PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (12) 2729-2736 December 2023

Histological and Toxicity Studies on Extracts of *Stachytarpheta Jamaicensis* on Liver, Kidney, Lungs and Heart of an Animal Models (Albino Rats)

***[1]OMORODION, NT; [2]IYARE, GI; [3]KOFI, NB**

**[1]Department of Medical Laboratory Sciences, School of Basic Medical Sciences, College of Medicine University of Benin, Benin city, Nigeria*
[3]Department of Medical Laboratory Science, Faculty of Applied Health Sciences, Edo State University, Uzairue
[3]River State College of Health Sciences and Management Technology, Port Harcourt, River State, Nigeria

**Corresponding Author Email: terry.omorodion@uniben.edu; ORCID ID: 0000-0002-5500-5293*
Co-Authors Email: iyareprince2@yahoo.com, jnwibana@gmail.com

ABSTRACT: The objective of this study was to investigate the histological and toxicity studies on extracts of *Stachytarpheta Jamaicensis* on liver, kidney, lungs and heart of an animal models (Albino Rats) using appropriate standard methods. The results of the acute toxicity study showed that the LD50 values for all extracts were higher than 5000 mg/kg, as there were no signs of illness or death. The sub-acute toxicity investigations demonstrated that there were no adverse effects observed in the haematological, biochemical, and histological parameters of the blood and vital organs, respectively. Nevertheless, a notable disparity ($p > 0.05$) was detected in the relative weights of organs and body weights when comparing the high dose of 2000 mg/kg to the control group. This study thus provides novel evidence that the oral administration of aqueous and ethanol extracts derived from the leaf and root of *Stachytarpheta jamaicensis* at low to medium doses over a period of 21 days does not induce any harmful effects.

DOI: https://dx.doi.org/10.4314/jasem.v27i12.9

Cite this paper as: OMORODION, N. T; IYARE, G. I; KOFI, N. B. (2023). Histological and Toxicity Studies on Extracts of Stachytarpheta Jamaicensis on Liver, Kidney, Lungs and Heart of an Animal Models (Albino Rats). *J. Appl. Sci. Environ. Manage.* 27 (12) 2729-2736

Dates: Received: 12 November 2023; Revised: 10 December 2023; Accepted: 15 December 2023 Published: 30 December 2023

Keywords: *Stachytarpheta jamaicensis*, liver, kidney, lung, heart

Medicinal compounds originating from botanical sources have been shown to be valuable assets in various civilizations and nations, consistently fulfilling significant roles in the provision of healthcare on a global scale. Indigenous practices of using herbs are an important part of cultural beliefs and the main way that people get better in developing cultures. According to Patil and Gaikwad (2010), the prevalence of counterfeit pharmaceuticals and the occurrence of adverse reactions have led to the widespread adoption of plant-based medicines, even in highly industrialized countries (Dias and Takahashi, 1994). However, recent research has indicated that numerous medicinal herbs have also exhibited negative consequences (Nath and Yadav, 2015). Hence, it is imperative to conduct toxicity investigations on any medicinal botanical extract that is expected to have preclinical or clinical utility. For several decades, medicinal plants have been employed as a natural reservoir of bioactive chemicals, which possess therapeutic properties and provide cost-effective remedies for a diverse range of ailments. The universal acceptance of utilizing medicinal plants as a substitute for chemically synthesized pharmaceuticals in disease treatment has been observed. Extensive research has consistently shown that medicinal plants possess secondary metabolites and exhibit a range of medicinal properties, such as analgesic, antidiarrheal, antimicrobial, antioxidant, antihypertensive, antinociceptive, and anti-inflammatory effects (Idu *et*

al., 2007; Ramakrishnan and Sivaranjani, 2013; Joshi *et al., 2010;* Meena and Pitchai, 2011; Rahmatullah *et al., 2011;* Vikrant and Arya, 2011). *Stachytarpheta jamaicensis* (L.) Vahl, a plant belonging to the Verbenaceae family, is widely recognised by various common names such as Gervao, Brazilian tea, Verbena cimarrona, rooter comb, or blue porter weed (Idu *et al., 2007;* Okwu and Ohenhen, 2010). The plant predominantly thrives in the tropical parts of the Americas, alongside the subtropical woods of Africa, Asia, and Oceania. *S. jamaicensis* holds significant importance as a medicinal plant due to its notable therapeutic capabilities within traditional and folk medical practices. According to Sivaranjani *et al.* (2014), the elderly have historically utilised this plant as a remedy for many ailments such as allergies, respiratory disorders, cough, cold, fever, constipation, digestive issues, dysentery, and to facilitate menstruation, among other uses. *Jamaicensis* is believed to have originated in Taiwan, where it was initially cultivated as an ornamental plant. Subsequently, its ornamental significance led to its distribution to several other nations. *Stachytarpheta jamaicensis* is a member of the Verbenaceae family and falls under the genus Stachytarpheta, which encompasses a total of 140 species. These species are primarily found in tropical and subtropical regions of the Americas, with limited distribution in tropical Africa, Asia, and Oceania (Stevens, 2008). *S. jamaicensis* is a shrub-like botanical species characterised by its erect growth habit, reaching a maximum height of 2 metres. The leaves of this plant are broadly elliptic to ovate in shape, exhibiting a glossy and dark green appearance. When fresh, the leaves are bullate on the upper surface and possess indistinct petioles with acute apices. Notably, the upper margins of the leaves feature divergent marginal teeth that are more than twice the length of those found on the lower margins. Upon drying, the leaves typically darken to a dark brown or blackish brown color. The flowers exhibit a dark purple-blue coloration, with the stamens being enclosed within and the style somewhat protruding. The style surpasses the anthers by a margin of 1 mm. The bracts are lanceolate in shape, possessing membranous margins below the midpoint. According to Shih-Huei and Ming-Jou (2003), the stems of the plant are characterised by their flexibility and slender shape, as well as the presence of a rachis. *Jamaicensis* is commonly used as an anti-helminthic agent in traditional medicine for the treatment of ulcers, venereal illnesses, dropsy, rheumatic/inflammatory conditions, stomach ailments, and fevers. The use of tea derived from the stem bark has been found to be beneficial in the management of dysentery and diarrhoea. Previous studies conducted on Stachytarpheta species have demonstrated the presence of many phytochemicals, including steroids, glycosides, flavonoids, phenylpropanoid, phenylethanoid, and iridoid glycosides, as well as aliphatic and phenolic compounds (Roengsumran *et al., 2002)*.

