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Research Article
Assessing the Haematological/Chemical Pathological Properties of a Polyherbal Formulation on Liver Fibrosis

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Abstract

Background and Objective: Hepatic fibrosis is a wound healing response to insults and as such affects the entire world population. In industrialized countries, the main causes of liver fibrosis include alcohol abuse, non-alcoholic steatohepatitis and chronic hepatitis virus infection which accounts for about 80% of liver fibrosis. A common denominator that is central to liver fibrosis is the activation of hepatic stellate cells, which is triggered by a plethora of signaling pathways. Liver fibrosis can progress into more severe stages, known as cirrhosis, when liver acini are substituted by nodules and further to hepatocellular carcinoma when the architecture of the liver is disfigured. This study was carried out to assess the hematological/chemical pathological and HBV DNA indices of a fibrosis liver in order to determine the efficacy of the extemporaneous polyherbal formulation. **Materials and Methods:** The safety and pharmacological properties of extemporaneous polyherbal preparation was evaluated. Acute toxicity study using Wistar rats revealed that oral administration of aqueous extract of homogenized herbal preparation of *Allium sativum*, *Zingiber officinale*, *Curcuma longa* and *Moringa Oleifera* was non-toxic and LD_{50} was found to be above 5000 mg kg^{-1}. Microbiological examination showed that these herbal products were free of microbial contamination. Sequel to the National Agency for Food and Drug and Administration and Control (NAFDAC) guidelines on the use of herbal preparations in Nigeria, five consenting adults were given an extemporaneous herbal preparation (2000 mg kg^{-1}) in divided dose 12 h for 3 months. The data obtained were analyzed using descriptive statistics, chi square test and independent t-test. **Results:** Results obtained from descriptive statistics, chi square test and independent t-test showed that there was a significant ($p<0.05$) improvement in aspartate aminotransferase (AST) to platelet ratio index (APRI) and hepatitis B (HBV) DNA values which were close to normal in most of the participants. **Conclusion:** It can succinctly be inferred that non-orthodox medications that are non-toxic and with suitable dosage forms and stability can be an alternative for the management of liver fibrosis.

Key words: Liver fibrosis, NAFDAC, herbal product, Hepatitis B, APRI, organoleptic property

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The liver is the largest gland and the second largest organ of the body, lying beneath the diaphragm in the right hypochondrium and upper part of the epigastric region[1]. The liver plays pivotal roles in metabolism of drug and xenobiotics, protein synthesis and in maintaining biologic equilibrium of organisms[1]. Hepatitis is a disease of the liver and is a serious public health issue[2]. Hepatitis is caused by viruses and these include; hepatitis A, B, C, D and E, respectively. Hepatitis G virus (a flavivirus) was recently identified and found structurally related to hepatitis C virus[3- 5]. Hepatitis can also arise from the inflammation of the liver caused by toxic substances or immunological abnormalities[6]. The prevalence of chronic hepatitis B infection is highest in Africa, Western Pacific and Asian countries where the virus is acquired mainly through perinatal transmission from the chronically infected mother[7].

Development of novel treatment to combat incidence of hepatitis is still a challenge particularly in developing countries where the prevalence of this disease is still very high. Complementary and alternative (herbal) preparations could prevent the staggering percentage of deaths each year from chronic hepatitis induced-liver fibrosis[8,9].

Preventing side-effects arising from the use of orthodox medications needs a global attention and resources must be channeled to tropical and sub-tropical African countries where the burden of the disease is greatest. One of such ways is to return to the folklore medicine or development of naturaceuticals, variously christened; herbal medicine, natural products, complementary and alternative medicine (CAM) or even traditional medicine[10].

An estimated 354.2 million adult's visit to practitioners of CAM every year in the United States and their cost represents $ 33.9 billion in 2007[11]. It was believed to have exceeded the number of visits to all primary healthcare physicians. Herbal therapy has been used extensively in Nigeria.

In Nigeria, herbal medicine products that are considered for registration or listing may be categorized as:

- Herbal medicinal products manufactured locally
- Imported herbal medicinal products
- Homeopathic herbal medicinal products

However, these guidelines do not apply to extemporaneous preparations. This means preparations that are made by the practitioner and given to the patient on a one-to-one within the locality of its preparation[12].

