Trop J Nat Prod Res, May 2021; 5(5):983-987

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antimicrobial and Toxicity Studies on Holisa Herbal Formulation

Abiodun H. Adebayo[1]*, Omolara F. Yakubu[1], Edvyne S. Ezekiel-Hart[1], Olajuwon Okubena[2]

[1]*Department of Biochemistry, College of Science and Technology, Covenant University Ota, Ogun State, Nigeria*
[2]*Health Forever Products Ltd, Lagos, Nigeria*

ARTICLE INFO

Article history:
Received 20 March 2021
Revised 12 April 2021
Accepted 12 May 2021
Published online 03 June 2021

ABSTRACT

Herbal plants have been reported to play crucial role in enhancing human health. The study evaluated the antimicrobial property, acute and sub-chronic toxicological effects of Holisa herbal formulation consisting of *Vernonia amygdalina*, *Rorippa madgasceriensis*, and *Securinega virosa* plants using biochemical and histopathological indices. Thirty (30) male Wistar rats of five groups (A-E) of 6 animals each were used. Animals in group A were given distilled water while those in B, C, D, and E received 20, 40, 60, 100 mL/kg of body weight of the formulation for 28 days. The zone of inhibition was determined with agar well diffusion method using seven bacterial and three fungal isolates. The acute toxicity was carried out with the use of Swiss mice which were observed for 72 hours. The zone of inhibition observed on both the bacterial and fungal culture media was very minimal (6-9 mm). Significant ($p<0.05$) increase in body weight was observed in groups B, C, and E and a significant ($p<0.05$) decrease in group D when compared to the control. Alkaline phosphatase, direct bilirubin, triglyceride, total cholesterol, low-density lipoprotein, high-density lipoprotein, creatinine, and total protein were increased significantly ($p<0.05$), while aspartate aminotransferases, alanine aminotransferases, albumin did not show significant differences. The histopathology conducted showed that the formulation showed congestion in the blood vessels and vascular constriction on the liver and kidney tissues respectively. The results revealed that lower doses of Holisa herbal formulation may not have any adverse effect on the liver and kidney tissues but high doses elicited toxicity.

Keywords: Holisa herbal formulation, Antimicrobial studies, Biochemical studies, Histopathology.

Introduction

The use of medicinal plants in the management of different ailments is because of their phytochemical constituents and goes back to antiquity. [1] Usage of herbal medicines can be traced back as far as 2100B.C.in ancient China (Xia Dynasty) and India (Vedic period). The first reports go back to 600 B.C. with the Caraka Samhita of India and the early notes of the Eastern Zhou dynasty of China that pushed toward turning out systematized around 400B.C. The use of herbs as a medication is the most seasoned form of healthcare known to humanity and has been used in all societies from the beginning of time.[2] Herbal products cannot be discounted as "natural" and subsequently, nontoxic components, as many consumers believe. Although selected products may have remedially valuable effects, many cause adverse effects[3] and drug interactions similar to those accomplished with conventional agents.[4] It is however a known fact that over 80% of the total populace depends on herbal medicines and products for healthy living.[5] This rise in the use of herbal products has also given rise to various forms of misuse and adulteration of the products leading to consumers' and manufacturers' mistakes and in some instances deadly outcomes. The challenge is boundless and monumental, making the global herbal market unsafe to be used in the region if there is sufficient knowledge about their safety and viability.