Despite the traditional usage of this plant, there has been a lack of investigation into its toxicological properties. Despite the numerous documented phytochemical benefits of *S. jamaicensis*, including its anti-helminthic properties and efficacy in treating venereal diseases, it is noteworthy that there are currently no regulations in place within Edo State, Nigeria, governing the utilisation of this particular plant. The objective of this study was to assess histological and toxicity studies on extracts of *Stachytarpheta Jamaicensis* on liver, kidney, lungs and heart of an animal models (Albino Rats).

MATERIALS AND MATERIALS

Plant materials: The identification and authentication of *Stachytarpheta jamaicensis* were conducted, after which the plant samples were harvested and processed into a fine powder. The resulting powder was then stored in airtight containers for future use.

Extraction Preparation: A quantity of 1 kilogramme of powdered leaves and roots was subjected to extraction using distilled water and ethanol solvents, respectively, employing Soxhlet equipment.

Animals Housing: Adult male and female individuals exhibiting albinism Wistar rats and Swiss albino mice were bred in a random manner within the Animal House of the Phytomedicine Unit, which is located in the Department of Plant Biology and Biotechnology within the Faculty of Life Sciences at the University of Benin, Benin City. The animals used for experimental purposes were housed in polypropylene cages, with a maximum of 6 mice or rats per cage. The cages were lined with wood shavings, which were replaced daily. The animals were provided with free access to tap water and pelletized top feed. The rats were provided with a regular pellet meal and had unrestricted access to water. The animals were subjected to a light-dark cycle of 12 hours and were managed in accordance with established protocols.

Study on Toxicity: The present investigation focuses on conducting an acute toxicity study. The acute toxicity research was conducted using the Organisation of Economic Co-Operation and Development (OECD) guideline 420, which outlines the testing procedures for chemicals. The study followed the guidelines established in 2008, with minor adjustments. A total of sixteen (16) groups, each

consisting of three male and three female mice, were subjected to different doses (50, 100, 500, and 5000 mg/kg per body weight) of four distinct extracts. The mice were closely monitored for 72 hours to identify any indications of toxicity, death, or morbidity.

A study on the sub-acute toxicity: The sub-acute toxicity research was conducted following the guidelines provided by the World Health Organisation (2000) and the Organisation for Economic Co-Operation and Development (OECD) (2008), with minor adjustments. A sub-acute toxicity investigation was conducted using the up-and-down method. A total of sixteen (16) groups, each consisting of three male and three female mice, were subjected to varying doses of four different extracts: 50, 100, 500, and 5000 mg/kg per body weight. The mice were closely monitored for a duration of 21 days to assess any potential indications of toxicity, mortality, and morbidity. After a period of 24 hours following the administration of the last doses, the animals were euthanized, and samples of tissues and blood were obtained. Various assessments were conducted, including a complete blood count, an analysis of metabolic parameters, and an examination of histology.

Tissue Processing: The tissues were then fixed in 10% formalin for histological processing (Omorodion et al., 2019).

Haematolxylin and Eosin Staining Technique: Deparaffinize, ethanol-treat, and water-wash. Stain the sample for two minutes at room temperature with Harris hematoxylin. Let the colour develop in the lukewarm water for 10 minutes. Carry out eosin staining at room temperature for 3 minutes. , Distinguish, drench, and clear. (Omorodion *et al.*, 2019).

Photomicrography: The sections were analysed via an Olympus binocular microscope® equipped with an integrated illumination system at magnifications of x40 and 100. The photomicrography of sections was afterwards conducted utilising a digital microscope camera, which was affixed to an Olympus trinocular microscope.

Statistical Analysis: Results from the studies were taken as the mean ± SEM. Statistical analysis was arrived at using graph pad prism 8 version software (UK). Comparisms amongst treated and control groups were analysed using one-way ANOVA by Dunnett's multiple comparisms test. $P < 0.05$ was regarded as indicating significant differences.

RESULTS AND DISCUSSION

*Acute and Sub-Acute Toxicity: Acute Toxicity***:** After 72 hours of administration of 50, 100, 500, 5000 mg/kg ethanol and aqueous leaf extracts respectively showed no mortality, loss of cognitive/ loss of agility or any physical morphology associated with toxicity was observed in all the treatment groups (Table 1). Also after 72 hours of administration of single dose of 50, 100, 500 and 5000 mg/kg aqueous and ethanol root extracts of *Stachytarpheta jamaicensis* revealed no mortality, loss of cognitive/ loss of agility or any physical morphology associated with toxicity was observed in all the treatment groups (Table 2). The lipid profile tests carried out on the sera after sacrifice showed significant difference in triglyceride, total cholesterol, HDL (High Density Lipoprotein) and LDL (Low Density Lipoprotein) Table 7 and 8. The weight index carried out at 7 days intervals showed significant loss of weight between control and treated groups (Table 9 and 10).