The hall mark of the documentation for registration or listing among other things includes a comprehensive certificate of analysis which encompasses the acute toxicity test, comparison of organoleptic properties and microbiological analysis[12].

Although more than 80% of the people in both the underdeveloped and the developed countries depend largely on herbal medicines for their medical needs[13] the major problem with herbal medicines in such countries still remains their poor and sometimes unhygienic presentation. A major aspect of the standardization process includes the assessment of the efficacy and safety, as well as development of suitable dosage forms and stability for this herbal medicines[14]. The extemporaneous polyherbal preparation investigated in the present study are used for various ethno-medicinal purposes including the management of liver fibrosis. The constituents of this extemporaneous herbal preparation include; *Allium sativum*, *Zingiber officinale*, *Curcuma longa* and *Moringa oleifera,* respectively. The medicinal uses of the constituents of this extemporaneous herbal preparation are well documented[15, 16].

Allium sativum (garlic) is used to treat a number of ailments and several studies have confirmed its therapeutic properties[17]. Garlic is rich in a variety of sulfur-containing compounds, including thiosulfinates, sulfoxides, dithins of which allicin and ajoene have been shown to have potent health-promoting effects[18].

Zingiber officinale Roscoe (Ginger) belongs to the family Zingiberaceae and has been used as a spice for over thousand years[15]. Its roots contain polyphenol compounds (6-Gingerol and shogaols) which have a high antioxidant activity[19,20]; Habib *et al.*[21] evaluated its antidiabetic, antihyperlipidimic and hepatic anticancer effect.

Curcuma longa (Turmeric) is a rhizomatous perennial herb that belongs to the family Zingiberaceae, native to South Asia and is commonly known as turmeric. Turmeric contains a mixture of phenolic compounds called curcumin and a volatile oil with tumerone and zingiberene, cineole and other monoterpenes; starch; protein and high amounts of vitamin A and other vitamins. The essential oil has potent stimulating effects on gall bladder (perhaps due to the p-tolylmethyl carbon) and also stimulates the liver to produce more bile and regulate it viscosity. Modern research also shows that the herb possesses anti-inflammatory and strong liver-protecting properties. Phansawan and Poungbangpho[22] and Sengupta *et al.*[23] reported its hepatoprotective activity against carbon tetrachloride toxicity.

Moringa oleifera (Moringa) leaves have been used in the treatment of liver disease. *M. oleifera* contains alkaloids, flavonoids, anthocyanins, proanthocyanidins, cinnamates, methionine; phenylalanine and threonine which enhance liver

production of lecithin, reduction of hunger pains, increases body metabolism and thwarts fat build up in the liver[24].

Identifying persons with cirrhosis or advanced CHB in need of treatment was generally based on the combined assessment of clinical features (including hepatomegaly and splenomegaly), the level and ratio of aminotransferases and other relevant tests, such as albumin and platelet counts, HBV DNA viral load, the degree of fibrosis and/or necro-inflammation on liver biopsy or NITs and liver imaging[2]. Liver biopsy was considered the gold standard method to stage liver disease and assess the degree of fibrosis but it was not widely used in resource-limited settings because of its high cost, invasiveness, patient discomfort, risk of complications, sampling error, as well as the need for expert histological interpretation[25].

Several non-invasive fibrosis tests based on blood or serum indices (APRI, FIB-4 and a commercial assay-fibro test,) or ultrasound principles are now available and increasingly used for evaluating and staging liver fibrosis, which reduces the need for liver biopsy in persons with an established cause of liver disease. The use of accurate and validated NITs in resource-limited settings could help with the optimal selection of persons with CHB for antiviral therapy[25].

This study was carried out to assess the hematological/chemical pathological and HBV DNA indices of a fibrous liver in order to determine the efficacy of the extemporaneous polyherbal formulation.

