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Toxicological and analgesic evaluation of *Solanecio biafrae* ethanol leaf extract

Akpan JL [1], Onwudiwe TC [2], Ikongshul Cecilia A [1], Igwe DO [3], Ofonakara U [4], Okam PC [5], Nwokike Matthew Onyemaechi [6, 7] and Akuodor GC [5, *]

[1] *Department of Pharmacology, Faculty of Basic Medicals Sciences, University of Calabar, Calabar, Nigeria.*
[2] *Department of Pharmacology and Toxicology, Madonna University, Elele Campus, Nigeria.*
[3] *Department of Physiology, David Umahi Federal University of Medical Sciences, Uburu, Nigeria.*
[4] *Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Health Sciences, Alex Ekwueme Federal Univrsity Ndufu-Alaike, Ikwo Ebonyi State, Nigeria.*
[5] *Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.*
[6] *Department of Pharmacology, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria.*
[7] *Department of Pharmacology, School of Medicine, Kabale, University, Uganda.*

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Abstract

Aim: The leaves of *S. biafrae* have been used ethnomedically across Sub-Saharan Africa for the treatment of different diseases. The toxicological assessment and analgesic effects of the ethanol leaf extract of *S. biafrae* was the focus of the present study which was necessitated by the traditional uses of the extract in folk medicine.

Methods: Acute and subacute toxicity testing were examined and parameters such as relative organ weight of both liver and kidney, changes in animal body weight, haematological indices as well as the examination of liver and kidney function parameters were all evaluated. For analgesic potential of the extract; acetic acid-induced writhing in mice and hot plate model were also evaluated.

Results: The result of acute testing showed that the extract has an LD_{50} value of 3,492mg/kg in rats. The subacute toxicity tests showed no significant changes in the body weight of the animals throughout the duration of the experiment. However, there was significant reductions in the relative organ weight of both the liver and the kidney of the extract. The haematological parameters showed the extract had reduced PCV levels in rats as well as the serum WBC levels. The acetic acid-induced writhing test showed that all the concentrations of the extract used showed significant increase in their protective ability against acetic acid-induced writhing in mice. The hot plate model showed that the extract only produced significant inhibition against pain the first 30minutes of testing

Conclusion: Findings show that, the ethanol leaf extract of *S. biafrae* though exhibited toxic potentials, it however demonstrated potent direct and central analgesic properties.

Keywords: *Solanecio biafrae*; Leaf extract; Toxicological profile; Pains: Rodents

1. Introduction

Medicinal plants are widely used in treating and preventing specific diseases and are known to play an important role in health care. The demand for herbal medicines for disease treatment is on the increase due to their efficacy, availability and affordability. More than three-quarters of the world's population rely upon complementary and alternative

* Corresponding author: Akuodor GC

medicine for health care [1], especially for millions of people in major areas of developing countries [2, 3]. In Nigeria, a vast range of medicinal plants have been used for the treatment of different ailments without scientific investigation of their therapeutic potentials. In the last few decades, researchers have evaluated numerous medicinal plants for their bioactive and pharmacological properties. These efforts are being continually taken to investigate the advantages of herbal medicine in modern science with the aim to adopt effectively potential medical practice and prevent harmful effects [4, 5].

Solanecio biafrae is a perennial climbing herb which naturally occurs in African forest zones, from Guinea to Uganda. *S. biafrae* is equally known for its therapeutic virtues, notably in Nigeria where it is used in the treatment of diabetes or pulmonary defects [6]. In Benin, Côte d'Ivoire, Congo, or Cameroon it is used in traditional medicine to treat many diseases such as bleeding from cuts, sore eyes, cough, heart troubles, rheumatic pain, or localized oedemas. In the Western and North-western Regions of Cameroon, ethnobotanical studies revealed its utilization in the treatment of cases of women infertility [7].

Despite the numerous medicinal benefits of the plant in traditional medicine, there is little or no scientific knowledge or evidence of its toxicological effects. The toxicological evaluations (acute and subacute) are aimed at harnessing the harmlessness or safety of chemical compounds and a possible mechanism of action through which they exhibit their effects. Acute and subacute systemic toxicity studies are used to for hazard disclosure and management of the risk involve in the handling of and use the compound in question [8]. Acute toxicity involves the administration of a single dose test that symptoms and the extent to which toxicity affects the

2. Materials and Methods

2.1. Collection and Identification of *S. biafrae*

Fresh leaves of *S. biafrae* were purchased from Watt market, Calabar south, Cross River State. The leaf was taken to department of plant and ecological techniques herbarium for identification by Effa Effa Amobeja.