Table 1: Acute effect of ethanol and aqueous leaf extracts of *Stachytarpheta jamaicensis* on albino rats after 72 hours administration of single dose (50,100, 500 and 5000 mg/kg) of extracts.

Group(s)	Dose (mg/kg)	Cognition	Agility	Signs of Toxicity such as: Grooming, nausea, writhing,	Mortality after 72 hours of administration
Control	2 ml/kg	Normal	Normal	None	0/6
ALESJ	50	Normal	Normal	None	0/6
ALESJ	100	Normal	Normal	None	0/6
ALESU	500	Normal	Normal	None	0/6
ALESJ	5000	Normal	Normal	None	0/6
ELESJ	50	Normal	Normal	None	0/6
ELESJ	100	Normal	Normal	None	0/6
ELESJ	500	Normal	Normal	None	0/6
ELESJ	5000	Normal	Normal	None	0/6

ALESJ= Aqueous Leaf Extract of Stachytarpheta jamaicensis; ELESJ= Ethanol Leaf Extract of Stachytarpheta jamaicensis

Histological study: The organs isolated also showed significant difference in the heart, liver and kidney weight when compared with control and treated groups (Figure 1, 2 and 3). The Histological study carried out on the heart, liver and kidney of the albino *Wistar* rats after 21 days treatment with the highest dose of 2000 mg/kg administered did not display any symptoms of toxicity (Plates 1-4).

Table 2: Acute effect of ethanol and aqueous root extracts of *Stachytarpheta jamaicensis* on albino *Wistar* rats after 72 hours administration of single dose (50,100, 500 and 5000 mg/kg) of extracts.

Group(s)	Dose (mg/kg)	Cognition	Agility	Signs of Toxicity such as: Grooming, nausea, writhing,	Mortality after 72 hours of administration
Control	2 ml/kg	Normal	Normal	None	0/6
ARESJ	50	Normal	Normal	None	0/6
ARESJ	100	Normal	Normal	None	0/6
ARESJ	500	Normal	Normal	None	0/6
ARESJ	5000	Normal	Normal	None	0/6
ERESJ	50	Normal	Normal	None	0/6
ERESJ	100	Normal	Normal	None	0/6
ERESJ	500	Normal	Normal	None	0/6
ERESJ	5000	Normal	Normal	None	0/6

ARESJ= Aqueous Root Extract of Stachytarpheta jamaicensis; ERESJ = Ethanol Root Extract of Stachytarpheta jamaicensis

Table 5: Effects of ethanol and aqueous leaf extracts of *Stachytarpheta jamaicensis* on liver function tests

		ALESJ				**ELESJ**			
Parameter	**Control**	**50 mg/kg**	**100 mg/kg**	**500 mg/kg**	**2000 mg/kg**	**50 mg/kg**	**100 mg/kg**	**500 mg/kg**	**2000 mg/kg**
ALP (IU/L)	62 ± 0.9	70±3.5*	64.2± 3.0	60± 4.6	63.8± 1.7	60.8±1.2	61.8± 1.2	60.8± 1.2	61.8± 1.2
ALT (IU/L)	21.0± 2.9	22.4± 1.7	21.0± 1.8	21.8± 2.3	20.8± 2.9	20.4± 1.9	21.2± 2.1	21.4± 2.3	20.8± 2.5
AST (IU/L)	48.6± 7.8	50.6± 2.9	51.4± 4.1	49.2± 6.3	48.6± 5.9	50.4±4.2	50.0± 3.4	49.4± 4.3	48.2± 9.3
Total Bilirubin	0.5±0.02	0.5±0.02	0.5±0.02	0.5±0.04	0.4±0.05	0.5±0.06	0.5±0.07	0.6±0.05	0.4±0.04
Conjugates	0.3±0.08	0.4±0.02	0.3±0.07	0.2±0.04	0.3±0.03	0.5±0.02	0.4±0.05	0.3±0.09	0.4±0.06
Albumin	3.4±0.06	3.0±0.04	3.4±0.05	3.2±0.08	3.3±0.09	3.1±0.07	3.2±0.02	3.1±0.08	3.3±0.16
Total protein	6.4±0.07	6.2±0.5	6.6±0.9	6.8±0.3	6.4±0.7	6.2±0.7	6.4±0.5	6.2±0.7	6.4±0.6
Globulin	3.3±0.2	3.8±0.3	3.4±0.8	2.7±0.9	3.0±0.4	2.8±0.5	2.9±0.6	3.0±0.4	3.4±0.5

Values are expressed in mean ± SEM (n=5), p<0.05 compared to control; ***Keys:*** *ALP- Alkaline Phosphates; ALT- Alanine amino transferase; AST- Aspartate amino- transferase; ALESJ= Aqueous Leaf Extract of Stachytarpheta jamaicensis; ELESJ= Ethanol Leaf Extract of Stachytarpheta jamaicensis*

TABLE 6: Effects of ethanol and aqueous root extracts of *Stachytarpheta jamaicensis* on liver function tests

