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Safety evaluation of an anti-haemorrhoid (pile) herbal recipe (locally called 'agbo jedi-jedi') of southwestern Nigeria using animal genetic assays

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Research Article

Keywords: Alstonia boonei, anti-haemorrhoid, herbal recipe, mutation, Syzygium aromaticum

Posted Date: October 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2113277/v1

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Abstract

Background

In this study, an anti-haemorrhoid herbal recipe containing bark of *Alstonia boonei* and dried cloves - *Syzygium aromaticum* was investigated for cytogenotoxicity, mutagenicity and histopathological effects following the micronucleus and sperm morphology assays using Swiss albino rats. The rats were administered anti-haemorrhoid herbal recipe at 25.0%, 50.0% and 100.0%, while distilled water and 0.05% sodium azide were the negative and positive controls, respectively. Phytochemicals in the herbal recipe were elucidated using the Fourier Transform Infrared (FTIR) and Gas Chromatography Flame Ionization Detection (GC-FID) techniques.

Results

In the treated rats, there was increase in body weight, cells division inhibition, micronucleated erythrocytes (MNPCEs & MNNCEs) and abnormal sperm cells were significantly induced at 100.0% ($P < 0.05$), whereas 25.0% promoted cell division. The herbal recipe at 50.0% and 100.0% did not cause any histopathological damages on the liver, kidney and testis except 25.0%. Polyphenolics, terpenoids and alkaloids were detected in the anti-haemorrhoid herbal recipe which could be cytotoxic, clastogenic and spermatotoxic at a high concentration (100.0%).

Conclusion

These results necessitate regulation and control of consumption of this anti-haemorrhoid herbal recipe by people through the authorized government agencies in Nigeria.

Background

The use of herbal medicines is a common practice by people of Southwestern Nigeria because of their efficacy and easy availability. Most herbal medicines are formulated with more than one plant part, or/and with non-plant materials. Herbal recipes for treating various diseases are being sold at different places such as commercial motor parks, commercial motor cycles and tricycles parks, asides those sellers who always hawk around the major and minor streets in Nigeria [30]. In Nigeria a significant number of people consume these herbal products because they erroneously believe that herbal medicines are natural and therefore safe for consumption [15].

Among several herbal recipes that are being sold at the aforementioned sale points, anti-haemorrhoid herbal recipe receives highest patronage from young people and adults including the aged [18]. A study showed that anti-haemorrhoid herbal recipes accounted for 52.2% of the total herbal medicines that were consumed in Lagos State [23]. Most people consume anti-haemorrhoid herbal recipes because of their

belief that they are effective as aphrodisiac, reduction of high blood glucose (hyperglycemic condition) that results from frequent consumption of carbohydrate-rich foods and for easy defecation. Most diseases are caused by what is eaten as either foods or medicines. The World Health Organization advises that herbal medicines must be scientifically established to be effective against diseases being claimed to cure, and toxicologically safe [26]. Although, the therapeutic effects of herbal medicines depend on phytochemicals in them, however, not all phytochemicals are therapeutic against diseases, a number of phytochemicals could be harmful to body health [7,10].

Phytochemicals causing diseases negatively affect the body cellular biomolecules, consequently changing the body physiology. A significant number of herbal medicines that are being sold in the Nigerian markets are not certified by the regulatory agencies such as National Agency for Food and Drug Administration and Control (NAFDAC) and The Nigeria Natural Medicine Development Agency (NNMDA) to be safe for consumption [27]. Herbal medicines for the prevention and treatment of a particular disease can be prepared with different formulations of plants which makes their effectiveness and toxicity to be different [5]. Some herbal medicines that were sold in Ilorin, Kwara State and Lagos State were reported to be contaminated with heavy metals and bacteria of public health concern [21, 22]. Toxicity of herbal medicines has been a major concern that necessitates their toxicological evaluation alongside with the scientific establishment or confirmation of their therapeutic potency [6].

Therefore, this investigation was aimed at safety evaluation of a formulation of anti-haemorrhoid herbal recipe containing bark of *Alstonia boonei* and dried seeds of cloves-*Syzygium aromaticum* for toxicity to somatic and reproductive cells in albino rats.

Materials And Methods

2.1 Preparation of anti-haemorrhoid herbal recipe

A formulated anti-haemorrhoid herbal recipe containing bark of *Alstonia boonei* (De Wild) and dried cloves-*Syzygium aromaticum* (L.) was purchased from a herbal seller at the college of health sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo state Nigeria. The formulation weighed 155.75g (bark of *A. boonei*) and 1.44 g (dried cloves). To this herbal recipe, 500 ml distilled water was added and left for 4 days for cold extraction of phytochemicals to occur. The extract was sieved to obtain anti-haemorrhoid herbal recipe, stored at 4^0 C. This absolute (100.0%) herbal recipe was diluted with distilled water to obtain 25.0% and 50.0% concentrations [12].

