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Sub-chronic Toxicological evaluation of extract of *Lavandula stoechas* on Liver, haematological parameters, and feed consumption of Wistar rats

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Abstract
Introduction: *Lavandula stoechas*are commonly used to treat various medical ailments. This study aims to determine its toxicological impact on Wistar rats' meal consumption, liver, and haematological markers.
Method: Animals of either sex were selected. Group 1 received normal saline (10 ml/kg), while groups 2, 3, and 4 received 50, 100, and 200 mg/kg, respectively, of Lavandula stoechas. The animals were kept in standard cages and given oral access to the extract for 28 days before being weighed and killed. A cardiac puncture was utilized to get blood, which was then immediately tested. The haematoxylin and eosin (H&E) staining technique evaluated the plant's histological hepatotoxic potential.
Result: Compared to the control, ethanol leaf extract of *Lavandula stoechas* generated a substantial ($P<0.05$) increase in body weight in the first, third, and fourth weeks but not in the second. There was a significant ($P<0.05$) drop in RBC, HGB, and MCV but no change in neutrophils, basophils, eosinophils, or platelets. At 100 mg/kg dose, *Lavandula stoechas* generated a substantial ($P<0.05$) rise in ALP and BILD. Other metrics did not differ substantially ($P<0.05$) across doses delivered. A histological examination revealed minor tubular deformation.
Conclusion: Though the plant is primarily safe, the study results show that it may have a minor effect on the liver, implying that it should be used cautiously for an extended period. A histological examination revealed minor tubular deformation.

Key: *Lavandula stoechas*, blood, rats, liver

Introduction

The liver is a significant metabolic organ found solely in vertebrates that performs several vital biological tasks, such as detoxification and the formation of proteins and biochemicals required for digestion and growth (Nosek 2016). It is positioned in the right upper quadrant of the belly, below the diaphragm, and is primarily protected by the lower right rib cage in humans. Other metabolic functions include carbohydrate metabolism, hormone generation, food conversion and storage (such as glucose to glycogen), and red blood cell disintegration. The liver also functions as an accessory digestive organ, producing bile, an alkaline fluid containing cholesterol and bile acids that emulsifies and assists in the digestion of dietary fat. In many mammals, including humans but not rats, is the gallbladder, a small hollow pouch located directly beneath the right lobe of the liver, which stores and concentrates bile produced by the liver, which is then discharged to the duodenum to aid digestion (Molina *et al.*, 2012).

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The liver, primarily through its hepatocytes, regulates a wide range of high-volume biochemical activities, including the creation and breakdown of tiny and complex organic compounds, many of which are required for proper essential functions. The overall number of functions performed by the organ is estimated to be around 500 (Molina *et al.,* 2012).

Long-term compensation for the absence of liver function is unknown, while liver dialysis techniques can be utilized in the short term. Without the liver, artificial livers have not been produced to promote long-term replacement. Liver transplantation is the only treatment for total liver failure as of 2018 (Nguyen-Lefebvre *et al.,* 2015). However, long-term clinical experience has often supported therapeutic plants, with little to no scientific evidence of their safety and efficacy (Pathan *et al.,* 2003). Many medicinal herbs are used as medicine based only on a long-standing, widespread traditional folk practice. With the upsurge in the use of herbal drugs, a thorough scientific investigation of these plants is imperative based on the need to validate their folkloric usage (Younossi*et al.,* 2023). Although herbs are generally assumed to be safe, many unsafe and fatal side effects have been reported. These could be direct toxic effects, allergic reactions, effects from contaminants and/or interactions with drugs and other herbs (Younossi*et al.,* 2023). Phytotherapeutic products are often mistakenly regarded as less toxic because they are 'natural'. Nevertheless, those products contain bioactive principles that can potentially cause adverse effects (Schweitzer *et al.,* 2015).