		ARESJ				**ERESJ**			
Parameters	**Control**	**50 mg/kg**	**100 mg/kg**	**500 mg/kg**	**2000 mg/kg**	**50 mg/kg**	**100 mg/kg**	**500 mg/kg**	**2000 mg/kg**
ALP	62.4± 0.9	62.2±2.8	63.2± 1.4	61.0± 2.1	62.6± 2.3	65.2± 2.3	64.2± 2.1	62.4± 3.2	65.6± 4.8
ALT	21.0± 2.9	21.4± 2.9	22.0± 2.3	23.2± 2.2	20.8± 2.9	23.4± 1.9	21.2± 2.0	23.4± 2.2	25.8± 2.5
AST	49.6± 7.8	51.6± 2.8	50.4± 4.6	49.2± 3.4	48.6± 2.5	49.4± 4.2	51.0± 3.3	49.4± 4.3	46.2± 2.2
Total Bilirubin	0.5±0.04	0.6±0.02	0.4±0.04	0.6±0.07	0.4±0.03	0.7±0.05	0.5±0.08	0.5±0.07	0.4±0.03
Conjugates	0.3±0.08	0.3±0.02	0.2±0.09	0.2±0.05	0.2±0.03	0.2±0.02	0.2±0.04	0.2±0.05	0.2±0.05
Albumin	3.4±0.07	3.0±0.04	3.4±0.05	3.2±0.06	3.3±0.07	3.8±0.04	3.6±0.02	3.4±0.06	3.3±0.9
Total protein	6.4±0.07	6.2±0.05	6.6±0.09	6.8±0.03	6.4±0.07	6.2±0.07	6.1±0.05	6.2±0.7	6.4±0.55
Globulin	3.3±0.21	2.6±0.10	2.7±0.11	2.9±0.18	3.0±0.10	2.5±0.28	2.5±0.29	2.7±0.29	2.8±0.29

Values are expressed in mean ± SEM (n=5), p<0.05 compared to control; ***Keys:*** *ALP- Alkaline Phosphates; ALT- Alanine amino transferase; AST- Aspartate amino- transferase; ARESJ= Aqueous Root Extract of Stachytarpheta jamaicensis; ERESJ= Ethanol Root Extract of Stachytarpheta jamaicensis*

TABLE 7: Effects of ethanol and aqueous leaf extracts of *Stachytarpheta jamaicensis* leaves and root on lipid profile tests

		ALESJ				**ELESJ**			
Treatment (mg/kg) Parameters	Control	50	100	500	2000	50	100	500	2000
Total cholesterol	84.2± 2.2	80.6± 1.9*	81.0.± 2.3	80.0± 2.6*	78.8±2.6*	80.6± 1.9*	80. 8± 2.9*	77.8 ± 2.5*	71.2± 2.8*
Triglyceride	64.2± 2.9	50.8± 3.2*	54.4± 3.4*	53.0 ± 3.4*	45.0±3.9*	56.0± 2.6*	53.8± 3.2*	51.0± 3.8*	49.0± 3.6*
HDL	46.6± 1.0	55.4± 1.4*	48.0± 1.4	48.4± 1.3	49.6± 1.5	53.2± 1.7*	49.8± 1.9	48.4± 1.8	47.8± 1.7
LDL	39.4± 2.1	20.8± 1.6*	22.6± 1.8*	23.2± 1.9*	25.6±2.1*	20.6± 2.2*	20.2 ± 2.6*	22.4± 2.7*	21.4± 2.3*

*Values are expressed in mean ± SEM (n=5), * =p<0.05 compared to control;* ***Keys:*** *HDL- High Density Lipoprotein; LDL- Low Density Lipoprotein; ALESJ= Aqueous Leaf Extract of Stachytarpheta jamaicensis; ELESJ= Ethanol Leaf Extract of Stachytarpheta jamaicensis*

Table 8: Effects of ethanol and aqueous root extracts of *Stachytarpheta jamaicensis* on lipid profile tests

		ARESJ				**ERESJ**			
Treatment (mg/kg) Parameters	Control	50	100	500	2000	50	100	500	2000
Total cholesterol	84.2± 2.3	78.2±1.5*	80.2±2.2*	76.6±2.8*	74.6±3.5*	79.6±1.3*	81. 8±1.6	80.4±1.8*	78.0±1.9*
Triglyceride	64.2± 2.3	53.0±3.2*	59.2±5.3*	55.6± 4.0*	53.8±3.2*	52.0±1.9*	55.0±4.5*	52.6±2.7*	50.2±3.9*
HDL	46.6± 1.0	57.6±1.2*	56.8±1.8*	56.2±2.1*	55.8±1.9*	59.6±4.1*	58.2±1.2*	50.2±2.2*	54.0±3.1*
LDL	39.4± 2.2	20.4±2.4*	28.6±2.3*	27.0±2.4*	23.8±2.3*	24.6±1.3*	21.4±2.5*	20.2±3.3*	18.2±2.4*

Values are expressed in mean ± SEM (n=5), * = p<0.05 compared to control; **Keys:** HDL- High Density Lipoprotein; LDL- Low Density Lipoprotein; ARESJ= Aqueous Root Extract of *Stachytarpheta jamaicensis;* ERESJ= Ethanol Root Extract of *Stachytarpheta jamaicensis;*

Table 9: Effect of Ethanol and Aqueous Leaf Extracts of *Stachytarpheta jamaicensis* Body weight index of albino rats after 21 days (3 weeks) treatment