2.2. Preparation of the leaf extract

The freshly purchased leaves were gently washed, to minimize squeezing and air dried at room temperature in the laboratory of the Department of Pharmacology. The dried leaves were grinded into powder form using a mechanical blender. The powdered material was weighed on a weighing balance and was emptied in a round bottom flask containing 2000 ml of petroleum Ether and was left for 48 h, then corked and homogenized. The extraction was done after 48 h. For the ethanol leaf extract, the pulverized material was soaked in a round bottom flask containing 2000 ml of ethanol, and was left for 48hrs. The ethanol leaf extract was filtered with Whattman filter paper No. 1 and was left for 24 h. To obtain the ethanol extract, the sample was dried and kept in a sample bottle and stored in the refrigerator at 4^0c until used for the experiment to avoid mold growing on it.

2.3. Experimental animals

A total number of 100 Wister rats of weight range 150-210 g of age 3 weeks- 1month were used for this study; 25 rats was used for both the Eddy's hot plate model and Tail-flick model studies while another 25 rats each was used for both the acetic acid-induced model and formalin-induced respectively. And finally, 25 rats were used in the study of the extract as a centrally-acting analgesic. They were obtained from animal house of the department of Pharmacology, University of Calabar, Calabar. A total of 5 animals per group was housed in standard cages each of the above animal house. The animals were allowed to acclimatize to the environment for 1 week under standard temperature and access to food and water ad-libitum. These animals are those that have not been used in any previous experiment.

The method as described by Aziz, [9) was adopted for the phytochemical analysis of the ethanol extract of M. sativa leaf. The metabolites that were assayed include tannins, saponins, alkaloids, flavonoids, terpenoids, steroids, anthraquinones, glycosides, reducing sugars and resins.

2.4. Toxicological evaluation

2.4.1. Acute Toxicity Study

The acute toxicity (LD_{50}) study was determined in mice, on the ethanol leaf extract of *S. biafrae*, using Lorke [10] method with slightly adjustments. A total of 13 mice, both male and female were fasted overnight prior to the study. In phase one, 9 mice were randomized into 3 groups of 3 per cage. Groups 1, 2, and 3 rats received 10 mg/kg, 100 mg/kg, 1,000 mg/kg of the extract administered orally. The animals were observed for signs of toxicity which includes; paw licking, weakness, feeling sleepy, respiratory distress, hyperactivity, coma and death for the first 4 hours, and subsequently 24 hours. Since no signs of toxicity were observed, the second phase was initiated. In this phase, 4 mice were also grouped into 4 with one mouse per cage. Doses was selected based on the results of the phase 1 since no death occurred, higher doses were selected and administered; 1600 mg/kg, 2900 mg/kg, 5000 mg/kg and 10 mL/kg distilled water. The animals were observed for signs of toxicity and mortality for 48 hours and thereafter 72 hours for late toxicity.

2.4.2. Subacute toxicity testing

For this experiment, twenty-five (25) male Wistar rats were used. They were allowed to acclimatize for a period of seven days. The rats were randomly selected and grouped into five groups of five rats each. The rats were weighed and marked for easy identification.

2.4.3. Experimental protocol

Treatment began on the first day of the experiment lasted for a period of 21 days in all the five groups of the animal using the method as described by Ezeokpo et al., [11].

- Group 1 received 1o mL/kg normal saline as treatment and was regarded as negative control.
- Group 2 received 100 mg/kg body weight of *S. biafrae* as treatment
- Group 3 received 200 mg/kg body weight of *S. biafrae* as treatment
- Group 4 received 400 mg/kg body weight of *S. biafrae* as treatment

All administration was done via oral gavage using oro-gastric tube

2.4.4. Termination of experiment and sample collection.

On the 21st day of the experiment, the animals were anaesthetized by chloroform inhalation method and sacrificed. The animals were carefully dissected and the blood was removed through cardiac puncture for haematological and biochemical analysis and the organs (liver and kidney) were harvested and weighed [12].

2.4.5. Haematological and biochemical analysis

The heamatological analysis was carried out using auto-analyzer machine for the determination of serum levels of white blood cell (WBC), Neutrophils, lymphocytes, monocytes, eosinophils, basophils and packed cell volume (PCV). While the biochemical analysis was done to determine the effect of the extract on lipid profile of the rats as well as liver function and kidney function parameters using digital automated analyzers.

2.5. Determination of analgesic activities of the extract

2.5.1. Acetic acid-induced writhing in mice.

The method of Akuodor et al., [13] and Okorie et al., [14] was adopted for this study. Twenty-five mice of weights ranging from 25-30g were randomly selected and put into five groups of five rats each. The mice were allowed to acclimatize for one week with access to food and water ad libitum after which they were administered. Group one was administered with normal saline, group two was administered with indomethacin (/kg/day), and groups three to five were treated with extract concentrations of 100 mg/kg, 200 mg/kg, and 400 mg/kg respectively. Thirty minutes after the administration, the mice were given 0.7% of acetic acid for pain induction. The analgesic activity of the extract was determined by the number of writhing experienced by the mice, which recorded for one hour at thirty minutes' interval. Also the percentage inhibition in writhing syndrome of the animals was calculated and compared to the standard drug. The percentage inhibition is the indication of percentage of protection against abdominal constriction and was taken as an index at analgesia.