2.2 Micronucleus test

The use of animals in this study conformed with the standard guidelines set for the care and usage of laboratory animals for experiment [24]. Twenty-five female rats were divided into treatment (25.0%, 50.0%, 100.0%) and control (distilled water and 0.05% sodium azide) groups containing 5 animals per group. The rats were orally administered the anti-haemorrhoid herbal recipe (0.1 ml per 10.0 g body weight) 24 hourly for 2 consecutive days. After 48 hours, they were sacrificed by euthanizing with diethyl ether. They

were dissected to remove their femoral bones from blood tissue was to flush out with freshly prepared Hank's balanced salt solution (HBSS) into a test-tube. Slides were prepared and scored as previously described [13].

2.3 Sperm morphology assay and histopathological study

Twenty-five mature male rats were administered the anti-haemorrhoid herbal recipe and controls (0.1ml per 10.0g body weight) once daily for 35 days [9]. The animals' weights were determined after every week using a digital electronic balance. After five weeks, they were sacrificed, the epididymis was removed and perforated for sperm cells to swim into phosphate buffer solution. The solution of sperm cells was stained with eosin yellow for 30 minutes. Preparation and scoring of slides were done as previously reported [11].

The testes, liver and kidneys of the treated rats were removed and fixed in 10.0% formalin for further processing. These organs were prepared for histological examination following the standard procedure [34].

2.5 Spectroscopic analyses

2.5.1 FTIR

The anti-haemorrhoid herbal recipe was prepared for FTIR analysis as previously described by Zahrah and Muchtaridi [35]. This analysis was carried out using a FTIR spectrophotometer (Agilent, Cary 630). The pelletized IR sample of the herbal recipe was scanned at the wave number range between 4000–700 cm^{-1}.

2.5.2 GC-FID

Phytochemicals in the anti-haemorrhoid herbal recipe were detected using the gas chromatography Hewlett Packard-HP SERIES II (5890) connected to a flame ionization detector. The carrier and combustion gases were nitrogen and hydrogen set at 20 ml/minutes and 45 ml/minutes flow rates, respectively. The initial temperature was 50°C while the injector and detector temperatures were 220°C and 270°C, respectively. The oven temperature was 240°C and maintained at 10°C/minute held for 2 minutes. The phytochemicals in the herbal recipe were identified by comparing their mass spectra with those of the standards present in the Chemistation / library (NIST 11) [4].

Results

Administration of the anti-haemorrhoid herbal recipe to rats caused body weight gain that was inversely proportional to the selected doses (Table 1). The animals in 25.0%, 50.0% and 100.0% doses had average body weight increase of 33.09%, 28.80% and 10.23%, respectively. It was

20.93% and 21.16% body weight gain for the rats given the negative and positive controls, respectively.

Table 1
Effect of anti-haemorrhoidherbal recipe on the weights of albino rats treated for 5 weeks

Dose (%)	Initial weight (g)	Final weight (g)	% Weight gain
Distilled water	225.11 ± 4.83	284.69 ± 14.61	20.93
Sodium azide (0.05)	230.51 ± 1.1	292.38 ± 23.4	21.16
25.0	185.23 ± 13.19	276.82 ± 30.07	33.09
50.0	213.87 ± 13.57	300.37 ± 43.04	28.80
100.0	228.47 ± 2.87	254.52 ± 46.46	10.23

Values with different superscript alphabet(s) are significantly different ($p < 0.05$).

Activity of the anti-haemorrhoid herbal recipe on micronuclei (MN) induction caused the same percent of MN at 25.0% and 50.0% doses, while 100.0% dose induced higher percent of MN than that recorded with the positive control. The least MN percent was recorded with the negative control (Fig. 1).

The ratio of PCE to NCE was inversely proportional to the dose as 100.0% < 50.0% < 25.0%. The negative and positive controls recorded the same proportion of PCE to NCE (Fig. 2).

The percent of abnormal sperm cells induced by the anti-haemorrhoid herbal recipe was non-dose dependent, having recorded higher percent of abnormal sperm cells at 25.0% than that at 50.0% dose. However, the highest percent of abnormal sperm cells was obtained at 100.0%. The negative control induced the least percent which was not significantly different from those recorded at other doses except 100.0% (Fig. 3).

Histopathological effects of the anti-haemorrhoid herbal recipe on the testes showed normal seminiferous epithelium and basement membrane, lumen and interstitial space with cells. However, there was a mild micromorphological alterations at 25.0% (Plate 1).

Plate 1: Histopathological effect of anti-hemorrhoid herbal recipe on the testis of rats

AJ means 'Agbo Jedi'

The hepatocytes, central venules, sinusoids and portal triad were normal in the negative control, 50.0%, and 100.0% groups (Plate 2). However, there was a significant observable morpho-cellular alterations such as dilation of the central venules with congestion, pyknotic hepatocytes, inflammation of hepatic parenchyma in the rats administered with 25.0% herbal recipe and positive control.

