and sub-chronic toxicity

evaluations of the herbal mixture. Hence, *Yagari* is a safe polyherbal formulation. However, further research with a large number of animals is principally necessary to denote safety and efficacy of the herbal mixture.

## 6. SIGNIFICANCE STATEMENT

This study discovered the presence of hypoglycaemic agents and positive erythropoiesis in *Yagari* that can be beneficial for the management of high blood sugar levels and to restore lost blood during excessive bleeding respectively. The study will help the researchers to uncover the critical areas of toxicology that many researchers were not able to explore. Thus a new theory on establishing safety and efficacy of herbal mixture may be arrived at.

## ETHICAL APPROVAL

All experimental protocols including the use of animal models were approved and followed the guidelines of the Faculty of Biological Sciences Ethical Committee of the University of Nigeria, Nsukka, Nigeria, with the Ethical Approval Number; UNN/FBS/2016-0623.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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