*Corresponding author. E mail:
abiodun.adebayo@covenantuniversity.edu.ng
Tel: +2348036357197

Citation: Adebayo AH, Yakubu OF, Ezekiel-Hart ES, Okubena O. Antimicrobial and Toxicity Studies on Holisa Herbal Formulation. Trop J Nat Prod Res. 2021; 5(5):xxxx doi.org/10.26538/tjnpr/v5i5.30

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Traditional medicines are inexpensive, readily available, and acceptable making them a reliable choice to modern medicines.[6] Besides, it is believed that there is better cultural acceptability, and improvement was achieved with the body.[7] Usage of most herbal formulations has been abused over time without a legitimate assessment of its maximum toxicity level. Hence there is a need to ascertain its safety. Extensive development and public interest in the use of herbal medicine have brought new attention and inspiration to medicinal plant research. Holisa herbal formulation is presently being used particularly in Africa because of the significant expense of western medications. However, there is no satisfactory and agreeable information on its relative safety since the majority of their use depends on essential information. There is no good logical documentation on the formulation to determine its toxicological consequences on the body. The study evaluated the anti-microbial properties, the effect on liver function, lipid profiles, and the kidney function.

Materials and Methods

Herbal formulation

Holisa herbal formulation in liquid form of 200 mL size bottle was obtained from Health Forever product Limitedtd (Ikeja Lagos, Nigeria).

Microorganisms

The selected bacterial strains for the study were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, *Serratia spp*, *Listeria monocytogenes*, and the fungi were *Saccharomyces spp*, *Rhizopus spp.* And *Aspergillus niger*. They were all obtained from the culture collection center, Department of Biological Sciences, Covenant University, Ota. All organisms were sustained on nutrient broth (NB) at 37°C and fungus on potato dextrose agar (PDA) at a temperature of 28°C.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Experimental animals
Twelve male Swiss mice were used and assigned into four groups of 3 mice each weighing between 10.50 g - 17.00 g and 30 male Wistar rats were used and assigned into five groups of six rats each weighing between 92g-149g obtained from Lagos University Teaching Hospital (LUTH) fed with a standard diet (obtained from Graceline feed, Ota, Ogun state). Animals were kept at an average temperature of 25^{o}C in ventilated cages and retained in the animal house of the Department of Biochemistry, Covenant University, Ogun State. All experimental animals were handled following standard protocols officially accepted by the Research ethics committee of the Biochemistry Department of Covenant University, Ota, Ogun State, Nigeria. The animals were acclimatized for seven days.

Antimicrobial studies
Agar well diffusion method was used for the antimicrobial studies according to Benkeblia.[8] In sterile Petri-dish, 6mL of Agar plates were inoculated with 0.1 mL of the broth culture of each bacterium and fungus isolate. The seeded plates had been corked and allowed to set for uniform isolate distribution. Using regular 5 mm diameters sterile cork borer, holes were bored on the plates and equivalent volumes (5 mL) of the herbal formulation were placed into the already well-created micropipette. The tests were performed in duplicates. The control disk was used for the bacteria-containing petri-dish Gentamycin 10 µg while Flocunadazole 10 µg was used for the fungi. The control disk was placed in the petri-dish center. The plates were allowed to stand at room temperature for one hour to permit proper diffusion of the herbal formulation. The bacterial plates were incubated at 37°C and the fungal plates were incubated at 25°C for 24 hours until noticeable potency of the herbal formulation was observed to inhibit the growth of the test isolates. The zones of inhibitions were measured in millimeters (mm) and the mean values were calculated and recorded.

Acute toxicity study
The acute toxicity study was performed using twelve (12) male Swiss mice. The animals were divided randomly into four treated groups, each comprising three animals per group. Based on their weights, the groups received 250, 500, 1000, and 2000 mL/kg body weight of the herbal formulation respectively, after the overnight fasting. They were administered the Holisa herbal formulation orally. The animals were monitor closely for the first 4 hours and then for each hour for the next 12 hours, followed by 6 hourly intervals for the next 56 hours (72 hrs observations) to identify any deaths or changes in general behavior and other physiological activities.[9]

Sub-chronic toxicity study
Thirty male Wistar rats were acclimatized for seven days in the laboratory. The animals were kept on regular animal feed and had access to water *ad libitum*. Weighed and divided into five groups of six animals each. The control group received a dose of 1.0 mL of distilled water orally once every day for 28 days, after fasting overnight. The following doses were given to the four treated groups respectively: 20, 40, 60, and 100 mL/kg body weight orally once a day for 28 days treated with the Holisa herbal formulation. From the beginning of the treatment, the animals were measured every seven days to note any difference in weight. The animals were starved overnight at the end of the experiment.[10]