MATERIALS AND METHODS

Sample collection and preparation: Plant materials from garlic, ginger, turmeric and Moringa were obtained from the open herbal market in Abuja, Maiduguri and Kano, Nigeria and appropriately identified as *Allium sativum*, *Zingiber officinale*, *Curcuma longa* and *Moringa Oleifera*, respectively in the Biological Sciences Department, University of Agriculture, Makurdi. The cloves, leaves and rhizomes of *A. sativum, M.Oleifera, Z. officinale* and *C. longa,* respectively were chopped into smaller pieces and properly air dried at room temperature for 3 weeks. The dried plant parts were pulverized separately using high capacity grinding machine, preserved in air tight container and sterilized at 180EC for 10 min[26]. Two grams of the extemporaneous herbal preparation was weighed and boiled in 100 mL distilled water for 30 min. The mixture was filtered; the filtrate was concentrated in-vacuo in water bath to yield an extract. The powdered samples were combined at different proportions to form the polyherbal preparation. The polyherbal preparations were then subjected to acute toxicity test. The experiment was carried out at the Medical Laboratory Science Department and Department of Pharmaceutical Microbiology, University of Calabar Teaching Hospital (UCTH) and University of Ibadan, Nigeria respectively between April and November, 2016.

Toxicological studies

Laboratory animals: Wistar rats (160-200 g) of either sex were acclimatized to laboratory conditions in the animal facility of the Biochemistry Science Department of University of Calabar. The rats were housed in wooden cages in a well-ventilated room (25±5°C), fed with standard rodent feed and allowed free access to drinking water. All experiments were carried out after ethical clearance was obtained from the ethics committee of the University of Calabar.

Acute toxicity test and stability test: The acute toxicity (LD_{50}) study of the extemporaneous herbal preparation was carried out using the method described by Lorke[27] with slight modifications. The study was carried out in two phases. In the first phase of the study, nine rats were randomly divided into three groups with three rats each. The poly-herbal extract was then orally administered (via intra-gastric cannula) to the rats at concentrations of 10, 100 and 1000 mg kg^{-1} b.wt. The rats were observed for the first 4 h after dosing and subsequently for 72 h for signs of toxicity. The following humane endpoints were used weight loss, hair loss and increase in rectal temperature, swellings and bleeding.

Based on the result of phase I, the concentration was varied to 1600, 2900 and 5000 mg kg^{-1} b.wt. and administered to another fresh set of three groups of three rats each. The rats were observed for signs of toxicity and mortality for the first 4 h and subsequently daily for 7 days. Thereafter, the animals were euthanized and the liver harvested and put in a coupling jar containing 10% formalin to preserve the architecture and to maintain the integrity of the organ. The selected pieces of tissue were processed into paraffin wax, sectioned at 5 µm and stained by the H and E method and Perl's Prussian blue method. The stained slides were examined under the microscope and photomicrographs needed were taken.

Encapsulation of polyherbal preparation: Weight and content uniformity were carried out using ten capsules selected randomly from the supplied batch and they were weighed individually and collectively on a digital weighing balance (Weight South WBT-100). The contents of each capsule were simultaneously determined. The mean weights and coefficient of variations were then calculated as specified in the European Pharmacopoeia[28].

The disintegration time of the capsules was determined as specified in the British Pharmacopoeia[29] using an Erweka ZT×20 series manual disintegration tester (Erweka, Germany). Three media (distilled water, potassium and sodium chloride) simulating three pH conditions (7.0, 7.2 and 7.5, respectively) of the gastrointestinal tract were used.

The extemporaneous herbal preparation was thermally degraded using Linkam TP 92, HFS 91/Hot stage plate with platinum resistor. The setup included a small aluminum dish in which the extemporaneous herbal powder was kept on the silver block in the hot stage. The herbal preparation for the study were heated at different temperatures ranging from 30-120°C with an increment of 10°C for a period of 1-6 h with an increment of 1 h at each temperature. Besides this, a fresh sample of the preparation was used at each temperature and retention time. Then, the thermally treated samples were cooled down to room temperature before measurements. The heating and cooling rate of the hot stage was maintained at 10°C min^{-1}.

Other parameters examined were pH, solubility, humidity, light, oxygen, moisture and other organoleptic properties.

Ash values: The total ash value was determined from air-dried samples using the procedure described by Khandelwal[30].

The total ash, acid insoluble ash and water-soluble ash values were determined from air-dried samples using the procedure described in the Indian Pharmacopeia (IP).

Procedure: About 2 g of powdered drug was weighed accurately into a tarred silica crucible, incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed.

Percentage of total ash was calculated using the following equation as described by Khandelwal[30].