Plate 2: Histopathological effect of anti-hemorrhoid herbal recipe on the liver of rats

AJ means 'Agbo Jedi'

The interstitial spaces, glomerulus, renal cortex, renal tubules, capsular spaces, mesangial cells of kidney of the rats treated at 50.0%, 100.0% and negative control appeared normal. However, the kidney tissues of rats in the positive control and 25.0% groups showed congested convoluted renal tubules at the proximal and distal ends, infiltrated renal parenchyma by inflammatory cells, sclerotic glomerulus and pyknotic parenchymal cells with a wider capsular margin (Plate 3).

Plate 3: Histopathological effect of anti-haemorrhoid herbal recipe on the kidney of rats

AJ means 'Agbo Jedi'

The peaks at 3854.1, 3565.2, 3541.0, and 3041.5 cm^{-1} are characteristic spectra of hydroxyl (OH) and amine (NH) functional groups. The absorbance spectra at 2264.4, 2208.4, 2178.6 and 2141.4 cm^{-1} correspond to unsaturated hydrocarbon groups of alkenes and alkynes of stretched and conjugated types. The bands at 1958.7, 1541.3 and 1507.7cm^{-1} (stretching) characterize double bonds aromatic hydrocarbon group. The absorbance spectrum showed at 795.8cm^{-1} (bending) corresponds to single bond aromatic hydrocarbon group (Fig. 4).

Figures 5a and 5b show the chromatograms of fourteen and eighteen phytochemicals detected in *Alstonia boonei* and *Syzygium aromaticum*, respectively. These contained alkaloids, terpenoids and polyphenolics.

Discussion

The body weight gain observed in the treated rats implies that they consumed and utilized the given feeds well. Polyphenolic phytochemicals with antioxidant capacity have been reported to cause body weight gain in farm animals and albino rats by increasing feeds utilization [31, 33]. In this study, the anti-haemorrhoid herbal recipe contained a number of polyphenolic phytochemicals such as gallic acid, eugenol, phloretin, gallocatechin, epigallocatechin, quercetin, kaempferol, myricetin, quaijaverin, pinocembrin which might be responsible for the observed weight gain.

The induction of higher PCE/NCE at 25.0% than the negative control means that this herbal recipe promoted cell division at low concentration, while becoming cytotoxic at high concentrations as evident in these ratios that were inversely proportional to the selected doses. Polyphenolics are good antioxidants which could be cytotoxic through their prooxidative mechanisms at certain concentrations and conditions. Epigallocatechin, -sitosterol, lupeol, and ursolic acid found in the anti-haemorrhoid herbal might be responsible for its cytotoxicity to cell division in the treated rats. These phytochemicals are capable of inhibiting uncontrolled proliferation and inducing apoptosis in neoplastic and cancerous cells [29].

The induction of non-dose dependent micronuclei indicates weak clastogenic or aneugenic potentials. This effect on the bone marrow cells was further corroborated with the effect of the anti-haemorrhoid herbal recipe on the sperm cells also suggesting weak mutagenicity. This is in accordance with our

previous report of weak mutagenic effect of rutin from aqueous fraction of *Myristica fragrans* on the sperm cells of Swiss albino mice [11]. The induction of significantly different micronuclei and abnormal sperm cells at 100.0% than the negative control indicates that the anti-haemorrhoid herbal recipe was toxic and should not be consumed at a high concentration because of its potential to cause mutations in dividing cells [28]. These could be due to oxidative phytochemicals capable of generating free radicals to cause damage in the DNA molecule and testicular tissue [3]. The polyphenolic phytochemicals in the anti-haemorrhoid herbal recipe could have behaved as prooxidants at 100.0% while behaving as antioxidant at 25.0% and 50.0%. These lower concentrations could be considered as not toxic due to induction of micronuclei and abnormal sperm cells that were not significantly different from those of the negative control. Some polyphenolics possess genotoxicity properties to induce micronucleated cells in the bone marrow of albino mice, rats and hamster fibroblast cells [20]. Oil extract of *Syzygium aromaticum* induced sperm cells abnormalities in adult male Wister rats due to point mutations, chromosome aberrations or errors that occur during spermatogenesis [14]. The morphology and motility of sperm cells can be improved by moiety of lignans, sitosterol, alkaloids and flavonoids phytochemicals capable of scavenging free radicals and reactive oxygen species thereby inhibiting the activity of lipid peroxidases, consequently protecting the integrity of the cell membrane of spermatozoa [19, 32].