	ALESJ				ELESJ			
Treatments (mg/kg)	**Initial weight**	**Weight at week 1**	**Weight at week 2**	**Weight at week 3**	**Initial weight**	**Weight at week 1**	**Weight at week 2**	**Weight at week 3**
Control	153±3.5	156±2.7	161±5.2	168±3.4	153±3.5	156±2.7	161±5.2	168±3.4
50	152±5.1	157±4.4	160±5.1	163±3.2	154±5.6	159±3.8	161±2.8	166±2.6
100	155±6.3	158±4.8	161± 4.7	164±2.5	152±6.2	159±5.7	161±4.0	167±2.9
500	153±2.3	160±4.1	162±3.3	165±3.7	153±3.1	160±1.9	165± 2.4	160±3.5
2000	153±5.0	161±6.0	150±4.3*	145±3.4*	152±6.6	164±5.9	152±6.5*	131±5.8*

*Values are expressed in mean ± SEM (n=5), *=p<0.05 compared to control; ALESJ= Aqueous Leaf Extract of Stachytarpheta jamaicensis; ELESJ= Ethanol Leaf Extract of Stachytarpheta jamaicensis;*

Table 10: Effect of Ethanol and Aqueous Root Extracts of *Stachytarpheta jamaicensis* Body weight index of albino rats after 21 days (3 weeks) treatment

	ARESJ				ERESJ			
Treatments (mg/kg)	**Initial weight**	**Weight at week 1**	**Weight at week 2**	**Weight at week 3**	**Initial weight**	**Weight at week 1**	**Weight at week 2**	**Weight at week 3**
Control	153±3.5	156 ±2.7	161±5.2	168±3.4	153±3.5	156 ±2.7	161±5.2	168±3.4
50	154 ± 5.2	157±2.2	158±1.4	160±2.4	155±4.7	157±5.1	159±4.9	161±3.2
100	153± 6.3	156± 2.9	159± 2.7	162± 2.4	155±4.3	158±2.4	159±3.2	160±2.6
500	152± 4.2	156± 4.5	158± 3.7	160± 3.1	156±4.2	159±1.3	160±1.7	161±4.1
2000	153± 4.4	157± 2.9	153± 4.5*	134± 3.8*	150±3.5	153±3.9*	143±3.0*	136±4.2*

*Values are expressed in mean ± SEM (n=5), *=p<0.05 compared to control; ARESJ= Aqueous Root Extract of Stachytarpheta jamaicensis; ERESJ= Ethanol Root Extract of Stachytarpheta jamaicensis;*

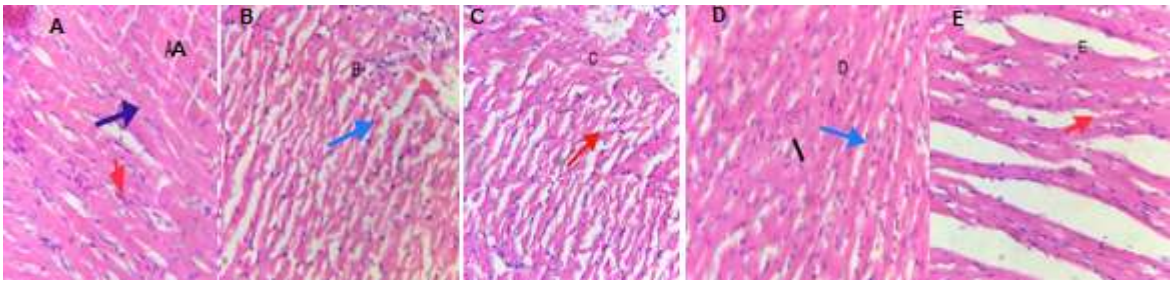

Plate 1: Photomicrograph of Heart of rats treated with *S. urticaefolia* leaf and root (Hx & E stain x400). Key: A= Control, B= ALESJ- Aqueous Leaf Extract of *Stachytarpheta jamaicensis*, C= ARESj- Aqueous Root Extract of *Stachytarpheta jamaicensi*, D= ELESj- Ethanol Leaf Extract of *Stachytarpheta jamaicensis*, E= EREJj- Ethanol Root Extract of *Stachytarpheta jamaicensis ;* Long arrows- coronary artery short arrows-myocardial fibres

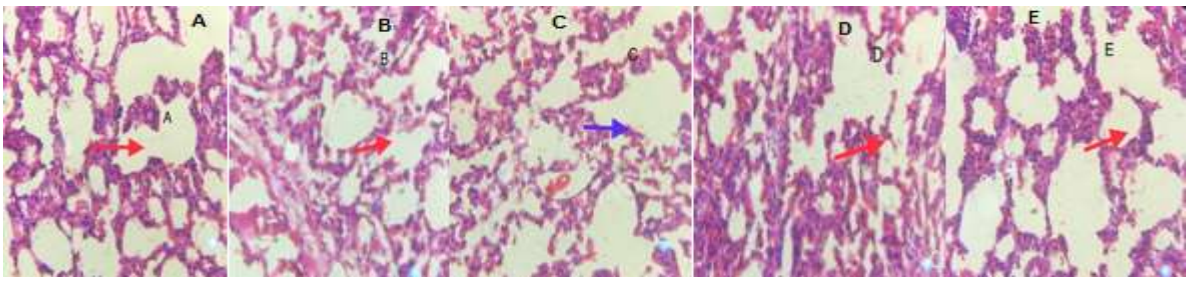

Plate 3: Photomicrograph of Lungs of rats treated with *S. jamaicensis* leaf and root (Hx & E stain x400). **Key:** A= Control, B= ALESJ- Aqueous Leaf Extract of *Stachytarpheta jamaicensis*, C= ARESU- Aqueous Root Extract of *Stachytarpheta jamaicensis*, D= ELESJ- Ethanol Leaf Extract of *Stachytarpheta jamaicensis*, E= ERESU- Ethanol Root Extract of *Stachytarpheta jamaicensis*, AW= Airway AS= Alveolar Space