It was calculated as ({Wc-Wt}/Wc) x 100.

Where Wc is the number of writhes of the control group

Wt is the number of writhes of the treated group.

2.5.2. Hot plate test.

The procedure described by Muhammad *et al*, [15] was followed to perform this test. Twenty-five Wister rats (n = 5) were used. Animals were subjected to pre-testing on a hot plate (Harvard apparatus) maintained at 55 ± 0.1°C. Animals having latency time greater than 15 seconds on hot plate during pre-testing were excluded. Animals was divided randomly into 5 groups, each containing of five rats. The group I was treated with normal saline (10 ml/kg), group II was treated with indomethacin (10 mg/kg, orally), and Group III-X were treated with oral doses of 50, 100 and 200 mg/kg of *S. biafrae* extracts respectively. Indomethacin was used as reference drugs for comparison. After 30 min of extract and drug administration, rats were dropped inside the cylinder onto the hot plate and the latency time (time for which rat remains on the hot plate without licking or flicking of hind limb or jumping) was recorded in seconds in order to prevent the tissue damage the cut off time of 30 seconds was set for all animals. The latency time was recorded for each group at 0, 30, 60, 90 and 120 min following drug administration. The percentage analgesia was calculated using the following formular.

$$\% \text{ Analgesia} = (\alpha-\beta/\alpha) \text{ x}100$$

Where α is the reaction time after treatment; β is the baseline value

2.6. Statistical Analysis

Results were expressed as means ± SEM and analyzed with statistical products and services solution (SPSS version 20) by using one-way analysis of variance (ANOVA). A difference in the mean $P<0.05$ was considered statistically signif

3. Results

3.1. Phytochemical constituents

Phytochemicals detected in the ethanol leaf extract of *S. biafrae* were alkaloids, tannins, terpenoids, saponins, steroids and glycosides, while flavonoids and reducing sugards were absent.

3.2. Acute toxicity test

The ethanol leaf extract of *S. biafrae* did not produce any lethality or visible signs of toxicity in rats up to the oral dose level of 5000 mg/kg body weight 24 hours after treatment. Further monitoring for seven days did not still yield mortality or visible toxic signs. Hence, the LD50 value was greater than 5000 mg/kg body weight

3.3. The effects of the ethanol leaf extract on the body weight of Wistar rats.

The body weights of the rats were taken at different day intervals during the course of the experiment and the results was recorded as shown in table 3. The results showed that there was no regular pattern in the changes in weight of the animals in the days of recorded weights, for instance, in the extract concentration of 400 mg/kg, there was a marked increase in the body weight of the rats on the tenth day of the experiment but that increase only lasted a few days because in the following few days there was a sharp reduction in body weight of the animals and this reduction continued till the end of the experiment. Again, the extract concentration of 200 mg/kg recorded a steady increase in the body weight of the animals, which continued to increase after an initial set back in the first week of the experiment. Also the negative control group witnessed very minimal changes in terms of weight.

3.4. The effects of the ethanol leaf extract of *S. biafrae* on the hematological parameters of Wistar rats

The hematological parameters measures the effects of the extract on the blood indices which includes the parameters white blood cell (WBC) and its differentials and the PCV (packed cell volume). The result is shown in Table 4. It showed that at 100 mg/kg, the extract significantly reduced the PCV levels of the animals, this reduction was also observed in the extract concentrations of 200 mg/kg and 400 mg/kg of body weight of rats though to a lesser extent. The white blood cell count (WBC) of the extract was significantly reduced in the 400 mg/kg and 200 mg/kg concentrations.

However, the positive control did not show any reduction in WBC values rather they were elevated but the values was not significant when compared to the negative control.

3.5. Liver function parameters

There was significant reduction in the value of aspartate transaminase (AST) enzyme in the positive control group and in the group treated with highest concentration of extract 400 mg/kg. these reductions were similar to that recorded in extract concentration of 200 mg/kg and they were all significant (p<0.05) in comparison with the negative control. Also, there was a decrease in the serum level of alkaline phosphatase (ALP) in the 200 mg/kg concentration of extract. However, when compared to the negative control, they were not significant and such was the case of the other liver enzymes considered in this study.

3.6. The effect of the ethanol leaf extract of *S. biafrae* on the kidney function parameters.

The kidney function parameters measure the extent of damage to the kidney caused by the presence of toxic substances. The effect of the extract of *S. biafrae* on the kidney function parameters is shown in Table 6. The parameters investigated includes the serum levels of urea, sodium, potassium, bicarbonate, chloride as well as creatinine. The results indicated that there was no significant difference in the values of the serum levels of the parameters tested. However, there was varying values across these parameters though no significant in comparison with the negative control.

3.7. Effect on vital organs

S. biafrae ethanol leaf extract did not produce any significant effect on the weight of different vital organs from rats after daily administration for 21 days (Table 5). All organs were macroscopically comparable to the control.