Sample preparation
The rats' weights were taken before they were sacrificed. Diethyl ether was used as anesthesia to euthanize. Blood was collected from the heart using syringes that are non-heparinized. Organs such as the liver and kidney were taken and rinsed with normal saline to remove contamination in the blood. The organs taken were stored in the freezer for overnight after which 0.2 g of each organ was homogenized in buffer with ice-cold phosphate, pH 7.4. The stored organs were cut into bits and using the homogenizer 0.2 g of the liver was homogenized in 1.8 mL of the homogenizing buffer. The resulting homogenate were centrifuged in a cold centrifuge at 3000rpm for 10 minutes to prevent denaturing the enzymes. The supernatant was collected for use in the biochemical analysis. Plasma was collected 10 minutes from the entire blood via the centrifugation cycle at 4000rpm.

Biochemical assays
Commercial tests kits obtained from Randox laboratories UK were used. The following biochemical parameters were carried out in plasma; alanine aminotransferase (ALT)[11], aspartate aminotransferase (AST)[12], alkaline phosphatase (ALP)[13], total bilirubin[14], total protein[15], urea,[16] creatinine[17,] and albumin.[18]

Histopathological studies
The method as described by Aliyu et al.[19] with slight modification was adopted. The liver and kidney were carefully dissected from the abdominal region. They were fixed in normal formal saline for 72 h and sliced into a thickness of 2.1 mm. The tissues were dehydrated with alcohol of graded concentrations. They were further treated with paraffin wax. Sections of the tissues were then cut on a microtome to 5µm. These were later fixed on a slide and allowed to dry. The sample slides were subsequently stained in hematoxylin-eosin and examined under a light microscope; photomicrographs of the samples were recorded.

Statistical analysis
Data were analyzed with one-way analysis of variance (ANOVA) and Tukey's test using the statistical package for the social sciences (SPSS), version 21.0 (SPSS Inc., Chigaco, IL, USA). The probability of *$p<0.05$ was considered to be statistically significant. All data were represented as mean ± standard error of mean for 7 animals graphically using Graph pad prism, version 5.0.

Results and Discussion

There is an increasing demand for herbal products as alternative medicines. Unfortunately, the use of herbal products is not strictly regulated in Nigeria thus making them freely available, a scenario that predisposes to possible abuse by consumers. Herbal medicines are being used by about 80% of the world population primarily in developing countries for primary healthcare. They have stood the test of time for their safety, efficacy, and cultural acceptability. These drugs are made from renewable resources of raw materials by eco-friendly processes and will bring economic prosperity to those growing these raw materials.[20] Herbal remedies may have recognizable therapeutic effects; they also may have toxic side effects. More so, many herbal preparations lack scientific facts to back up acclaimed medicinal benefits. In rural communities, the exclusive use of herbal drugs, prepared and dispensed by herbalists without formal training, for the treatment of diseases is still very common requiring that experimental screening methods be established to ascertain the safety and efficacy of these herbal products as well as to establish their active components.[21] Toxicity studies have always been considered a vital integral component of drug development bearing in mind that herbal medicines are often used indiscriminately without recourse to the potential side-effects which could vary from mild, moderate, and severe, to life-threatening.[21] The acute toxicity studies of the formulation revealed no alterations in animals' actions. The mice used in the experiment showed no adverse gastrointestinal effects and all the mice which received 2000 mL/kg BW of the extract died within 4 hours while two of the animals that received 1000 mL/kg BW dose died within 12 hours.[21, 22] The LD_{50} of the herbal formulation is greater than 2000 mg/kg/day BW. The effects of the drug on the bodyweight variation of the tested animals were remarkable only on Group B, C, and E that received the lowest and highest dose of the formulation (20mL/kg, 40mL/kg, and 100 mL/kg BW respectively) because there was a significant increase in their weights after 28 days. A significant decrease in weight was observed in group D that received 60 mL/kg compared to the control. Compared to the control there were no morphological changes in organs (liver and kidney). Histopathological tests showed vascular congestion in the liver and kidney of group A to E had except for group D which was normal (Plate 1&2). The vascular