Lavender (*Lavandula stoechas*), a member of the Labiatae family, is utilized in herbal medicine for various cosmetic and medicinal uses (Aviram, 2000). Inhaling lavender essential oils reduced cholesterol plaques in rabbits with atherosclerotic disease but did not affect serum cholesterol levels (Huxley & Neil 2003). In rats, lavander has a hypolipidemic impact (Sesso *et al.,* 2003). Furthermore, lavender aromatherapy has been shown to have vasodilatory effects and to increase coronary blood flow in humans (Fuhrman & Aviram, 2001). A lavender flower extract protected Isolated rat hearts from ischemia-reperfusion (IR) injury. In our recent study, lavender oil showed neuroprotective activity and antioxidant properties in an experimental stroke model (Cavanagh & Wilkinson, 2002). In a just-completed study, therapy with lavender essential oil after MI decreased ischemia injury in rats. This research sought to evaluate the sub-chronic effect of the extract of Lavandula stoechas on feed consumption and the liver and haematological parameters of Wistar rats.

Methods

Study design: This experimental study used laboratory animals (Winstar rats in a confined and controlled environment) as subjects. The study was conducted in the Animal House of Bringham University in Nigeria, West Africa.

Figure: Location of the study site

Study animals: 198 male and 239 female Wistar rats were acquired from Bingham University's Animal House. They were fed standard animal pellets purchased from Grand Cereals Limited and given unlimited water. The Animal Ethics Committee of Bingham University College of Health Sciences issued rights and approval for the studies (BU/2021/1132). The rats were randomized to treatment groups with six animals (n = 6). The care and handling of the animals followed established public health guidelines in the Guide for Care and Use of Laboratory Animals (2011).

Plant collection: Fresh leaves of *Lavandula stoechas*were were taken from its natural habitat in the adjacent Karu village, Nasarawa State, Nigeria. The Department of Botany at Bingham University validated the plant and assigned it a voucher number (BU1177).

Plant extraction: The *Lavandula stoechas*leaves were shade-dried for two weeks. The dried plant material was then crushed and reduced to little pieces. Extraction was carried out with 70% ethanol at a ratio of 200g of extract per litre of ethanol by percolation at room temperature. The liquid filtrates were concentrated and evaporated to dryness in a vacuum at 40°C using a rotary evaporator. The ethanol extract was kept at -4°C until it was needed.

Animal study: The Organization for Economic Development (OECD) guideline no. 425 for analysis of Chemicals was employed for this study (OECD 2008). Twenty-four (24) rats of either sex (weighing between 190 and 289g) were randomly chosen. The extract was given to rats in groups 2, 3, and 4 at doses of 50, 100, and 200 mg/kg, respectively, while group 1 served as the control group and received normal saline (10 ml/kg). The weights of the rats were recorded at the start of the experiment and once a week thereafter. The day of sacrifice was designated as D29, whereas the initial day of dosing was defined as D0.

Haematological analysis: The rats were sacrificed using diethyl-etherbythe protocol on the 29th day of the trial. Blood samples were slowly obtained through cardiac puncture. Blood was drawn into sample bottles containing EDTA for haematological analysis, including haemoglobin concentration, white blood cell counts (WBC), differentials (neutrophils, eosinophils, basophils, lymphocytes, and monocytes), red blood cell counts (RBC), platelets, and haemoglobin (Hb) concentration. This was accomplished with the help of an automated haematology machine (Cell-Dyn, Abbott, USA).

Food and water consumption: The difference between the daily supply of feed and water and the amount still available after 24 hours was used to compute the daily feed and water consumption. The rats were sacrificed on the 29th day of the experiment, and their organs were removed for further gross and histo-pathological investigation.

Biochemistry analysis: The second portion of the blood was collected into a plain bottle, allowed to clot, and centrifuged at 300rpm for 10 minutes. The serum collected was used to estimate biochemical parameters.
Histological study: The liver of the animals was surgically removed and weighed, and a part of each was fixed in 10% formaldehyde for histological processes.
Statistical analysis: Data were expressed as the Mean, standard Error of the Mean (SEM). Data were analyzed statistically using one-way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons between the control and treated groups. Values of $P \leq 0.05$ were considered significant.