Percentage total ash = Total ash value of the sample = (Z-X)/Y×100

Where:

X = Weight of empty dish
Y = Weight of the drug taken
Z = Weight of the dish+ash (after complete incineration)

Microbial load determination

Fungal load: For fungal load, 2 g of the well-blended polyherbal preparations were sterilized by 2% sodium hypochlorite solution for 10 min and washed 3 times with sterilized distilled water, then placed on petri dishes containing potato dextrose agar (PDA, HiMedia) incorporated with 0.5 mg chloramphenicol/mL (HIMEDIA) for suppressing the bacterial growth. These plates were incubated at 25°C for 7 days and samples were examined daily after 4th day for fungal growth.

Bacterial load: WHO guidelines[31] were used for the assessment of bacterial load and their identity was confirmed with different biochemical methods described in the aforesaid document.

Total aerobic count and coliform count were determined using most probable number (MPN) technique on soybean casein digest medium (HIMEDIA) and *Enterobacteriaceae* enrichment broth (HIMEDIA), respectively. The MPN is given by the root of the following equation:

$$nv = \frac{sv}{1 - \exp(-v\delta)}$$

Where:

sv = Small volume of the powdered preparation
nv = Number of small volume
exp = Exponential
vδ = Density of the sample

For *Escherichia coli*, homogenized polyherbal preparation was transferred to MacConkey broth, incubated at 45°C for 24 h. Broth was then transferred to MacConkey agar (HIMEDIA), incubated at 45°C for 24 h. Presence of *E. coli* was confirmed by Indole test using Kovac's reagent.

For *Salmonella*, homogenized polyherbal preparation was transferred to tetrathionate bile brilliant green broth (HIMEDIA), incubated at 45°C for 24 h, broth was then transferred to plates of deoxycholate citrate agar (HIMEDIA), xylose, lysine deoxycholate agar (HIMEDIA) and brilliant green agar (HIMEDIA) incubated at 37°C for 48 h. The positive plates were sub cultured on triple sugar iron agar (HIMEDIA) slants using a deep inoculation technique for identification.

For *Pseudomonas aeruginosa*, the homogenized pretreated polyherbal preparation was transferred to soybean-casein digest medium (HIMEDIA) incubated at 37°C for 48 h, then placed on cetrimide agar (HIMEDIA) and incubated at 37°C for 48 h. Presence of *P. aeruginosa* was confirmed by oxidase test using N, N, N, N-Tetra methyl-phenylenediamine dichloride (HIMEDIA).

Enrolment of study participant for treatment with polyherbal formulation: A total of five volunteers between the ages of 21 and 63 were enrolled in the current study. The

inclusion and exclusion criteria for enrolment and eligibility for treatments were based on the positive outcomes of the following test conducted on each individual.

Brief history of participant A: An apparently healthy 63 years old male participant residing in Calabar presented with HBsAg seropositive with no corresponding antibody after observing some ache in the lower abdomen. Further tests which included HBV DNA, liver function test and the complete blood count were carried out as specified by Elitech Chemistry Analyzer, Coulter AcT2 Hematology Analyzer and Flex Cycler Standard PCR manuals in order to determine the eligibility for treatment using the noninvasive test (NIT).

Brief history of participant B: The following tests were conducted, liver function test, complete blood count, HBsAg/HCVab as specified by Elitech Chemistry Analyzer, Coulter AcT2 Hematology Analyzer and Flex Cycler Standard PCR manuals on an apparently jaundice 25 years old female prison inmate with a palpable discoloration of the sclera, fingertips, tongue and skin.

Brief history of participant C: A 35 years old businessman had an observable increase in vein visibility and bruises went for a general medical checkup and he tested positive to HBsAg. However, further tests including liver function test (LFT), complete blood count (CBC) and HBV DNA were conducted as specified by Elitech Chemistry Analyzer, Coulter AcT2 Hematology Analyzer and Flex Cycler Standard PCR manuals to determine his eligibility for treatment with the polyherbal formulation.

Brief history of participant D: A university Professor who also works as a pastor with a known case of portal hypertension went for an evangelism in a remote village in the vicinity of the campus, when suddenly a call came in requesting for a blood donor to save a woman who was in labour and in dire need of blood. The professor decided to volunteer and after the preliminary screening he was found to be positive to HBsAg. The participant came to the university teaching hospital and his positive status was confirmed. The participant however went for HBV DNA in addition to the LFT and the CBC as specified by Elitech Chemistry Analyzer, Coulter AcT2 Hematology Analyzer and Flex Cycler Standard PCR manufacturers' specifications.