The histopathological effects of the anti-haemorrhoid herbal recipe on the testicular, liver and kidney tissues suggest that 25.0% dose was mildly toxic, while 50.0% and 100.0% were not toxic. This could mean that higher doses contained sufficient amounts of phytochemicals capable of protecting the liver, kidney and testes of the treated rats. Our results are in accordance with the report on the protective activity of oil extracted from *Syzygium aromaticum* on the liver, kidney and testes of rats exposed to acrylamide toxicity [16]. In the present study, the effects of the anti-haemorrhoid herbal recipe on the testes, liver and kidney were different from the effect of aqueous extract of *Alstonia boonei* which was reported to damage the testicular and kidney tissues of experimental animals [17]. Antioxidants in *Syzygium aromaticum* have been reported to protect kidney and liver against dagame caused by diabetes, carbon tetrachloride and thioacetamide, respectively, through free radical scavenging mechanisms to prevent lipid peroxidation of the cell membrane [25, 2]. Phytochemicals in the *A. boonei* and *S. aromaticum* might have acted synergistically to produce the observed effects in this study. This explains the concept of medicinal herbal preparations in the Southwestern Nigeria where most of these preparations are in combination of more than one plant. It is equally possible that phytochemicals in herbal preparations may act antagonistically to harm the consumers rather than to cure them from diseases. This therefore makes the toxicological screening of medicinal herbal preparations important in order to prevent toxicity of phytochemicals.

Conclusion

This study evaluated possible toxicity of a commonly sold anti-haemorrhoid herbal recipe on the street of Ogbomoso, Southwestern Nigeria to the somatic and reproductive cells of Swiss albino rats following the micronucleus and sperm morphology assays. The herbal recipe was observed to promote body weight gain and induced cytotoxicity, with the least dose promoting cell division better than the negative control.

At 100.0%, it induced micronuclei in the bone marrow and abnormal sperm cells that were significantly different from those recorded for the negative control. Some mild morphological alterations in the liver, kidney and testicular tissues were observed only at 25.0%. The Polyphenolics, terpenoids and alkaloids phytochemicals found in this anti-haemorrhoid herbal recipe were suspected to behave as antioxidants at low doses and prooxidants at a high dose to cause the observed effects in this study. Therefore, this formulated anti-haemorrhoid herbal recipe should not be consumed at a high concentration to avoid possible clastogenic/aneugenic and spermatotoxic effects.

Declarations

Acknowledgement

Professor O.S. Bello of Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso is appreciated for his effort in scanning the manuscript for preliminary plagiarism check.

Authors contributions

AA designed, coordinated and supervised the conduct of the experiment, analyzed the data and wrote the manuscript.

IOA proofread the manuscript

MJ carried out the experiment and collection of data on micronucleus test

OH carried out the experiment and collection of data on sperm morphology test

AR carried out the experiment and collection of data on histopathology and spectroscopic studies

Funding

This investigation was not funded by any organization.

Availability of data and materials

The dataset used and/or analyzed in this study are available from the corresponding author on reasonable request.

Declarations

Ethic approval and consent to participate

The animal ethics committee approval number is FBMS/AEC/B/073/22".

However, the animals used in this study were strictly handled following the guide for the care and use of laboratory animals, 2011.

Consent for publication

Not applicable

Competing interest

Authors declared no competing interest

References

Abhishek KJ, Alok KP (2019) In Vivo Micronucleus Assay in Mouse Bone Marrow, in *Genotoxicity Assessment*: *Methods and Protocols, Methods in Molecular Biology* (Alok D, Mahima B. eds) Springer Science Business Media, LLC part of Springer Nature pp. 2019 – 2031.

Abtahi-Eivari SH, Shokoohi M, Ghorbani M, Halimi M, Hajizadeh H, Pourlak T, Bahrami J, Ghoreishi Z (2021) Effects of Hydroalcoholic Extracts of Cloves (*Syzygium aromaticum*) on the Serum Biomarkers, Antioxidant Status, and Histopathological Changes of Kidneys in Diabetic Rats. Cres J Med & Biol Sci. 8(4): 269–275.

Adamkovicova M, Toman R, Martiniakova M, Omelka R, Babosova R, Krajcovicova V, Grosskopf B, Massanyi P (2016) Sperm motility and morphology changes in rats exposed to cadmium and diazinon. Reprod Biol & Endocrinol. (14): 42, 1-7.

Adeleke GE, Ogunmola IA, Berena GA (2022) Spectroscopic and Chromatographic Characterization of *Anacardium occidentale* nut Shell Extract and its Enzyme Responses in *Periplaneta americana* (Cockroach). Int J Curr Res Acad Rev 10(03): 1-14.

Aderolu Z, Patrick I, Olawore A, Bamidele A (2020) Effects of herbal mixtures (*Jedi, Gbewutu* and *Opa-eyin*) on the health status of juvenile african catfish (C*larias gariepinus*). Egypt J Aquat Biol Fish 24(1): 31 – 48.