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

congestion observed is not responsible for the drug because group A was given only distilled water also had vascular congestion as seen in the photomicrograph. Nevertheless, there were also significant changes in the weights of different organs particularly in the animals that received the highest dose of the drug in the kidney and liver. Creatinine is excreted in the kidneys and used as an indicator for renal failure.[23] The concentration of creatinine plasma increases significantly suggesting an underlying effect of the herbal formulation on renal filtration mechanism. There was no significant increase in the plasma protein (Table 2) of the experimental animals which explains that the kidney did not compromise its function. The specific markers of hepatic injury and hepatocellular necrosis are Aspartate aminotransferase (AST), Aminotransferase (ALT), and Alkaline phosphatase (ALP), and an increase in their plasma concentrations indicates hepatic and cardiac damage.[24] An increase in serum ALP may be as a result of an enhanced synthesis of the enzyme to increase biliary pressure.[25] There was no significant change in the levels of both enzymes which further explains that the herbal formulation had no effect on the liver and this is confirmed by the histological study where the hepatic tissue morphology indicated no pathological changes and has no toxic effect on the liver and kidney tissue. Since ALP is also synthesized by other tissues of the body, a dose-related rise in ALP activity may not be due to liver damage. [26, 27] This result is supported by the histological assessment of the liver tissue where no obstructive jaundice and intrahepatic cholestasis as a consequence of a high level of ALP was observed. It has been reported that lipids play a key part in the pathological changes associated with disease conditions[28] A rise in triglyceride has been associated as a risk factor for atherosclerosis and shows the presence of hyper-lipidemic agent; this was observed in groups administered 40 and 60 ml/kg, HDL-cholesterol levels showing the presence of an anti-atherogenic agent in all the experimental animals treated with the herbal formulation suggesting that cardiovascular risk factors that contribute to the death of rats were present.[29]

Table 1: Zone of inhibition (mm) of Holisa herbal formulation against bacteria and fungi isolates

Microorganisms	Holisa formulation	Gentamycin	Flocunadazole
Pseudomonas aeruginosa	9	13	-
Staphylococcus aureus	8	23	-
Salmonella typhi	10	17	-
Escherichia coli	17	23	-
Bacillus subtilis	6	15	-
Serratia spp.	8	22	-
Listeria monocytogenes	7	20	-
Saccharomyces spp	6	-	19
Rhizopus spp.	7	-	9
Aspergillus niger	6	-	10

Table 2: Effect of Holisa Formulation on Liver Function Parameters in Wistar Rats

GROUPS	AST(U/I)	ALT (U/I)	ALP (U/I)	ALB (mg/dL)	T.PRO (g/dL)	T.BIL(mg/dL)	D.BIL(mg/dL)
A(1mL d.H_2O)	297.1 ± 43.30	30.6 ± 0.33	10.24 ± 0.04	0.7 ± 0.03	1.3 ± 0.04	2.0 ± 0.34	15.3 ± 1.43
B (20mL/kg)	380.0 ± 26.77	19.5 ± 1.50	10.52 ± 0.04	0.9 ± 0.02	1.7 ± 0.19	1.1 ± 0.12*	14.8 ± 0.66
C(40mL/kg)	486.0 ± 42.18*	23.7 ± 0.47	10.62 ± 0.04	1.1 ± 0.04*	1.5 ± 0.09	1.2 ± 0.29*	16.2 ± 0.49
D(60mL/kg)	486.0 ± 47.02*	23.2 ± 2.78	10.25 ± 0.05	1.1 ± 0.04*	1.2 ± 0.7	1.4 ± 0.27*	15.7 ± 1.59
E(100mL/kg)	426.0 ± 29.63*	20.7 ± 1.65	10.01 ± 0.04	0.9 ± 0.03	1.4 ± 0.06	1.3 ± 0.11	18.6 ± 0.60*