Brief history of participant E: A 21 years old midwifery student, a serial blood donor decided to be screened after a recurrent constipation and diarrhea. The result came out positive to HBsAg. The participant immediately went for LFT and CBC, quantitative HBV DNA as specified by Elitech Chemistry Analyzer, Coulter AcT2 Hematology Analyzer and Flex Cycler Standard PCR manufacturers' specifications.

Ethical consideration: The acute toxicity test and the stability test were carried out to ascertain that the extemporaneous herbal preparation is not harmful for human consumption and the organoleptic properties were stable for a relatively length of time. This was the core requirement by the National Agency for food and Drug Administration and Control (NAFDAC) before any herbal preparation can be consumed in Nigeria[12].

Ethical approval was sought from the state Ministry of Health before embarking on the research work (CRS/MH/HREC/016/VOL.V/214). Informed consent was sought from each respondent before the herbal preparations were administered. The privacy, dignity and autonomy of the respondents were maintained accordingly throughout the conduct of the study. Those found positive for HBsAg were initially tested with the five panel ELISA test kit to determine the replicability or otherwise of the virus before carrying out the APRI and HBV DNA.

Statistical analysis: The data obtained were subjected to descriptive statistics (using SPSS version 24) such as frequency distribution, percentages and means and corresponding standard deviations, inferential statistics such as chi-square test for testing the association between categorical variables and independent t-test for examining differences between group means of categorical and continuous data variables. The statistical significance of all results was considered when the p value is <0.05[32].

RESULTS

Acute toxicity test and stability test: From the result of the acute toxicity test, the animals did not show any sign of toxicity and mortality at all dose l levels. This was evident in the palpable normal architectural presentation of the liver as evident in the normal canaliculi, central vein and the lobules under the microscope (Fig. 1 and 2)

Evaluation of microbial contamination in polyherbal preparation: The polyherbal preparation used in the present study had fungi and total aerobic bacteria loads below the WHO[31] permissible limits for non-sterile dosage form (Table 1). A further screening using selective media revealed that all the specific bacterial such as *E. coli, Salmonella* spp. and *P. aeruginosa* were absent.

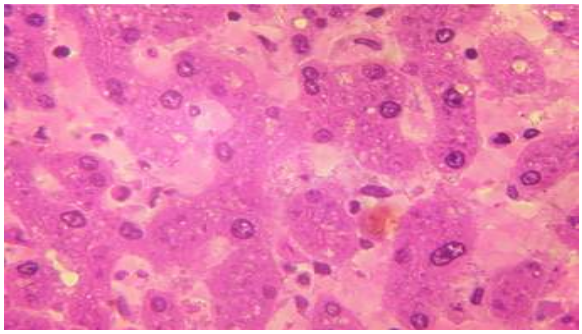

Fig. 1: Normal liver (H and E, 40X) with well differentiated canaliculi, central vein and lobules

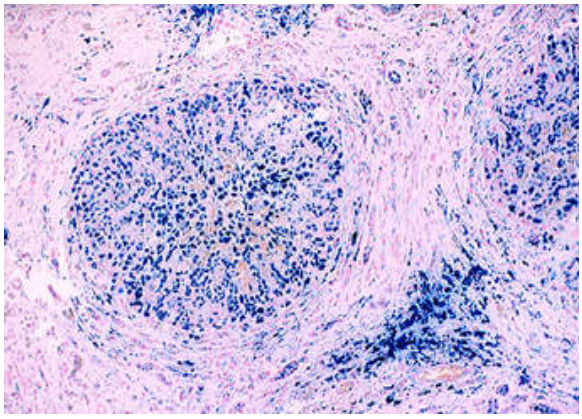

Fig. 2: Normal liver (Perl's Prussian Blue, 40X)

Table 1: Microbial load of the extemporaneous herbal preparation

Organism	Count (CFU g^{-1})	WHO[31] Limit (CFU g^{-1})
Fungi	40	10^4
Total aerobic bacteria	10	10^3
Coliforms	ND	50
Escherichia coli	ND	10^2
Salmonella spp.	ND	10^2
Pseudomonas aeruginosa	ND	10^1

CFU: Colony forming unit, ND: Not detected

Encapsulation parameters: No changes were observed in all the organoleptic properties of the polyherbal product for the period of 3 months (Table 2). The pH of the product was constant throughout the period of 3 months. In terms of solubility, the product was soluble after 3 months. There were no significant changes in thermal stability of the polyherbal product after three months. Analysis of the ash content of the polyherbal product showed no significant loss in drying. All the properties evaluated for the capsules used in this study were relatively stable for the period of three months (Table 2).