Adeyemi OO, Alabi AS, Adeyemi OA, Talabi OT, Abidakun OM, Joel IY, Stonehouse NJ (2021) Acute gastroenteritis and the usage pattern of antibiotics and traditional herbal medications for its management in a Nigerian community. PLoS One,
16(10): 1-16, e0257837.

Akinboro A, Bakare AA (2007) Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* (Linn). J Ethnopharmacol, 112:470-475.

Akinboro A, Kamaruzaman BM., Mohd Zaini A, Shaida Fariza S, Othman Ahmad S (2011) Antioxidants in aqueous extract of *Myristica fragrans* (Houtt.) suppress mitosis and cyclophosphamide-induced chromosomal aberrations in *Allium cepa* L. cells. J Zhejiang Univ Sci B, 12(11): 915-922.

Akinboro A, Bakare AA (2013) Spermatotoxic, cytotoxic and genotoxic evaluation of aqueous extract of *Ocimum gratissimum* in albino mice. Open Access Med Aromat Plants, 4(1): 10-14.

Akinboro A, Adedosu OT, Famurewa FO, Olowe T, Oyewole OO (2017) Antioxidant, proximate and cyto-genotoxic evaluation of anti-typhoid fever herbal recipe in *Allium cepa* cells, Glob J Bio-Sci Biotechnol 6 (1):24-29.

Akinboro A, Kamaruzaman M, Zaini A (2020) Effects of rutin in *Myristica fragrans* Houtt. against cyclophosphamide-induced genotoxicity in sperm cells, on liver and kidney tissues, in Swiss albino mice, Am J Physiol, Biochem and Pharmacol 10(1):7–17.

Akinboro A, Rufai MA, Ogunbameru AD, Oladosu BE, Taiwo MO (2021a) Evaluation of an antimalarial herbal mixture and each extract for DNA and chromosomal mutations in Swiss albino mice and *Allium cepa* cells, Asian J Med Biol Res 7 (3): 249-259.

Akinboro A, Rufai MA, Adeagbo RA, Akanbi AO, Ogunkola AS, Okunola DO, Adeyeye TD, Akanbi YR (2021b) Evaluation of an Antimalarial Herbal Recipe and its Individual Plant Extracts for DNA Fragmentation and Clastogenicity Potential in Swiss Albino Mice, Niger J Basic Appl Sci 29(2): 89-101.

Akinwande DV, Adeyemi JA, Olawuyi ST, Akinola BK, Adedire CO (2019) Protective effects of *Camellia sinensis* on *Syzygium aromaticum*- or chlorpyrifos induced reproductive toxicity in male Wistar rats, J Basic Appl Zool, 80:48, 1-9.

Amaeze OU, Acdef OA, Olugbakeef M, Lawal ABC (2020) Knowledge of Herbal Medicines and Herb-drug Interaction Among Medical and Pharmacy Students of the University of Lagos, Nigeria. Niger J Pharm Res, 16 (1):61-70.

Ashraf E., Mohamed A., Samar I., Yasmine A. (2018) Protective effects of *Syzygium aromaticum* oil (Clove) against acrylamide induced hepatic, renal, and testicular toxicity in rats. Int J Pharmacol Toxicol 6 (1): 12-17.

Awodele O, Fajemirokun O, Oreagba I, Samuel TA, Popoola TD, Coulidiaty AGV, Emeka PM (2018) Herbal Medicines Use: Remedies or Risks, West Afr Pharm, 29 (2): 1 – 18.

Azeez A., Isiugo-Abanihe U.C. (2019) Disease Diagnosis and Management: The Experiences of Hemorrhoid Herbal Vendors and Customers in Oyo State. Nigeria, J Soc Behav Health Sci, 13(1): 82–97.

Bassam A A (2020) Effect of Herbal Medicine on Fertility Potential in Experimental Animals – an Update Review. Mater Sociomed, 32(2): 140-147.

Bardoloi A, Soren AD (2022) Genotoxicity induced by medicinal plants. Bull Natl Res Cent, 46:119, 1-11.

Bello R.H, David MS, Olutayo AO, Abdulrazaaq KO, Aliyu A, Olufadi AHY, Salami OT, Lawal BA, Akerele JO (2019) Bacteriology of Some Liquid Herbal Products Sold in IlorinKwara State Nigeria. J Sci Prac Pharm, 6 (1): 324-330.

Bolawa OE, Oyeka DS, Akindele AJ (2020) Quantitation of Heavy Metals in Herbal Medicinal Drinks and Herbal Alcoholic Drinks in Lagos, Nigeria. Earth & Environ Sci Res & Rev 3 (1): 44-50.

Brimah P, Adigun R (2014) Jedi-jedi: Towards a formal medical classification of a sugar problem in Africans. Res Guild, 2 (1):1-8.