3.8. The effects of the ethanol leaf extract of *S. biafrae* on the lipid profile of Wistar rats

The lipid profile test is carried for identify abnormalities in lipids of the body like cholesterol and triglycerides. The result of the lipid profile is given in Table 5. The results indicate that the total cholesterol levels was elevated in the extract concentration of 400 mg/kg, the effect was not significant when compared with the normal saline group (negative control). Again, there was no significant difference in the values of high density lipoprotein (HDL), low density lipoproteins (LDL), and very low density lipoprotein (VLDL) among all the treatment groups in this study when compared with the controls and the triglyceride level though elevated in the 400 mg/kg of extract, did not show significant difference (p<0.05) among the extract concentrations when compared with the both the negative controls.

3.9. Acetic acid-induced writhing in mice

The results of the acetic acid-induced writhing in mice showed that the group treated with 50mg/kg of *S. biafrae* had early on-set of activity which began in the first 20 minutes. The analgesic effect was significantly higher (p<0.05) than the positive control treated with 10mg/kg of indomethacin, this effect followed the same pattern in the 40 minutes and 60 minutes' intervals. However, the group treated with 100 mg/kg had a slow on-set of activity. Again, at the 60 minute, all the concentrations of the extract (100 mg/kg, 200 mg/kg, 400 mg/kg) showed significant (p<0.05) increase in protective activity against acetic acid-induced writhing in mice with values of 53.33, 66.67 and 66.67 percent respectively compared to 33.33 percent observed in the control group treated with indomethacin 10mg/kg.

3.10. Hot Plate Model in Mice

Table 1 The effect of the ethanol leaf of *S. biafrae* extract on the body weight of Wistar rats (g)

Treatment	Day 1	Day 7	Day 14	Day 21
Normal saline (20 mL/kg**)**	143.43±3.08	144.96±20.47	140.28±13.18	144.35±14.3
S. biafrae 100 mg/kg	184.75±13.15	186.43±16.42	180.4±14.20	174.83±7.93
S. biafrae 200 mg/kg	175.5±17.70	172.67±11.67	171.43±10.23	184.23±9.03
S. biafrae 400 mg/kg	164.48±46.40	163.03±50.98	174.73±48.70	162.33±27.30

The values represent mean SEM., n=5; *Significantly different from normal control p<0.05;

The analgesic effects of the extract of *S. biafrae* using hot plate model was examined and monitored at 30 minutes' intervals until the 120th minute. The results obtained was used to calculate the percentage inhibition of pain. From the results it was observed that in the first 30minutes, all the concentrations of the extract alongside indomethacin (10

mg/kg) produced inhibition effects that was significantly higher (p<0.05) than that observed in the normal control group treated with normal saline. However, in the 60th minute, there was no significant difference (p<0.05) in the activities between the extract, positive and normal control groups. Similar patterns were also observed in the 90th and 120th minute interval only that the positive control showed significant increase (p<0.05) in both time intervals.

Table 2 The effect of the ethanol leaf of *S. biafrae* extract on the hematological parameters of Wistar rats

Treatment	PCV	WBC	NEUTROPHILS	LYMPHOCYTES	MONOCYTES	EOSINOPHILS
Normal saline (20 mL/kg)	55.20±2.10	7.80±1.90	35.10±8.70	60.11±13.60	2.45±0.50	3.50±1.00
S. biafrae 100 mg/kg	42.36±12.20	5.00±4.90	33.45±11.20	63.51±14.50	1.67±0.02	3.54±0.50
S. biafrae 200 mg/kg	45.28±6.10	6.10±3.80	33.45±11.2	68.10±19*	1.28±0.01	1.67±0.50
S. bifrae 400 mg/kg	45.56±2.60	3.50±1.80	32.48±16	65.70±6.30	1.22±0.05	2.10±0.20

The values represent mean SEM., (n=5); *Significantly different from normal control p<0.05

Table 3 Effects of ethanol leaf extract of *S. biafrae* on liver function parameters in rats

Treatment	AST (µL)	ALT (µL)	ALP (µL)
N/saline (20 mL/kg)	33.20 ±2.38	20.40±1.35	45.18±2.55
S. biafrae (100 mg/kg)	32.15±1.50	18.42 ± 2.10	50.20±1.61
S. biafrae (200 mg/kg)	33.11±2.35	22.40±2.40	52.09 ± 2.32
S. biafrae (400 mg/kg)	30.14±2.33	23.28±2.62	51.13±1.56

Values present means SEM; n=5

Table 4 Effects of the ethanol leaf extract of *S. biafrae* on kidney function parameters of Wistar rats

Treatment	Urea mm/L	Sodium mm/L	Potassium mm/L	Bicarbonate mm/L	Chloride mm/L	Creatinine mm/L
Normal saline (20 mL/kg)	6.20±3.00	136±8.50	3.40±0.5	22.30±0.91	102±11.8	132±7.80
S. biafrae 100 mg/kg	6.40±1.20	136±4.70	3.50±2.10	21.32±8.10	100±7.50	136±11.30
S. biafrae 200 mg/kg	6.00±3.20	134±1.80	3.30±0.20	22.25±1.70	98±9.30	135±11.45
S. biafrae 400 mg/kg	6.40±1.70	136±12.50	3.40±0.70	21.35±1.90	96±11.60	132±12.90