Results

Effect of 28 days oral administration of *Lavandula stoechas* on body weight (g) in rats: At 100 mg/kg dose level, ethanol leaf extract of *Lavandula stoechas* caused a significant ($P<0.05$) increase in body weight in the first, third, and fourth weeks, but not in the second week when compared to the control (Table 1).

Effect of 28 days oral administration of *Lavandula stoechas*on haematological parametersin rats *Lavandula stoechas* induced a substantial ($P<0.05$) drop in red blood cell, hemoglobin, and platelet levels at 100 mg/kg dose and a significant ($P<0.05$) rise in mean corpuscular hemoglobin concentration in rats at 50 mg/kg dose compared to the control. However, mean corpuscular hemoglobin concentration did not affect basophiles, neutrophils, eosinophils, or lymphocytes ($P<0.05$). (Table 2).

Effect of 28 days oral administration of *Lavandula stoechas*on hepatic indices in rats: *Lavandula stoechas* significantly ($p<0.05$) increased ALP and BILD at 100 mg/kg dose. The other parameters' level was not significantly affected across doses administered ($p<0.05$).

Effect of 28 days oral administration of ethanol leaf extract of *Lavandula stonemason* histology Liver of rats: The liver showed vascular congestion, slight hepatic necrosis with slight sinusoidal congestion and lymphocyte hyperplasia at 100 mg/kg and 200 mg/kg, Sinusoidal congestion at 100 mg/kg and Moderate hepatic necrosis and vascular congestion at the control (10ml/kg normal saline) (Fig. 4).

Table 1: Effect of 28 days of oral administration of *Lavandula stoechas* on body weight (g) in rats

Treatment (mg/kg)	Week 1	Week 2	Week 3	Week 4
DW (10ml/kg)	195.11±6.7	197.10±6.3	194.61±5.3	195.72±9.1
50 mg/kg	199.13±10.7	197.65±15.8	197.58±3.1	172.60±5.8
100 mg/kg	206.26±19.5*	215.61±22.5	210.83±13.5*	212.10±20.40*
200 mg/kg	200.64±11.3	201.08±8.1	197.47±6.1	203.54±8.1

*Significantly different from the distilled water (DW) control at p<0.05. DW = distilled water

Table 2: Effect of 28 days oral administration of ethanol leaf extract of *Lavandula stoechas* on haematological parameters in Wistar rats

			Treatment (mg/kg)	
Haematological parameters	DW(10ml/kg)	LS (50)	LS (100)	LS (200)
WBC (×10^9/L)	8.17±0.77	6.74±1.42	3.70±0.67*	7.20±1.85
RBC (×10^12/L)	8.30±0.35	8.65±0.664	6.17±0.55*	7.74±0.25
HGB (g/dL)	15.90±0.56	15.24±0.66	11.36±0.87*	14.58±0.36
HCT (g/dL)	55.2±2.02	56.6±3.7	34.7±3.1*	53.4±1.8
MCV (fL)	66.7±0.94	65.4±1.45	57.1±0.3*	69.6±1.7
MCH (pg)	19.1±0.2	17.8±1.9	18.8±0.7	18.80±0.2
MCHC (g/dL)	29.1±0.2	27.4±1.3	32.1±0.6*	27.1±0.7
PLT (×10^9/L)	620.8±52.8	567.0±96.8	252.0±50.4*	670.4±55.8*
LYM (%)	86.8±4.6	85.0±4.3	82.8±5.8	86.41±3.1
NEUT (×10^9/L)	10.8±3.6	10.8±3.7	15.4±5.6	11.0±3.2
EOSI (×10^9/L)	1.5±0.3	2.4±0.8	1.8±0.44	1.2±0.2
BASO (×10^9/L)	1.0±0.28	2.0±0.5	2.5±1.5	3.3±2.2

Data presented as Mean ± SEM: n = 6, One way ANOVA, followed by Dunnett's post hoc for multiple comparison
*significantly different from the distilled water (DW) control at p<0.05. DW = distilled water (WBC = white blood cells, RBC = red blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT = platelet, LYM = lymphocyte, NEUT = neutrophils, EOSI = eosinophils, BASO = basophils).