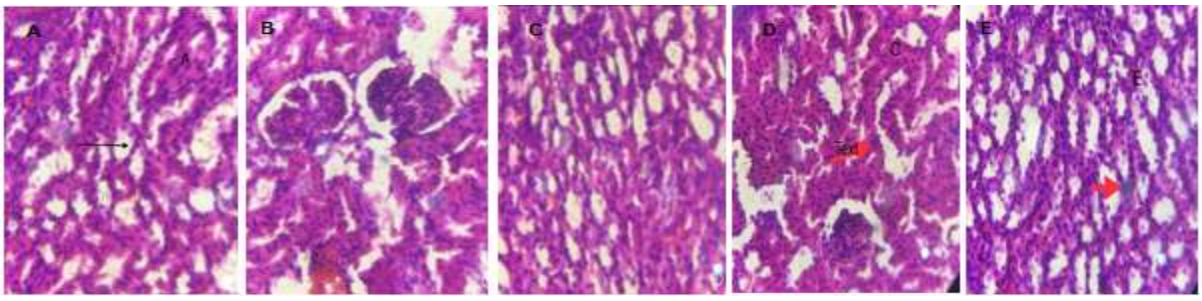

Plate 4: Photomicrograph of Kidney of rats treated with *S. jamaicensis* leaf and root (Hx & E stain x400). Keys: A= Control, B= ALESU- Aqueous Leaf Extract of *Stachytarpheta jamaicensis*, C= ARESJ- Aqueous Root Extract of *Stachytarpheta jamaicensis*, D= ELESU- Ethanol Leaf Extract of *Stachytarpheta jamaicensis*, E= ERESJ- Ethanol Root Extract of *Stachytarpheta jamaicensis*, NT-Normal Tubules NG- Normal Glomerulus

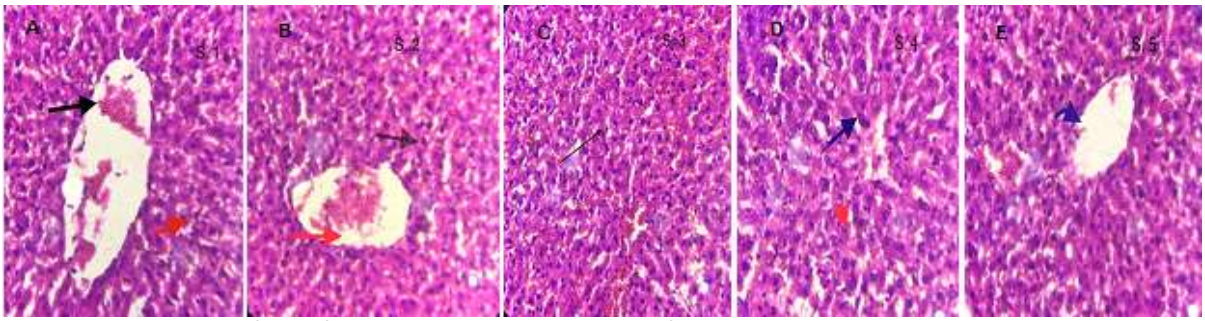

Plate 5: Photomicrograph of Liver of albino rats treated with *S. jamaicensis* leaf and root (Hx & E stain x400). Keys: A= Control, B= ALESU- Aqueous Leaf Extract of *Stachytarpheta jamaicensis*, C= ARESJ- Aqueous Root Extract of *Stachytarpheta jamaicensis*, D= ELESJ- Ethanol Leaf Extract of *Stachytarpheta jamaicensis*, E= ERESJ- Ethanol Root Extract of *Stachytarpheta jamaicensis*, Short arrows= Normal Hepatocytes long arrows= Distinct centriole

Phytochemical preparations have long been regarded as both safe and efficacious during various periods, owing to their few adverse effects. These hypotheses may have significantly influenced the indiscriminate use of these preparations among the general public, perhaps leading to bias. The aforementioned preparations are frequently administered over an extended period of time without adequate monitoring by experts and a lack of awareness regarding the potential harmful effects that may arise from prolonged administration (Eran *et al., 2016).* The importance of considering oral toxicity from a logical perspective should not be underestimated. This approach not only aids in the classification of dosages for future reference but also reveals the potential clinical manifestations induced by the chemicals being studied. Therefore, the current study was conducted to evaluate the acute and sub-acute toxicity of ethanol and aqueous extracts derived from the root and leaf of *Stachytarpheta jamaicensis* on an animal model. Prior to conducting experiments on the phytotherapeutic properties of herbal remedies, it is crucial to assess their toxicological characteristics. In the process of doing an assessment, the initial step involves obtaining the LD50 determination, if available. The acute toxicity evaluation offers insights into the mechanism of harmful action, serves as a foundation for classification and labelling, and aids in finding the optimal doses of herbal remedies through the utilisation of animal models. However, in the event of exposure to a large dose, such as 5000 mg/kg, animals have demonstrated the ability to survive. Therefore, no further acute analysis will be conducted (Zhu *et al., 2009).* The administration of ethanol and aqueous extracts of *Stachytarpheta jamaicensis* roots and leaf at varying doses (50, 100, 500, and 5000 mg/kg) did not result in any observable signs of toxicity or mortality in the rats subjected to the extracts. This lack of adverse effects was observed throughout a 72-hour observation period. The findings of this experiment indicate that the administration of various extracts derived from the root and leaf of *S. jamaicensis* does not provide any detrimental effects at the dosage examined. Moreover, the LD50 value, which represents the lethal dose required to cause mortality in 50% of the test subjects, exceeds 5000 mg/kg. According to the OECD guidelines, the chemical classification and labelling of ethanol and aqueous extracts derived from the leaves and roots of *S. jamaicensis* can be assigned to class 5 grade (LD50 > 5000 mg/kg), as the acute toxicity investigation did not result in any fatalities or indications of toxicity.