The values represent mean SEM., n=5; *Significantly different from normal control p<0.05

Table 5 Effect of ethanol leaf extract of *S. biafrae* on vital organ weights in rats

Treatment	Heart	Lungs	Kidneys	Liver	Spleen	Testicular
Normal saline 20 mL/kg	0.23 ± 0.11	0.50 ±0.13	0.48 ± 0.06	4.40 ±0.20	0.42 ± 0.07	1.36 ± 0.30
S. Biafra 100 mg/kg	0.30 ± 0.22	0.65 ±0.15	0.45 ± 0.03	3.80 ±0.16	0.48 ± 0.04	1.30 ± 0.10
S. biafrae 200 mg/kg	0.34 ± 0.02	0.60 ±0.08	0.44 ± 0.07	3.50 ±0.24	0.45 ± 0.03	1.40 ± 0.06
S. bifrae 400 mg/kg	0.33 ± 0.06	0.63 ±0.18	0.45 ± 0.06	3.55 ±0.20	0.42 ± 0.03	1.35 ± 0.05

Values present means SEM n=5.

Table 6 Effects of the ethanol leaf extract of *S. biafrae* on the lipid profile of Wistar rats

Treatment	Total cholesterol	HDL	LDL	VLDL	Triglyceride
Normal saline (20 mL/kg)	1.70±1.0	0.40±0.02	1.10±0.04	0.20±0.01	0.50±0.71
S. biafrae 100	1.60±0.15	0.40±0.03	1.00±0.05	0.20±0.02	0.40±0.02
S. biafrae 200	2.0±0.02	0.6±0.02	1.10±0.03	0.30±0.02	0.60±0.01
S. biafrae 400	1.50±0.03	0.40±0.01	0.90±0.08	0.20±0.02	0.50±0.03

The values represent mean SEM., n=5; *Significantly different from normal control $p<0.05$

Table 7 Effect of methanol leaf extract of *S. biafrae* on acetic acid-induced writhing in mice

Treatment	Dose (mg/kg)	Mean no of writhes	% Inhibition
N/saline	20 mL/kg	96.67±3.87	-
Indomethacin	10	3.40±1.91	91*
S. biafrae	100	28.63±2.71	75*
S. Biafra	200	12.32±1.82	85*
S. biafrae	400	5.96±3.80	90*

The values represent mean SEM., n=5; *Significantly different from positive control $p<0.05$;

Table 8 Effect of ethanol leaf extract of *S. biafrae* on hot plate in mice time (Time Interval in min)

Treatment	Dose (mg/kg)	0	30	60	90	120
N/saline	20 mL/kg	10.17±0.45	10.00 ± 0.58	10.67±0.33	10.55±0.22	10.50±0.20
Indomethacin	10	7.50±0.62	17.50±0.72	19.17±0.60	21.17±0.87	23.33±0.95
S. biafrae	100	6.50±0.43	8.50±0.50	10.83±0.83	11.67±0.71	13.17±0.60
S. biafrae	200	7.50±0.76 b	9.33±0.76	11.83±0.70	13.50±0.56	16.80±0.68
S. biafrae	400	8.00±0.52	10.17±0.79	12.50±0.88	14.67±0.56	17.50±0.34

The values represent mean SEM., n=5; *Significantly different from normal control $p<0.05$;

4. Discussion

Natural goods, such as plant extracts, have been a dependable source of medicines for a variety of illnesses for several centuries [16]. The evaluation of the pharmacological effectiveness of these natural products has received a lot of attention, but the toxicological analyses of these plant products are just significant. The current study assessed the ethanol extract of *S. biafrae* toxicity potentials and looked into its analgesic properties.

Acute toxicity testing is one of the initial steps in determining a substance's overall harmful effect. The acute toxicity study, which is typically administered orally as a single dose or over a period of 21 days, assesses the detrimental effects of chemicals to the recipient organism. In the current investigation, 3429 mg/kg of *S. biafrae* ethanol leaf extract was found to be acutely safe to Wistar rats. This was similar to the findings of Adelakun et al. [17], who also found that in male Sprague-Dawley rats, the LD50 of *S. biafrae* was slightly over 3000 mg/kg.

Additionally, a subacute toxicity research using a 21-day experiment on Wistar rats was conducted to assess the toxicity of the ethanol leaf extract of *S. biafrae*. By continuously exposing test compounds to relatively moderate dosages, short-term toxicity assessment helps assess the physiological and metabolic effects of the compound as well as the cumulative toxicity of the agent in the organ of interest. Additionally, a range of negative effects from the short-term toxicity trials can be identified, and this can serve as the foundation for predicting the compound's long-term safety or lack thereof. In order to determine whether the extract was generally safe for rats—a prerequisite for evaluating its application in humans and other higher animals—a number of factors were examined in this study.