Dowling J, Vandenbergh J (eds) (2011) Guide for the care and use of laboratory animals (8th edition), The National Academies Press, Washington, D.C. 1-246.

Gaber ESB, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK (2020) *Syzygium aromaticum* L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. Biomolecules, (10),202: 1-16.

Ghazali Y, Bello I, Mustapha AK (2019) The use of herbal medicines amongst outpatients at the University of Ilorin Teaching Hospital (UITH), Ilorin, Kwara State – Nigeria. Complement Ther Med 42: 158–163.

Igwillo UC, Abdullahi MM, Etatuvie SO, Mbaoji CO, Chukwuemeka AE, Leo AJ (2019) Applications of Natural Medicine (Traditional Medicine/Complementary and Alternative Medicine) in Nigerian Healthcare Delivery System. Int J Recent Res Life Sci (IJRRLS) 6(3):16-22.

Luzhna L, Kathiria P, Kovalchuk O (2013) Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. Front Gen (4): 131, 1-17.

Maher T, Raus RA, Daddiouaissa D, Ahmad F, Adzhar NS, Latif ES, Abdulhafiz F, Mohammed A (2021) Medicinal Plants with Anti-Leukemic Effects: A Review. Molecules, (26): 2741, 1-25.

Okunji KE, Galadima M, Jigam AA (2012) Toxicological Evaluation of Five Herbal Drugs hawked in Minna, Niger State. J Appl Pharm Sci 2 (12):167-171.

Qin S, Hou DX (2017) The Biofunctions of Phytochemicals and Their Applications in Farm Animals: The Nrf2/Keap1 System as a Target. Eng, (3):738–752.

Roozbeh N, Amirian A, Abdi F, Haghdoost S (2021) A Systematic Review on Use of Medicinal Plants for Male Infertility Treatment. J Family Reprod Health, (15): 74-81.

Sarbast AM, Aveen RK, Abdulsamih MT, Hero AQ, Payam MA, Kurdo BC (2021) Effects of *Allium siculum* in ethylene glycol induced kidney stone in male albino rats. Kuwait J Sci, 48 (3): 1-11.

Slaoui M, Fiette L (2011) Histology procedures: From tissue sampling to histological evaluation, Drug Safety Evaluation: Methods and Protocols. Methods in Molecular Biology, Jean-Charles Gautier (ed.), 691:69-82.

Zahrah A, Umillah R, Muchtaridi M (2018) Analysis of quality and quantity control of herbal compounds using Fourier-transform infrared spectroscopy: A review journal. Drug Invention Today, (10): 2734-2742.

Figures

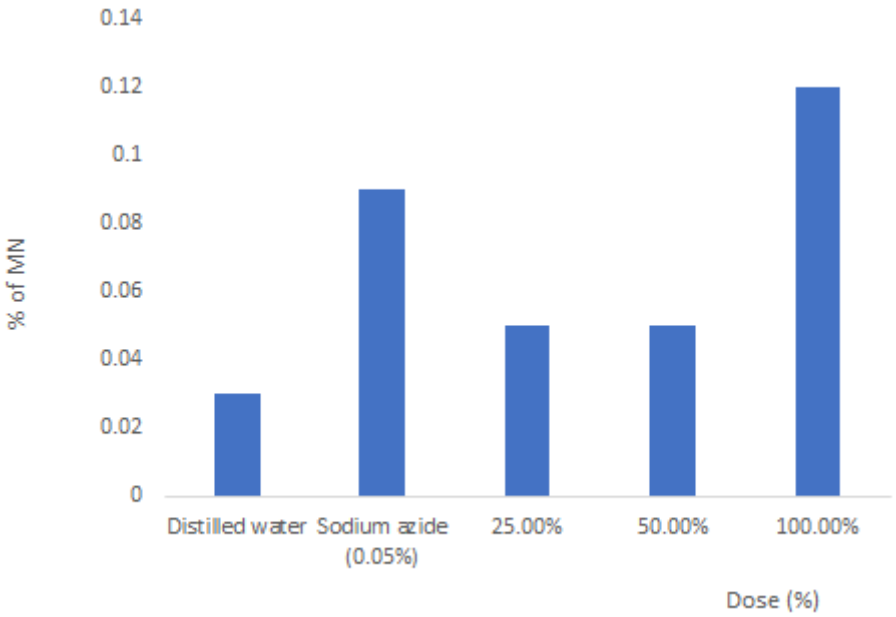


Figure 1

Induction of micronuclei by anti-hemorrhoid herbal recipe in bone marrow cells of albino rat