Table 3: Effect of 28 days of oral administration of *Lavandula stoechas*on hepatic indices in wistar rats

		Treatment (mg/kg)		
Hepatic indices	DW(10ml/kg)	LS (50)	LS (100)	LS (200)
ALB (g/L)	38.5±1.7	44.2±1.0	35.2±1.7	43.2±1.3
ALP (IU/L)	113.2±6.7	152.0±8.2	370.0±43.7*	125.5±6.3
ALT (IU/L)	62.8±3.4	70.0±10.2	94.2±10.4	87.5±1.9
AST (IU/L)	299.4±9.9	297.3±7.6	175.2±3.8	233.0±1.7
BILD (mol/L)	0.32.1±0.1	0.3±0.7*	0.6±0.2*	0.6±0.1
BILT (mol/L)	2.3±0.5	2.9±0.2	3.4±0.2*	2.8±0.2
TP (g/L)	69.6±3.1	66.1±2.7	61.3±5.1	82.2±2.1

Data presented as Mean ± SEM: n = 6, One Way ANOVA, followed by Dunnett's post hoc for multiple comparison *significantly different from the distilled water (DW) control at p <0.05. DW = distilled water (ALB = albumin, ALP = alanine phosphatase, ALT = alanine transaminase, BILD = unconjugated bilirubin, BILT = conjugated bilirubin, TP = total protein).

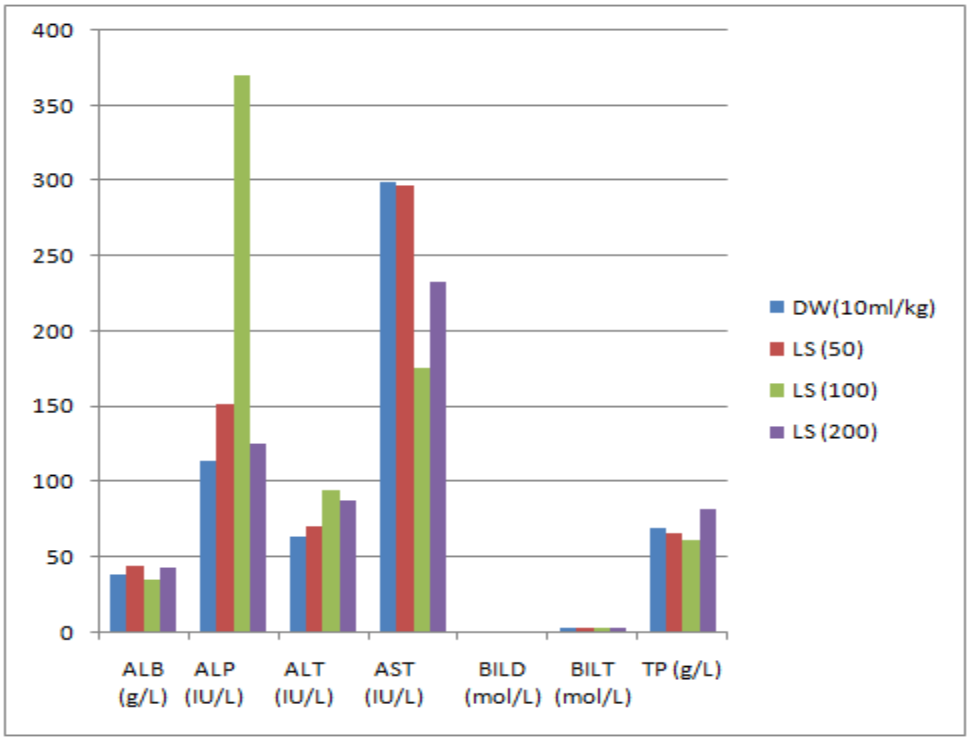

Figure 2: Effect of 28 days oral administration of *Lavandula stoechas*on hepatic indices in Wistar rats