One of such parameters evaluated is the body weight changes of the animals. Vahalia *et al,* [18] had distinctively informed that changes in the body weight of animals is an important indicator in the assessment of the toxicity effect of chemicals as well as drugs. In this study, the weights of the animals were measured from the beginning of the experiment to the day of sacrifice, and these recordings were done on a 3-day intervals. When the trial came to a close, it was found that the rats in the groups treated with extract concentrations of 200 mg/kg and 400 mg/kg had gained weight rather than losing it when compared to the negative control group given standard saline treatment. This could mean that, in terms of the weight loss indication, the extracts at various doses showed minimal harmful effects.

The relative organ weight, which is defined as the percentage of the weight of the organ in question in relation to the animal's total body weight, is another significant parameter measured in this study. Lasic et al., [19] have shown that organ weights are markers of the pathological and physiological well-being of the animals. Some herbal products may have a toxic effect on the body's vital organs, such as the liver and kidney [20]. This toxic effect can be measured using the relative organ weight as a direct indicator of the toxic effect on body weight. The study's analysis of the liver's relative organ weight revealed that the extract groups receiving 100 mg/kg and 200 mg/kg had smaller livers overall. By comparison, the 400mg/kg had no such effect as the negative control. Similar to what was seen in the weight of the liver organs, the positive control group produced the greatest weight reduction effect in the kidney as well. In the study, the relative weight of the kidney was observed at 400 mg/kg of extract, which yielded values comparable to those of the normal control. Doses of 200 and 100 mg/kg also produced weight reduction effects.

Heamatological indices is also another parameter investigated in this study. The resultant anaemia following the administration of a chemical agent may bring about hemolysis, inhibition of hematopoiesis as well decrease in the hematological parameters, this effects may be from the activity of the bioactive compounds in the compound. [21]. The function of the blood as the transport of both nutrients and foreign substances exposes it to high doses of toxins and this in turn may produce changes in the normal values of its constituents, so the measurement of these parameters give a hint in the effect of the such chemical on the blood [22].

Hematological indicators are yet another metric that this study looks into. Anaemia that results from the administration of a chemical agent may cause hemolysis, block hematopoiesis, and lower hematological indices. These effects may be attributed to the compound's bioactive components [21]. The blood's role in carrying nutrients and foreign substances exposes it to high levels of toxins, which can alter the normal values of its constituents. Therefore, measuring these parameters can provide insight into how a chemical may affect the blood [22].

When compared to the normal control, all extract concentrations in the current investigation showed lower packed cell volume (PCV) values, including the positive control. The blood's capacity to carry oxygen from the lungs to other areas of the body is determined by the PCV, or proportion of red blood cells in the blood. Additionally, the values of white blood cells (WBC) were lower in the extract at all concentrations as well as in the positive control when compared to the normal control. Because white blood cells have a specific role in protecting the body from external invaders, their

decreased quantity as a result of extract administration may contribute to a weakened body defense system and increased toxicity. Across all blood parameters evaluated in the study, there was no statistically significant variation.

In addition, a biochemical analysis of the rats' serum was done in this investigation. The analysis comprised testing the kidneys, determining the liver function parameters, and assessing the rats' lipid profile. Total cholesterol as well as other kinds of cholesterol, such as high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and triglycerides, were measured as part of the lipid profile test. It was noted in the study that the 400 mg/kg of extract had a somewhat lower result for total cholesterol. While the other cholesterols and triglycerides did not change, there were comparable decreases in LDL and HDL. Since the liver is known to be the location of both cholesterol synthesis and breakdown as well as removal, even a small drop in serum cholesterol levels may be a marker of liver damage [23]. Enzymes, however, are the real indicators to determine the early harmful effects of foreign chemicals given to experimental animals [24]. When it comes to the liver, problems are typically indicated by the levels of alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT). While the transaminases (ALT & AST) are cytosolic enzymes, ALP is a membrane-bound enzyme. Under normal physiological conditions, these enzymes are located in large quantities in the liver; however, in certain pathological circumstances, such as liver toxicity, these enzymes may only be present in the serum in significant amounts when the liver's cell membranes become leaky or completely ruptured. The correlation between an increase in blood levels of liver enzymes and a corresponding drop in these enzymes' tissue levels, as a direct indicator of damage to the liver and kidney, has also been documented [25]. The 200 mg/kg and 400 mg/kg of extract in this investigation showed higher serum levels of ALT and ALP, which may again be a sign of harmful chemicals in close proximity to the liver. The renal function analysis, however, did not reveal any appreciable variation in the values of the kidney function markers examined.