Values are expressed as mean ± standard error of mean.* $p < 0.05$ significant when compared to control. AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; T.BIL: total bilirubin; D.BIL: direct bilirubin; Alb: Albumin; T.PRO: total protein. A significant increase ($p < 0.05$) in AST was observed in the groups treated with 40, 60, and 100 mL/kg body weight of the formulation, while the level of direct bilirubin in the group treated with 100 mL/kg body weight of the formulation was also significantly increased ($p < 0.05$). The total protein showed no significant change across all groups.

Table 3: Effect of Holisa Formulation on lipid profile parameters in Wistar rats

GROUPS	Triglycerides (g/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
A(1mL d.H_2O)	120.6 ± 0.51	63.1 ± 4.72	91.3 ± 0.04	115.2 ± 1.25
B (20mL/kg)	94.5 ± 2.65*	50.2 ± 2.99	111.5 ± 0.18	64.7 ± 1.55*
C (40mL/kg)	215.2 ± 0.45*	40.3 ± 2.14*	111.6 ± 0.06	89.7 ± 6.02*
D (60mL/kg)	190.3 ± 0.50*	51 ± 2.64*	121.2 ± 0.09*	110.9 ± 5.48
E (100mL/kg)	135.9 ± 0.70*	62 ± 2.05*	130.7 ± 0.04	151. 4 ± 4.65*

Values are expressed as mean ± standard error of mean. *$p < 0.05$ significant when compared to control. HDL: high-density lipoprotein; LDL: low-density lipoprotein. Elevated levels of lipid profile may predispose to cardiovascular-related disorders. A significant increase ($p < 0.05$) in triglyceride was observed in the groups treated with 40 and 60 mL/kg body weight of the formulation. LDL shows an increase in the groups treated with 100 mL/kg body weight of the formulation.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