The summary results for participant A are presented in Table 3. The participant was administered 2000 mg of the herbal capsules in divided doses, 12 h for 3 months. There was a significant decrease in the APRI score and the HBV DNA after 3 months of treatment with the herbal product. After 3 months of treatments with herbal product, participant A showed a positive improvement in almost all the parameters tested which fall below the normal range. The exceptions were direct bilirubin and APRI which were above the normal range.

Table 2: Organoleptic properties and other parameters of the extemporaneous herbal preparation

	Dates	
Parameters	15-04-2016	14-07-2016
Color	Light brown	Light brown
Odor	Vegetable	Vegetable
Taste	Mild bitter	Mild bitter
Texture	Smooth	Smooth
pH		
Water	7.03	7.03
Buffer (7.4)	7.30	7.30
Solubility		
Water	Slightly soluble	Slightly soluble
Buffer (7.4)	Sparingly soluble	Sparingly soluble
0.1N HCl	Sparingly soluble	Sparingly soluble
UV spectrum (nm)		
Water	304	305
Buffer (7.4)	305	306
0.1N HCl	304	305
0.1N NaOH	305	306
Thermal stability		
To (°C)	54.3	55.3
Tp (°C)	61.1	62.1
Te (°C)	67.9	66.1
ΔH (J g^{-1}×K)	0.996	0.996
Ash values		
Ash content (% w/w)	1.55	1.55 (Max. 2.0%)
Loss on drying (% w/w)	5.5	6.0 (Max. 15%)
Acid insoluble ash (% w/w)	0.02	0.02 (Max. 0.1%)
Capsule properties		
Weight variation (%± SD)	0.02±0.02	0.02±0.02
Content uniformity (mg±SD)	0.11±0.10	0.11±0.10
Disintegration time (Sec± SD)	3.49±0.01	3.49±0.01

There was a significant decrease in the value of HBV DNA of participant B after 3 months of the treatment (Table 4). The participant had more than 90% unlikely to develop fibrosis going by the APRI score.

It can succinctly be said that participant C was likely to have severe fibrosis or cirrhosis going by the varying APRI score greater than 1.5 and the palpable value of the HBV DNA post treatment (Table 5).

The participant D exhibited significant improvement in all the parameters screened post treatment most of them were below the normal range (Table 6). The significant drops in the values of APRI and the HBV DNA post treatment makes the participant D very unlikely to develop fibrosis.

Table 3: Pre and post treatment report for participant A

Parameters	Initial values	Post treatment values	Normal ranges
Platelet count (L^{-1})	102×10^9	215×10^9	$150\text{-}450^9$
SGPT/ALT(IU L^{-1})	52.1	40.3	5-45
SGOP/AST (IU L^{-1})	77	28	0-40
Total bilirubin (μmol L^{-1})	56.9	27.7	5-21
Direct bilirubin (μmol L^{-1})	34.7	11.0	0-3.4
APRI	1.89	0.33	$\leq$0.5
HBV/DNA (IU mL^{-1})	17271	1200	>2000

APRI score $\leq$0.5 is not consistence with cirrhosis and does not require treatment. Treatment can be deferred with HBV/DNA of less than 2000 IU mL^{-1}

Table 4: Pre and post treatment report for participant B

Parameters	Initial values	Post treatment values	Normal ranges
Platelet count (L^{-1})	72×10^9	300×10^9	$150\text{-}450^9$
SGPT/ALT (IU L^{-1})	216.1	44.7	5-45
SGOP/AST (IU L^{-1})	300	30.1	0-40
Total bilirubin (μmol L^{-1})	47.1	47.1	5-21
Direct bilirubin (μmol L^{-1})	26.70	0.7	0-3.4
APRI	10.42	0.25	$\leq$ 0.5
HBV/DNA (IU L^{-1})	970271	27000	>2000

APRI score $\leq$0.5 is not consistence with cirrhosis and does not require treatment. Treatment can be deferred with HBV/DNA of less than 2000 IU mL^{-1}