After that, a sub-acute toxicity assay was done to see if the ethanol and water extracts from the root and leaf of *S. jamaicensis* could hurt animals over the course of 21 days. The sub-acute assay gives information about how much of a drug to give, how toxic it is to target organs, and how to spot potentially harmful signs of toxicity that could shorten the lives of lab animals. So, over the course of 21 days, the rat models were given 50, 100, 500, and 2000 mg/kg of ethanol and water-based extracts from the roots and leaves of *S. jamaicensis*. According to Mayur et al. (2017), the body mass index (BMI) served as a gauge of the rats' general health condition. Following a period of 21 days during which the extracts were orally administered, it was seen that all rats exhibited the expected and typical rise in body weight, with the exception of those rats who were subjected to a dosage of 2000 mg/kg.

This information is presented in Tables 9 and 10. The findings indicate that the administration of ethanol and aqueous extracts derived from the roots and leaves of *Stachytarpheta jamaicensis* did not adversely affect the normal metabolic processes in rats when administered at doses of 50, 100, and 500 mg/kg. Likewise, there were no notable alterations observed in the weight of several organs, such as the lung and spleen. Significant variations in the heart, liver, and kidney were observed in the experimental study, as depicted in figures 1–3. These findings indicate that the sub-acute oral administration of ethanol and aqueous extracts derived

from the root and leaf of *S.* jamaicensis elicits therapeutic effects that may be beneficial in addressing conditions such as inflammations, arteriosclerosis in the heart, diabetes, and liver diseases (Hilaly *et al., 2004).* This finding was additionally corroborated through the histological examination conducted on the liver, kidney, and heart. Olorunnisola et al. (2012) say that changes in the amounts of ALT, AST, and ALP in the leaves of *Stachytarpheta jamaicensis* are good signs of how well the liver is working. This means that giving the animals sub-acute aqueous and ethanol extracts from the leaves and roots of *Stachytarpheta jamaicensis* improved the function of their hepatocytes and got their metabolic processes back to normal.

The assessment of serum levels of ALT, AST, and ALP did not reveal any indications of liver damage, consistent with the findings reported by Andrade *et al.* (2007). Their study showed that liver injury is typically observed when the levels of ALT, AST, and ALP exceed three times and twice the upper limit of normal, respectively. In this study, it was observed that the administration of therapeutic doses of the aqueous leaf extract, as well as the aqueous and ethanol extracts of *Stachytarpheta jamaicensis* leaves and roots, did not lead to any significant alterations in hepatorenal cellular indices. This finding is supported by the data presented in tables 5 and 6. A serum biochemistry assessment was conducted to determine potential changes in renal and hepatic functions influenced by the extract. The levels of total protein, albumin, globulin, and total bilirubin, which are known to impact the hepatocellular and secretory activities of the liver, were found to be within the normal ranges as indicated in Tables 7 and 8.

The above observations were supported by the histological study of different organs, including the spleen, brain, heart, ileum, lungs, kidney, and liver, which can be seen in plates 1, 2, 3, 4, 5, and 5. The histological analysis showed that all of the experimental groups that were given a dose of 2000 mg/kg had significant changes in their internal organs.

Conclusion: In conclusion, it can be inferred that the findings of this study support the hypothesis and contribute to the existing body of knowledge. The histological examination findings suggest that the aqueous and ethanol extracts derived from the leaves and roots of *S. jamaicensis* showed a lack of toxicity and were deemed safe, even when administered at a dosage of 2000 mg/kg body weight. Therefore, it can be utilized for pharmacological and phytotherapeutic applications. Furthermore, no observable symptoms were observed as a result of their administration.

REFERENCES

Álvarez, E; Leiro, JM; Rodríguez, M; Orallo, F (2004). Inhibitory effects of leaf extracts of *Stachytarpheta jamaicensis* (Verbenaceae) on the respiratory burst of rat macrophages. *Phytotherapy Res.* 18(6):457–462. doi: 10.1002/ptr.1442

Andrade, BJ; Robles, M.; Fernandez-Castaner, A; Lopez-Ortega, S; Lopez-Vega, MC; Lucena, MI. (2007). Assessment of Drug Indued Hepatoxicity in Clinical Practice: A Challenge for Gastroenterologists. *World J. of Gastro*. 13 (3): 329 – 340.

Dias, FD; Takahashi, CS (1994). Cytogenetic evaluation of aqueous extracts of the medicinal plants *Alpiniamutans* rose (Zingerberaceae) and Pogostemum hyneanus benth (labitae) on *Wistar* rats and *Allium cepa* (Liliaceae) root tip cells. *Braz. J. Genet.* 17, 175–180.

Eran, B.A.; Noah, S.; Lee, HG; Kamer, M.; Suha, O; Elad, S (2016). Potential risks associated with traditional herbal medicine use in cancer care: A study of Middle Eastern oncology health care professionals. *Cancer*, 122, 598–610.

Hilaly, J.; Israili, H; Lyoussi, B. (2004). Acute and chronic toxicological studies of Ajuga iva in experimental animals. *J. of Ethnopharm*. 91, 43–50.