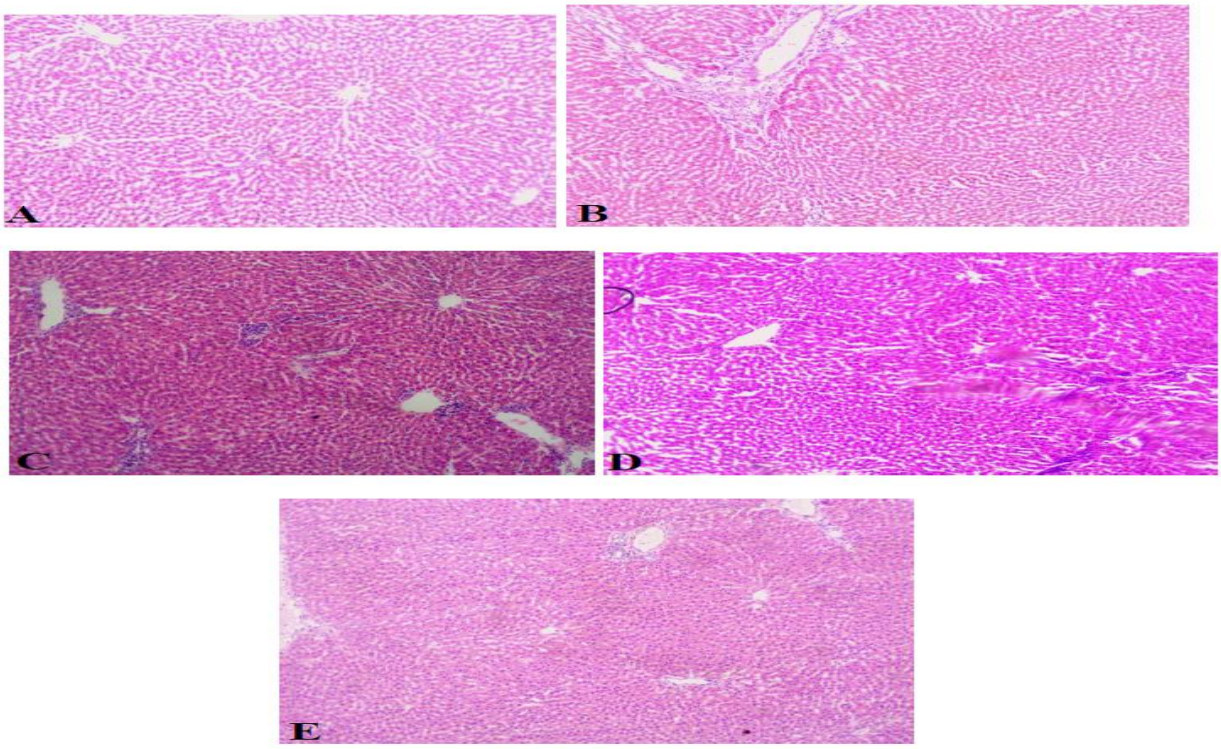

Plate 1: Photomicrograph of the liver tissues (H&E X100) of rats administered (a) distilled water (b) 20 mL/kg (c) 40 mL/kg (d) 60 mL/kg (e) 100 mL/kg body weight of Holisa herbal formulation.