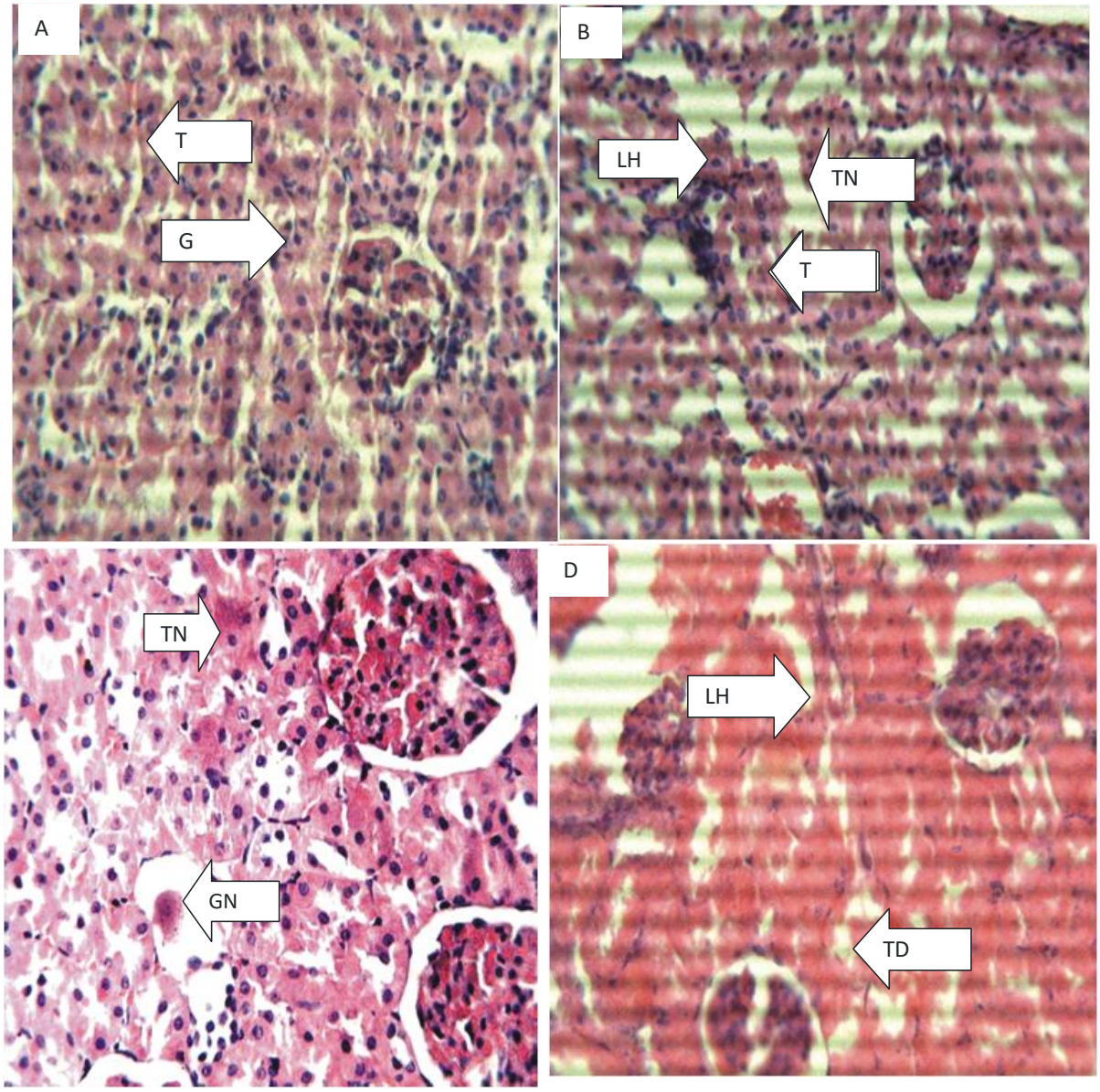

Figure 3: figure of the liver (Hematoxylin and eosin. H and E ×100). (a) Control group, shows normal hepatocyte (H). (b) LS 50 mg/kg, (c) LS 100 mg/kg, (d) LS 200 mg/kg.