Furthermore, the analgesic activity of the ethanol extract of *S. biafrae* was investigated using various investigating models. The analgesic effect is rated as the pain killing ability of the extract. First, the acetic acid-induced writhing in mice was used, it is a chemical method used to induce pain of peripheral origin by injecting the substance known for its irritant capabilities in mice. The analgesic activity of the test substance will be inferred by its ability to reduce the number of writhing by the animal. In this study the animal responds to the injection of acetic acid with a characteristic stretching called writhing [26]. Also any writhing activity such as extension of the hind leg, contraction of the abdomen or turning of the trunk is considered a positive response [27] The results obtained showed that the extract of *S. biafrae* reduced more writhings in mice and thereby produced a better percentage protection than the control drug (Indomethacin 10mg/kg) in the all the time intervals of the study.

Another method, the hot plate was used to really ascertain the extract's efficaciousness as analgesic agent. The hot plate test is to test the response of animals to pain and subsequent effectiveness of analgesics by observing them react to heat-induced pain. Again, the results from this model showed that the extract of *S. biafrae* produced similar effect with the positive control drug (Indomethacin 10mg/kg) after 2 hours of observation which maintained a high level of inhibition at 120th minutes. This is in agreement with that reported by Prempeh & Mensah-Attipoe [28, 29] who studied the analgesic activity of crude aqueous extract of *Z. xantholixyloides* using indomethacin as control. Their study showed that indomethacin peaked at 120th minute.

5. Conclusion

This research work investigates the toxic effects and analgesic potentials of the ethanol leaf extract of *Solenacio. biafrae* and different parameters were measured to evaluate these potentials. The results of the changes in body weight of animals and the relative organ weight indicates a possible toxic effect of the extract, the hematological parameters also hinted on potential toxicity. Biochemical investigations which included lipid profile, kidney and liver function tests. The lipid profile and liver function results further gave convincing evidence on the toxic effect hinted on by the earlier parameters. However, there was no indication of toxic effects of the extract from the results of the kidney function parameters. However, the analgesic potential of the extract was also tested using the hot plate and acetic acid-induced writhing tests in mice. The hot plate model represent central-acting analgesia while the acetic acid test represent peripheral analgesia in both phases of the experimental time intervals. Therefore, it can be said that ethanol leaf extract of *S. biafrae* exhibited toxicity and analgesic effects on experimental animal models. Moreover, this toxic effect tends to be more of hepatotoxicity than nephrotoxicity, while the analgesic activity showed both peripheral and central analgesia.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper

Statement of ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Also, all procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

References

[1] Edirne T, Arica SG, Gucuk S, Yildizhan R, Kolusari A, Adali E, Can M. Use of complementary and alternative medicines by a sample of Turkish women for infertility enhancement: a descriptive study. Complementary and Alternative Medicine 2010; 10:11

[2] Müller JC, Giuliana GK, Botelho Aedra CB, Boareto CA, Rattmann DY, Martins ES, Cabrini DA, Otuki MF, Paulo RD. *Morinda citrifolia* Linn (Noni): *In vivo* and *in vitro* reproductive toxicology. J Ethnopharmacol. 2009; 121: 229-233.

[3] World Health Organization. "WHO traditional medicine strategy 2014-2023." (2013):76.

[4] Essien AD, Akuodor GC, Ajoku GA, Megwas AU, Anele DO, Ezeunala MN, Okezie AO. Antimicrobial and toxicological evaluation of ethanol leaf extract of *Salacia lehmbachii.* Journal Interdiscip Toxicol. 2017; 10(4): 163–167.

[5] Akpan JL, Wokota PN, Ohadoma SC, Onwudiwe TC, Okoroama CE, Iganga ON, Nwokike MO, Akuodor GC (2024c). Evaluation of nephrotoxicity effects of the methanol leaf extrac of A. angustifolia in Wistar rats. Int J Basic Clin Pharmacol. 13(3):307-314.

[6] Adebayo AG. Inventory of antidiabetic plants in selected districts of Lagos State, Nigeria. J Ethnopharmacol, 2009;121: 135-139.

[7] Focho DA, Nkeng EAP, Lucha CF, Ndam WT, Afegenui A. Ethnobotanical survey of plants used to treat diseases of the reproductive system and preliminary phytochemical screening of some species of malvaceae in Ndop Central Sub-division, *Cameroon.* J Medicinal Plant Res. 2009; 3: 301-314.

[8] Li Y, Zhuang Y, Tian W, Sun L, 2020. In vivo acute and subacute toxicities of phenolic extract from rambutan (Nephelium lappaceum) peels by oral administration. Food Chem. 2020; 320,

[9] Aziz MA. Qualitative phytochemical screening and evaluation of anti-inflammatory, analgesic and antipyretic activities of Microcospaniculata barks and fruits. Journal of Integrative Medicine. 2015; 13(3):173-84

[10] Lorke D. 1983. A new approach for acute toxicity testing. Archieve of Toxicology. 1983; 54:275- 287.