Table 5: Pre and post treatment report for participant C

Parameters	Initial values	Post treatment values	Normal ranges
Platelet count (L^{-1})	98×10^9	205×10^9	$150\text{-}450^9$
SGPT/ALT (IU L^{-1})	111.1	57.7	5-45
SGOP/AST (IU L^{-1})	273.2	37.9	0-40
Total bilirubin (μmol L^{-1})	30.7	10.4	5-21
Direct bilirubin (μmol L^{-1})	15.2	0.70	0-3.4
APRI	6.97	0.46	$\leq$0.5
HBV/DNA (IU mL^{-1})	180270	14800	>2000

APRI score $\leq$0.5 is not consistence with cirrhosis and does not require treatment. Treatment can be deferred with HBV/DNA of less than 2000 IU mL^{-1}

Table 6: Pre and post treatment report for participant D

Parameters	Initial values	Post treatment values	Normal ranges
Platelet count (L^{-1})	109×10^9	400×10^9	$150\text{-}450^9$
SGPT/ALT (IU L^{-1})	40.1	27.2	5-45
SGOP/AST (IU L^{-1})	77.1	28.00	0-40
Total bilirubin (μmol L^{-1})	16.1	9.9	5-21
Direct bilirubin (μmol L^{-1})	1.02	0.3	0-3.4
APRI	1.77	0.18	$\leq$0.5
HBV/DNA (IU mL^{-1})	57100	1270	>2000

APRI score $\leq$0.5 is not consistence with cirrhosis and does not require treatment. Treatment can be deferred with HBV/DNA of less than 2000 IU mL^{-1}

Table 7: Pre and post treatment report for participant D

Parameters	Initial values	Post treatment values	Normal ranges
Platelet count (L^{-1})	100×10^9	370×10^9	$150\text{-}450^9$
SGPT/ALT (IU L^{-1})	30.1	15.7	5-45
SGOP/AST (IU L^{-1})	97.0	40.0	0-40
Total bilirubin (μmol L^{-1})	14. 0	7.0	5-21
Direct bilirubin (μmol L^{-1})	2.0	0.88	0-3.4
APRI	2.43	0.27	$\leq$0.5
HBV/DNA (IU mL^{-1})	1271	260	>2000

APRI score $\leq$0.5 is not consistence with cirrhosis and does not require treatment. Treatment can be deferred with HBV/DNA of less than 2000 IU mL^{-1}

The APRI/HBV DNA values post treatment for participant D was significantly reduced (Table 7). All the other parameters tested fall within the normal range post treatment.

DISCUSSION

From the result of the acute toxicity test, the animals did not show any sign of toxicity and mortality at all dose l levels.

This was evident in the palpable normal architectural presentation of the canaliculi, central vein and the lobules under the microscope. The general check of the animals revealed that there was no weight loss, hair loss, swellings and nose bleeding indicating that all the animals were in good health conditions throughout the course of the study. Therefore, the LD_{50} of the sample was estimated to be >5000 mg kg^{-1}. The absence of toxicity at 5000 mg kg^{-1} extract orally indicated that the extemporaneous herbal preparation is practically non-toxic and can therefore be safely used.

The stability testing on the other hand was carried out to check the quality of the extemporaneous herbal preparation which varies with the time under the influence of environmental factors, such as pH, solubility, thermostability, humidity, light, oxygen, moisture and other organoleptic properties. The results after the 3 months of the stability test were in conformity with the standard requirements for herbal products[31].

The total ash, acid insoluble ash and water-soluble ash values were found to be within the standard requirements for herbal products[32]. The analysis of the ash content was simply the burning away of organic content, leaving the inorganic minerals.

The physical appearance, weight variation, content uniformity as well as the disintegration times of the sampled capsules were found to be satisfactory as they met official compendia specifications[29]. All the capsules evaluated showed low weight variation and high degree of content uniformity indicating that the method of formulation used was acceptable for preparing good quality extemporaneous herbal preparation.

The viability of a medication blend can be reliant on the rate at which the case breaks down in the patient's gastrointestinal tract. The ability to interact strongly with water is also known to be essential for disintegration. In the present study all the capsules disintegrated in less than 15 min irrespective of the pH of the investigating media, implying that pH may not have detrimental effect on the disintegration of the extemporaneous herbal preparation.

The microbial load determination which encompasses fungal and bacterial load count was all found to be within the acceptable limit as specified by WHO[31].