Discussion

Herbal medicines are essential in Oriental medicine (OM), which has been practised for over 2000 years (El-Hilaly*et al.,* 2003). Herbal remedies include dietary supplements containing herbs, either alone or in combination. They are sometimes called botanicals because of their fragrances, flavors, and/or therapeutic properties (Furman 2015). Herbal drugs are widely used worldwide and available in health food stores without a prescription (Toygar *et al.*, 2020). Over the past decade, there has been an increased global interest in traditional systems of medicine and herbal medicinal products. This surge has partly been due to the rare or non-existent access to modern medicine in developing countries and large populations' acceptance of herbal medicines in developed nations (Setacci*et al.*, 2020).

Haematological parameters are functional indices that can be employed to assess the toxic potentials of plant extracts in living systems (Hüser*et al.,* 2020). They can also explain the related functions of chemical compounds/plant extracts. Herbal plants have been used for various medicinal and health benefits. Medicinal plants are often consumed locally without a graded dose or expected duration of use (Liamis*et al.*, 2014). This can precipitate unexpected side effects on the tissue, organ or body system. In this study, the impact of the plant on the liver and vascular parameters was evaluated (Maric *et al.*, 2022).

Ethanol extract of *Lavandula stoechas*resulted in a significant decrease in the red blood cells, haemoglobin and platelet compared to the control group of rats. This indicated that the plant may either suppress the production of red blood cells, decrease the lifespan of red blood cells or cause problems with how the body uses iron. Anemia, or decreased red blood cell count, can induce weariness and weakness. When a person's red blood cell count is lower than expected, their body needs to work harder to get enough oxygen to the cells. A low RBC count can result in some symptoms and health concerns. Hemoglobin is required for oxygen transmission in the blood from the lungs to the tissues. In muscle cells, myoglobin receives stores, transports, and releases oxygen (Archer *et al.,* 2003). The extract did not affect the levels of basophiles, neutrophils, eosinophils, or lymphocytes. This suggests that the plant does not affect the body's immune system. It could also imply that the plant has immunomodulatory properties.

To determine liver function, the activity and concentrations of serum ALT, AST, ALP, bilirubin (total and direct), total cholesterol, total protein, and albumin, all of which originate in the cytoplasm, were measured (Spampinato *et al.,* 2020). When there is hepatopathy, these enzymes and chemicals leak into the bloodstream, acting as a marker for liver disease (Spampinato *et al.,* 2020). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most often utilized indicators of liver (hepatocellular) injury. Because AST is detected in various organs, such as the heart and skeletal muscle, ALT is thought to be a more specific sign of liver inflammation (Algieri *et al.,* 2016).

Normal serum ALT, AST, total protein, and albumin levels in extract-administered rats indicate that the plant has little to no effect on inducing liver injury. The most common test for detecting biliary blockage is alkaline phosphatase estimate. Bilirubin is the primary bile pigment in humans, and when it is increased, it causes the yellow colouring of the skin, known as jaundice. Bilirubin is formed primarily from the breakdown of a substance called heme found in red blood cells. It is taken up from the blood, processed, and then secreted into the bile by the liver. Healthy individuals usually have a small amount of bilirubin in the blood (<17µmol/L). Conditions that produce increased bilirubin synthesis, such as red blood cell death, or decreased bilirubin removal from the bloodstream, such as liver failure, may result in a modest increase in bilirubin levels in the blood (Spampinato *et al.,* 2020). The study found that ALP, BILD, and BILT levels increased dramatically. These findings imply that the plant extract may damage red blood cells, mildly block the bile duct, and/or limit the evacuation of RBC from the bloodstream. A histological examination that reveals minor hepatic necrosis and other typical liver features agrees with other measures.

Conclusion

Although the *Lavandula stoechas* plant is generally safe, the study's findings reveal that it may have a minor impact on the liver, implying that it should be used cautiously over an extended period. A histology examination reveals only minor tubular distortion.

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