[11] Ezeokpo BC, Akuodor GC, Erejuwa OO, Akpan JL, Nnolim BI, Ogiji ED, Nwobodo MU,Ezeonu CT. Assessment of acute and subacute toxicity of ethanol extract of *Pseudocedrela kotschyi* leaf in Wistar rats. Journal of Biological Studies. 2017; 3(2): 48-57.

[12] Akuodor GC, Okorie AU, Ekenjoku JA, Megwas AU, Aja DOJ, Irogbeyi LA, Okezie OA Acute and sub-acute toxicity effect of the ethanol leaf extract of Justicia insularis in wistarrats. Saudi Journal of Med and Pharm Sciences. 020;6 (2): 225-231.

[13] Akuodor GC, Ohadoma SC, Ofor CC, Megwas AU, Chukwu LC, Ramalan MA, Okoroafor DO, Chilaka KC (2021b). Anti-nociceptive, Anti- inflammatory and antipyretic activities of the ethanol root bark extract of *Salacia lehmbachii* in rats and mice. International Journal of Basic and Clinical Pharmacology, 10 (6):614 – 620.

[14] Okorie O , Akuodor GC, Ifediba CE, Ofor CC, Afonne OJ. In Rodents, Methanol Extract from Ritchiea capparoides leaf has Analgesic, Anti-Inflammatory, and Antipyretic Properties. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2024; 920: 2

[15] Muhammad N, Saeed M, Khan H. Antipyretic, analgesic and anti-inflammatory activity of *Viola betonicifolia* whole plant. BMC Complement. Altern. Med. 2012; 12:59.

[16] Dias DA, Urban S, Roessner U. A Historical Overview of Natural Products in Drug Discovery. Metabolites. 2012; 2(2): 303–336.

[17] Adelakun SA, Ogunlade B, Omotoso OD, Oyewo OO. Role of Aqueous Crude Leaf Extract of ***Senecio Biafrae*** Combined With Zinc on Testicular Function of Adult Male Sprague Dawley Rats. J Fam Reprod Health 2018; 12(1): 8-17.

[18] Vahalia MK, Thakur KS, Nadkarni S, Sangle VD. Chronic toxicity study for Tamra Bhasma (A Generic Ayurvedic Mineral Formulation) in laboratory animals. Recent Res in Science and Tech. 2011; 3(11): 76-79.

[19] Lazic1 LE, Semenova E, Williams DP. Determining organ weight toxicity with Bayesian causal models: Improving on the analysis of relative organ weights. Scientific Report. 2020; 1-11

[20] Akuodor GC, Okorie AU, Ekenjoku JA, Megwas AU, Aja DOJ, Irogbeyi LA, Okezie OA Acute and sub-acute toxicity effect of the ethanol leaf extract of Justicia insularis in wistar rats. Saudi Journal of Med and Pharm Sciences. 2020; 6 (2): 225-231.

[21] Wu Z, Ma Y, Zhao L, Cai S, Cheng G. Acute and subchronic toxicities of the ethanol and hot-water extracts from Chinese sumac (*Rhus chinensis* Mill.) fruits by oral administration in rats. Food Chem. Toxicol. 2018; 119:14–23.

[22] Mukinda J.T., Syce J.A. Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. J. Ethnopharmacol. 2007;112 :138–144.

[23] Benrahou K, Doudach L, Guourrami O. Acute toxicity, phenol content, antioxidant and postprandial anti-diabetic activity of *Echinops spinosus* extracts. Int. J. Second Metab. 2022;9:91–102

[24] Adeyemi OS, Akanji MA. Biochemical changes in the kidney and liver of rats following administration of ethanolic extract of Psidium guajava leaves. Human and Experimental Toxicology. 2010; 30(9) 1266–1274

[25] Li Y., Zhuang Y, Tian W, Sun L. In vivo acute and subacute toxicities of phenolic extract from rambutan (*Nephelium lappaceum*) peels by oral administration. Food Chem. 2020;320

[26] Essien AD, Edidara Thomas, Essiet GA, Akuodor GC. Anti-inflammatory, antipyreticand anti-nociceptive activities of theethanol stem bark extract of *Salacia lehmbachii.* British Journal of Pharmacology and Toxicology. 2017; 8(2):9-16.

[27] Ohanme EO, Etu KE, Akuodor GC,Nwakelu B. Ofor CC. Evaluation of Anti-Inflammatory, Anti-Nociceptive andAntipyretic Properties of the Ethanol LeafExtract of Celosia Leptostachya in Ratsand Mice. International Journal ofPharmacy and Pharmaceutical Research.2022;25(4):284-298.

[28] Prempeh A, Mensah-Attipoe J. Analgesic activity of crude aqueous extract of the root bark of zanthoxylum xanthoxyloides. Journal of Ghanian Medicine. 2008; 42(2):79-84.

[29] Chandran, R.; George, B.P.; Abrahamse, H. Anti-Proliferative, Analgesic and Anti-Inflammatory Properties of *Syzygium mundagam* Bark Methanol Extract. Molecules 2020; *25*, 2900.