Idu, M.; Erhabor, JO; Odia, EA (2009). Morphological and anatomical studies of the leaf and stem of some medicinal plants: *Stachytarpheta jamaicensis* (L.) Vahl. And *S. cayennensis* (LC Rich) schau. *Ethnobot. Leaflets.*13 (11):1417–1425.

Idu, M; Omogbai, EKI.; Aghimien, GE; Amaechina, F; Timothy, O; Omonigho, SE (2007). Preliminary phytochemistry, antimicrobial properties and acute toxicity of *Stachytarpheta jamaicensis* (L.) Vahl. Leaves. *Trends in Med. Res.* 2(4):193–198. doi: 10.3923/tmr.2007.193.198.

Idu, M; Omogbai, EKI; Amaechina, F; Ataman, JE (2006). Some cardiovascular effects of the aqueous extract of the leaves of *Stachytarpheta jamaicensis* L. Vahl. *Inter. J. Pharma.* 2(2):163–165. doi: 10.3923/ijp.2006.163.165

Mayur Porwal; Najam Ali Khan; Kamal Kishore, M (2017). Evaluation of Acute and Subacute Oral Toxicity Induced by Ethanolic Extract of

Marsdenia tenacissima Leaves in Experimental Rats. *Sci. Pharmaceut.* 85(29): 1-11.

Nath, P; Yadav, KA (2015). Acute and sub-acute oral toxicity assessment of the methanolic extract from leaves of Hibiscus rosa-sinensis L. in mice. *J. Intercult. Ethnopharmacol*. 4, 70–73.

Okwu, DE; Ohenhen, ON. (2010). Isolation and characterization of steroidal glycosides from the leaves of *Stachytarpheta Jamaicensis* Linn Vahl. *Der Chemica Sinica.* 1(2): 6–14.

Omorodion, NT; Atoigwe-Ogeyemhe, BE Achukwu, PU; Odigie, EB.(2019). Histopathological changes associated with exposure of some viscerals and testicular tissues to bisephenol A and di (2-ethylhexyl) phthalate *Trop. J. Pharma. Res*. 18 (6): 1213-1218

Patil, UH; Gaikwad, DK (2010). Phytochemical profile and antibacterial activity of stem bark of *Anogeissus latifolia*. *Pharm. J*., 2, 70–73.

Rahmatullah, M., Jahan, R., Azam, F. M. S., Hossan, S., Mollik, M. A. H. and Rahman, T. (2011).Folk medicinal uses of verbenaceae family plants in Bangladesh. *Afr. J. Trad. Complemen. Alternate. Med.* 8 (5):53–65. doi: 10.4314/ajtcam.v8i5s.15.

Ramakrishnan, K.; Sivaranjani, R (2013). Pharmacognostical and phytochemical studies on stem of *Stachytarpheta jamaicensis* (L) Vahl. *Interna. Res. J. Pharm.* 4(10):44–47.

Rodríguez, LAG; Tacconelli, S; Patrignani, P (2008). Role of dose potency in the prediction of risk of myocardial infarction associated with nonsteroidal anti-inflammatory drugs in the general population. *J. Ameri. College Cardiol.* 2008; 52(20):1628–1636. doi: 10.1016/j.jacc.2008.08.041.

Roengsumran S; Sookkongwaree, K; Jaiboon, N; Chaichit, N; Petsom, A (2002). Crystal structure of ipolamiide monohydrate from *Stachytarpheta indica*. *Anal Sci*.18: 1063 - 1064.

Shih-Huei, DS; Ming-Jou, AF (2003). Remarks on the species of *stachytarpheta* (Verbenaceae) of Taiwan. *J. Bot. Bull. Acade. Sci*. 44: 167-174.

Sivaranjani, R; Ramakrishnan, K; Bhuvaneswari, G (2014). Pharmacognostic studies on root of *Stachytarpheta jamaicensis*. *Inter. Let. Nat. Sci.* 8(2):100–105.

Sivaranjani, R; Ramakrishnan, K; Bhuvaneswari, G (2014). Evaluation of in vitro antioxidant activity and estimation of total phenol and flavonoids contents of various extracts of *Stachytarpheta jamaicensis*. *Internat, J. Innov. Drug Discov.* 4(1):31–37.

Stevens, PF (2008). Angiosperm Phylogeny Website. https://www.mobot.org/MOBOT/research/APweb/ [Accessed November 12, 2020].

Sulaiman, M. R., Zakaria, Z. A., Chiong, H. S., Lai, S. K., Israf, D. A. and Azam Shah, T. M.(2009). Antinociceptive and anti-inflammatory effects of *Stachytarpheta jamaicensis* (L.) Vahl (Verbenaceae)in experimental animal models. *Med. Principe. Prac.* 18(4):272–279. doi: 10.1159/000215723.

Suneetha, P; Poornima, S; Sumana, K; Nidhi, H; Puttaraju, HP. (2013). Comparative studies on antimicrobial and antifungal efficacy from *Bixa Orellana* L., *Lantana Camara* L., *Stachytarpheta jamaicensis* (L.)Vahl, *Hyptis Suaveolens* (L.) poit. With triclosan. *CIBTech J. Microb.* 2(2):15–23

Vikrant, A; Arya, ML. (2011).A review on anti-inflammatory plant barks. *Inter. J. Pharmacy Techological Res.* 3(2):899–908.

World Health Organization (WHO). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine; WHO: Geneva, Switzerland, 2000; p. 35.

Zhu, H.; Martin, TM; Ye, L; Sedykh, A.; Young, DM; Tropsha, A (2009). QSAR Modeling of Rat Acute Toxicity by Oral Exposure. *Chemo Resource. Toxic.* 21-22:1913-1921.