A total of five consenting adults participated in the study. They were drawn from the academia, the prisons and higher institutions and from the general public.

The participant A was presented with a mild increase in the aminotransferases and the HBV DNA. This is evident in the slight increase in the APRI score suggesting a mild liver fibrosis and requiring treatment. After the 3 months regimen with the extemporaneous herbal preparation, there was a remarkable reduction in the transaminases and the quantity of the HBV DNA resulting in the observed low APRI/HBV DNA suggesting a great suppression of the virus and the treatment was subsequently deferred.

Table 5 shows a prison inmate with greater than 90% likelihood that she had cirrhosis. This is in agreement with Chou and Wasson[33] and Lin *et al.*[34] which reported that the lower the APRI score (less than 0.5), the greater the negative predictive value (and ability to rule out cirrhosis) and the higher the value (greater than 1.5) the greater the positive predictive value (and ability to rule in cirrhosis). With the staggering value of her quantitative HBV DNA, the participant was a ready candidate for treatment as she was swiftly administered with the extemporaneous herbal capsules of 2000 mg in divided doses of 12 h for 3 months. The results after the treatment was encouraging as her APRI reduced to a level which placed her highly unlikely to have fibrosis. The HBV DNA nonetheless reduced but not to the acceptable limit where treatment can be deferred.

From the foregoing assertion by Chou and Wasson[33] and Lin *et al.*[34], it can succinctly be said that the pre-treatment data for participant in Table 6 was likely to have severe fibrosis or cirrhosis going by the varying APRI score greater than 1.5,[2] hence treatment was recommended. He was subsequently administered with the extemporaneous herbal capsules of 2000 mg in divided doses of 12 h for 3 months. The results after the treatment was encouraging as his APRI reduced to a level which placed her very highly unlikely to have significant fibrosis. The HBV DNA nonetheless reduced but not to the acceptable limit where treatment can be deferred or discontinued otherwise extended for additional 3 months.

The underlining denominator associated with the participant in Table 7 was the palpable reduction of the APRI and HBV DNA values which places the participant very unlikely to develop cirrhosis post treatment based on the APRI/HBV DNA values $\leq$2/ 2000 IU mL^{-1}, respectively. Treatment can even be discontinued provided the client was carefully monitored in a long term to avoid reactivation of the conditions.

Treatment generally is not recommended and be deferred in persons without clinical evidence of cirrhosis (or based on APRI score $\leq$2 in adults) and persistently normal ALT levels and low levels of HBV DNA replication (HBV DNA 2000 IU mL^{-1}). However, participant reported in Table 7 treatment couldn't be deferred as the participant presented with APRI/HBV DNA values that qualified for treatment. The participant was nonetheless administered with

the extemporaneous herbal preparation for 3 months of 1000 mg 12 h. The indices and parameters were all reduced to normal suggesting discontinuation of treatment with constant monitoring.

The thrust of the documentation for registration or listing among other things required by NAFDAC before any herbal drug can be marketed in Nigeria includes a comprehensive certificate of analysis which encompasses the acute toxicity test, assessment of the organoleptic properties and the microbiological analysis[12].

CONCLUSION

Owing to the increase in the cost of treating viral hepatitis and the attendant liver fibrosis, it becomes pertinent to look into the complementary and alternative medicaments. It has been opined that chronic diseases are better managed with natural products compare with the orthodox medications and it attendant side effects leading to an uncontrolled release of free radicals. The polyherbal products used in this study was found to be non-toxic for human consumption based on the acute toxicity study. The organoleptic analysis and other parameters evaluated revealed that this polyherbal product is stable for a relatively length of time.

This extemporaneous herbal preparation has been found to be effective in the management of chronic liver disease and general improvement of the immune system when taken 2000 mg 12 h for 3 month. The present study has shown that this polyherbal product could be ideal alternative treatment regimens for people with chronic liver conditions. However, in-depth studies are needed to elucidate the long term benefit/side-effects of this polyherbal product.

SIGNIFICANCE STATEMENTS

This study discovers that the extemporaneous polyherbal preparation has been found to be effective in the management of chronic liver disease and general improvement of the immune system when taken for 12 h daily 2000 mg for 3 months. The present study has shown also that this polyherbal product could be ideal alternative treatment regimens for people with chronic liver conditions. However, in-depth studies are needed to elucidate the long term benefit/side-effects of this polyherbal product.

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