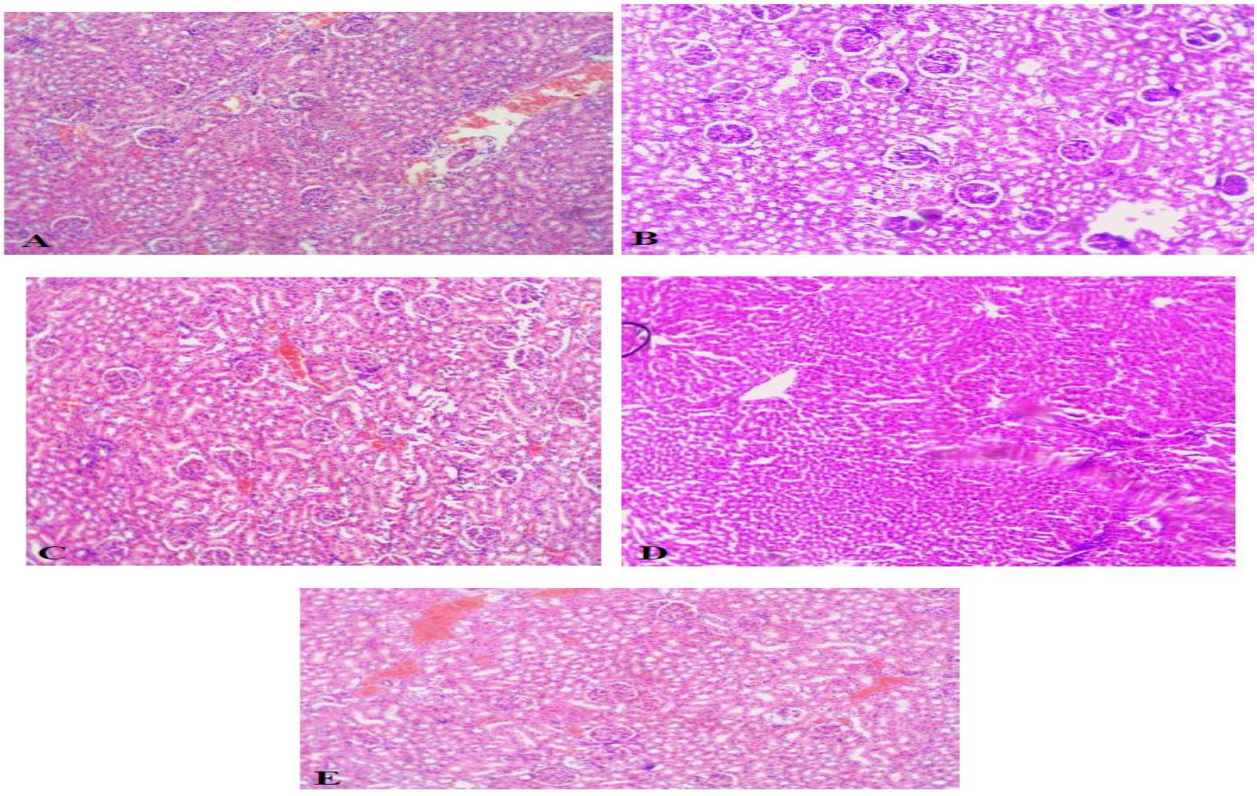

Plate 2: Photomicrograph of kidney tissues (H&E X100) of rats administered (a) distilled water (b) 20 mL/kg (c) 40 mL/kg (d) 60 mL/kg (e) 100 mL/kg body weight of Holisa herbal formulation. Histopathological tests showed vascular congestion in the liver and kidney of group A to E except for group D which was normal. The vascular congestion observed is not due to the drug because group A was given only distilled water also had vascular congestion as seen in the photomicrograph

Conclusion

The study provides scientific credibility to the use of holisa herbal formulation as an antimicrobial agent and may therefore serve as potential source of safe and effective drug formulation.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

We acknowledge the effort of the technical staff of the Department of Biochemistry for the laboratory support. We also thank Covenant University Center for Research Innovation and Development (CUCRID) for funding the publication of this paper.