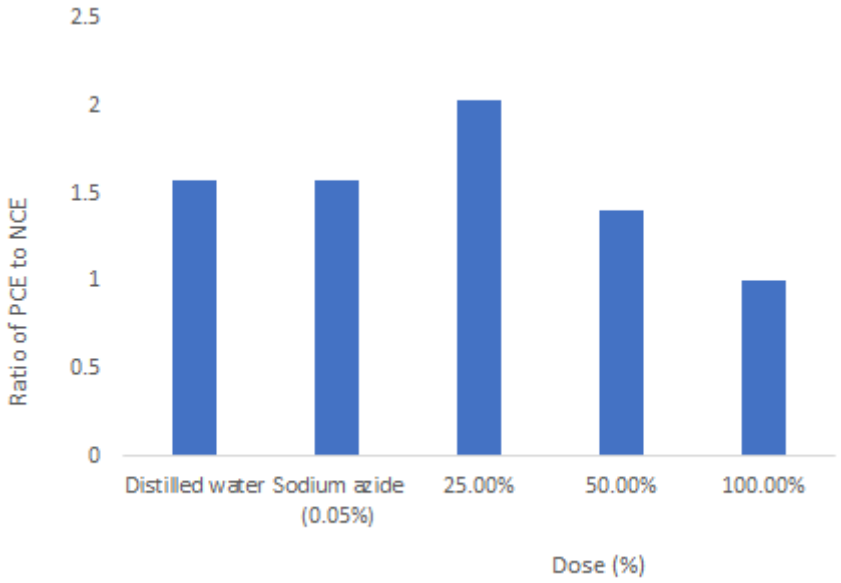


Figure 2

Ratio of PCE to NCE induced by the anti-haemorrhoid herbal recipe in the bone marrow of albino rats

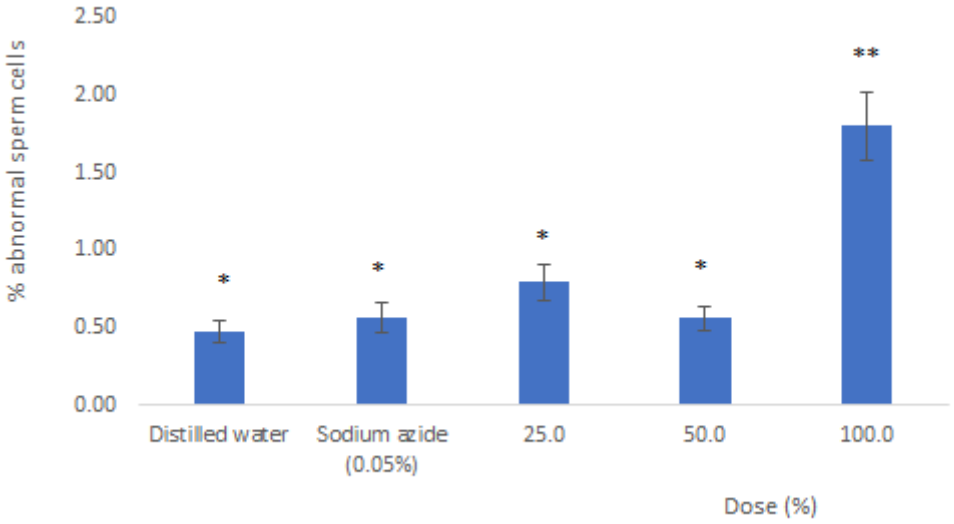


Figure 3

Induction of abnormal sperm cells by the anti-hemorrhoid herbal recipe in male albino rats

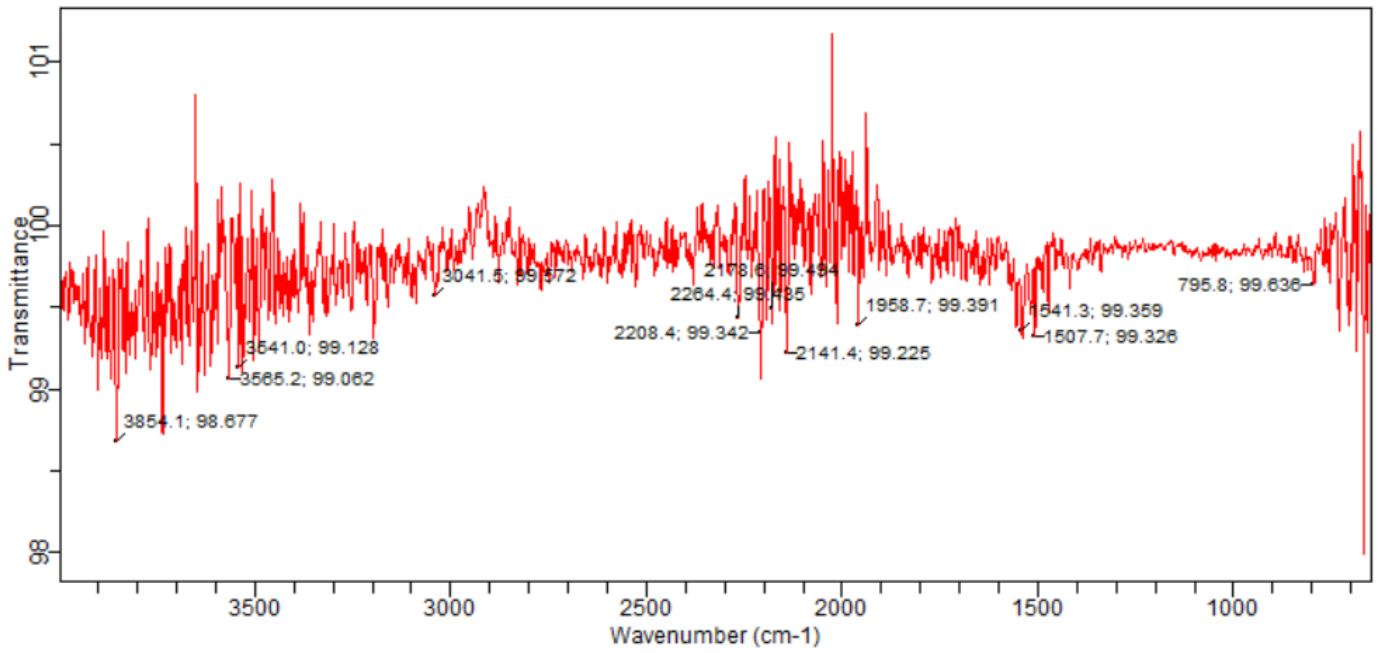


Figure 4

FTIR spectra of functional group of phytochemicals detected in anti-hemorrhoid herbal recipe

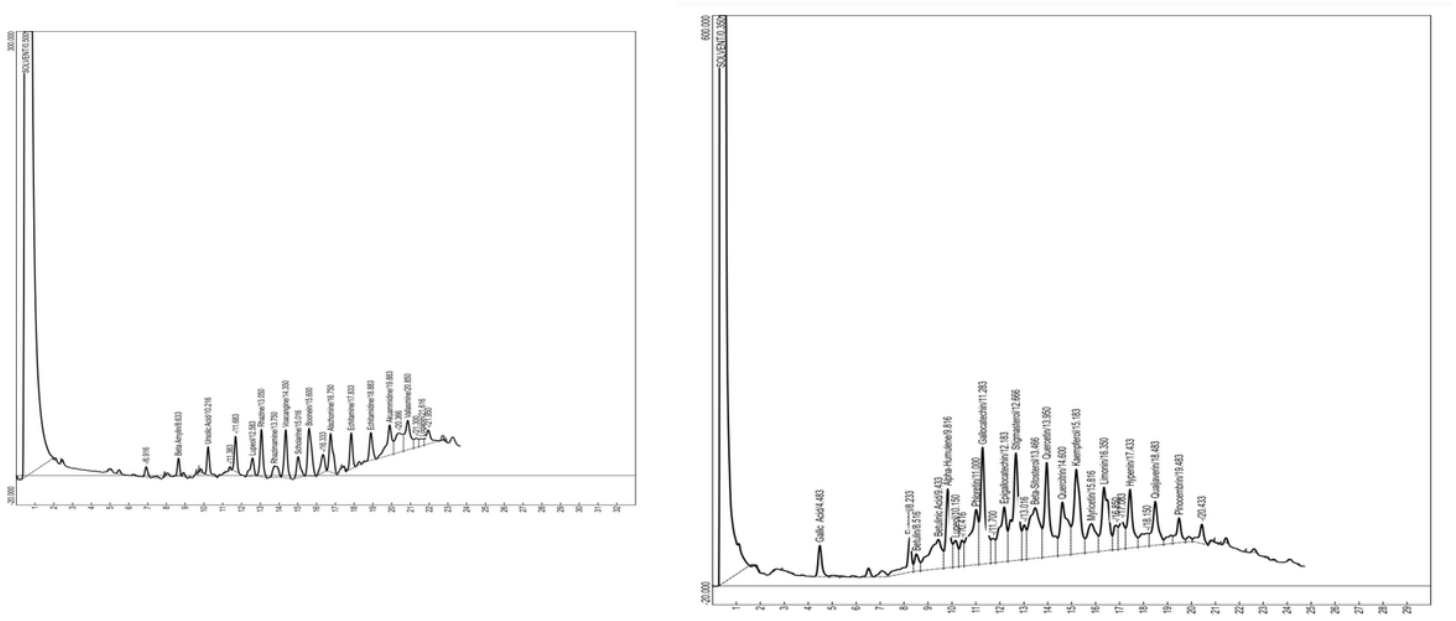


Figure 5

a: Chromatograms of phytochemicals detected in *Alstonia boonei* by GC-FID

b: Chromatograms of phytochemicals detected in *Syzygium aromaticum* by GC-FID

Supplementary Files

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