References

1. Alamgir ANM. Phytochemisty and Bioactive Compounds In: Therapeutic Use of Medicinal Plants and their Extracts. Springer Intl. 2018. 74 p.
2. Fitzgerald M, Heinrich M, Booker A. Medicinal Plant Analysis: A Historical and Regional Discussion of Emergent Complex Techniques. Frontiers in Pharm. 2020; 10:1480-1494.
3. Kumar S, Dobos G, Rampp T. The significance of Ayurvedic medicinal plants. J Evid-Based Compl Altern Med. 2017;22(3): 494-501.
4. Damyanov CA, Maslev IK, Pavlov VS, Avramov L. Conventional Treatment of Cancer Realities and Problems. Ann Compl Altern Med. 2018; 1(1):1002.
5. Ezekwesili-Ofili JZ and Okaka ANC. Herbal Medicines in African Traditional Medicine, Herbal Medicine, Philip F. Builders, IntechOpen, 2019; 10:191-214.
6. Yuan H, Ma Q, Ye L, Piao G. The traditional Medicine and Modern Medicine from Natural products. Molecules. 2016; 21:588-606.
7. WHO global report on traditional and complementary medicine. Geneva: World Health Organization; 2019. Licence: CC BY-NC-SA 3.0 IGO.
8. Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). Leb. Wiss Technol. 2004; 37:263-268.
9. Upadhyay P, Shukla R, Mishra SK. Acute and subacute toxicity study of hydro-alcoholic leaves of *Reinwardtia indica* in rats. Biomed Pharmacother. 2019; 111:36-41.
10. Yakubu OF, Adebayo AH, Famakinwa TO, Adegbite OS, Ishola TA, Imonikhe LO, Adeyemi OA, Awotoye OA, Iweala EE. Antimicrobial and toxicological studies of Ricinodendron heudelotii (Baill.). Asian J Pharm Clin Res. 2018; 11:299-305.
11. Bergmeyer HU, Hørder M, Rej R. International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical Section: Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2). J Clin Chem Clin Biochem. 1985; 24:481-495.
12. Bergmeyer HU, Hørder M, Rej R. International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical Section: Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1). J Clin Chem Clin Biochem. 1986; 24:497-510.
13. Tietz NW, Rinker AD, Shaw LM. International Federation of Clinical Chemistry. IFCC methods for the measurement of catalytic concentration of enzymes, Part 5. IFCC method for alkaline phosphatase (orthophosphoric-monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1). J Clin Chem Clin Biochem. 1983; 21:731-748.
14. Doumas BT, Perry BW, Sasse EA, Straumfjord JV. Standardization in bilirubin assays: evaluation of selected methods and stability of bilirubin solutions. Clin Chem. 1973; 19:984-993.
15. Weichselbaum TE. Biuret method of serum total protein estimation. Am J Clin Pathol 1946; 16:40.
16. Krieg M, Gunsser KJ, Steinhagen-Thiessen E, Becker H. Comparative quantitative clinico-chemical analysis of the characteristics of 24-hour urine and morning urine. J Clin Chem Clin Biochem. 1986; 24:863-869.
17. Larsen K. Creatinine assay by a reaction-kinetic principle. Clin Chim Acta. 1972; 41:209-217.
18. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta. 1971; 31:87-96.
19. Aliyu R, Adebavo AH, Gatsing D, Garba IH. The effects of ethanolic leaf extract of Commiphora africana (Burseraceae) on rat liver and kidney functions. J Pharmacol Toxicol. 2007; 2:373-379.
20. Bušić A, Marđetko N, Kundas S, Morzak G, Belskaya H, Ivančić Šantek M, Komes D, Novak S, Šantek B. Bioethanol Production from Renewable Raw Materials and Its Separation and Purification: A Review. Food Technol Biotechnol. 2018; 56(3):289-311.
21. Ogbonnia SO, Odimegwu JI, Enwuru VN. Evaluation of hypoglycaemic and hypolipidaemic effects of aqueous ethanolic extracts of Treculia africana Decne and Bryophyllum pinnatum Lam. and their mixture on streptozotocin (STZ)-induced diabetic rats. Afr J Biotechnol. 2008; 7:2535-2539.
22. Ogwal-Okeng W, Obua C, Anokbonggo W. Acute toxicity effects of the methanolic extract of Fagara zanthoxyloides (Lam.) root-bark. Afri Health Sci. 2003; 33:124-126.
23. Adebayo AH, Abolaji AO, Oputa TK, Adegbenro IK. Effects of ethanolic leaf extract of Chrysophyllum albidum G. on biochemical and hematological parameters of albino Wister rats. Afr J Biotechnol. 2010; 9(14):2145-2150.
24. Martha LC and Rolando H. "Is liver enzyme release really associated with cell necrosis induced by oxidative stress?". *Ox. Med and Cell. Long.* 2016; 3529149:12.
25. Javinani A, Aghaei Meybodi HR, Kavosi H. Extremely elevated serum alkaline phosphatase level upon treatment with teriparatide: a case report. J Med Case Rep. 2020; 14:87.
26. Arika WM, Nyamal DW, Osano KO, Ngugi MP, Njagi ENM. Biochemical Markers of *In vivo* Hepatoxicity. J Clin Toxicol. 2016; 6:296-304.
27. William V. Stenberg. Periodontal Problems in Children and Adolescents, Editor(s): Arthur J. Nowak, John R. Christensen, Tad R. Mabry, Janice A. Townsend, Martha H. Wells, Pediatric Dentistry (Sixth Edition), Elsevier. 2019. 371-378 p.
28. Ogunlana OO, Ogunlana OE, Adeneye AA, Udo-Chijioke O, Dare-Olipede T, Olagunju JA, Akindahunsi AA. Evaluation of the toxicological profile of the leaves and young twigs of *Caesalpinia bonduc* (Linn) roxb. Afr J Trad Compl Altern Med. 2013; 10:504-512.
29. Wang Z, Wang Z, Wang L, Qiu M, Wang Y, Hou X, Guo Z, Wang B. Hypertensive disorders during pregnancy and risk of type 2 diabetes in later life: a systematic review and meta-analysis. Endocrin. 2017; 55:809-821.