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ABSTRACT

Erytherina senegalensis leaf extract has been reportedly used in Nigerian studies for treatment of malaria, gastrointestinal disorders, fever, dizziness, secondary sterility, diarrhoea, jaundice, nose bleeding, pain and administered for venereal diseases. In view of its usage, the study aimed to investigate the effect the leaf extract of Erytherina senegalensis would have on biochemical parameters of the liver and histology of the liver of albino rats. Fresh mature leaf samples of E. senegalensis were collected, ethanolic extract was prepared and the effect of sub-chronic oral administration of the leaf extract of E. senegalensis was studied using twenty-five albino wistar rats. The oral administration of the leaf extract was done four times per week, for 60 days at doses of 2000mg/kg for group 1, 4000mg/kg for group 2, 6000mg/kg for group 3, 8000mg/kg body weight for group 4 and group 5 is the control group, received distilled water and food alone. The rats were anaesthetised by chloroform in a close jar and blood samples were collected for biochemical test, experimental rats were sacrificed by cervical dislocation and the liver harvested. The result reveals the significant increase in the serum activity level of Serum Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and Total Bilirubin, at p<0.05 and significant decrease in Direct Bilirubin at p<0.05. Also, a significant rise in total and decrease Conjugated Bilirubin was also observed, also result of the Histopathological analysis of the liver showed no alteration on the liver architecture of low dose group, but showed mild portal congestion on medium dose and high dose groups and this may suggest that the leaf extract of Erytherina senegalensis may be considered toxic on liver architecture and biochemical indices when consumed for long period of time in treatment of diseases.

Keywords: Erytherina senegalensis, liver enzymes, liver histology

INTRODUCTION

The wide use of medicinal plants among both urban and rural population in Nigeria could be attributed to cultural acceptability, efficacy against certain kind of diseases, physical accessibility and economic affordability as compared to orthodox medicine. Although, traditional medicine still remains the main resource for a large majority (80%) of the people in this region for treating health problems (Addis et al., 2001), there is also a strong belief that because herbal remedies are derived from nature, they are devoid of adverse or toxic side effects often associated with drugs used in convectional orthodox medicine (Adebayo et al., 2010). However, herbal preparations are assumed not to be safe because they may contain contaminants such as pathogenic microbes (Kneifel et al., 2002), heavy metals (Abou-Arab and Abou-Donia, 2000) and aflatoxins (Kneifel et al., 2002), Due to the manner in which they are prepared as well

as that majority of individuates use herbal medicine without considering the dosage effect.

Erythrina senegalensis is an example of a medicinal plant commonly known as 'Senegal coral' which belongs to the family Fabaceae. According to Kone *et al.*, (2004) the plant extract of *Erythrina senegalensis* has been useful in traditional medicine as antibacterial agents against some antibiotic-resistant strains.

The leaf and bark of Erytherina senegalensis extract have been reported used in Nigerian treatment of studies for malaria. gastrointestinal disorders, fever, dizziness, secondary sterility, diarrhoea, jaundice, nose bleeding, pain and administered for venereal diseases (Togola et al., 2008). A decoction of the bark has been used for the treatment of coughs bronchial infection, and throat inflammation. The pounded bark and leaves of Erytherina Senegalensis are used in soups administered after delivery, also in Nigeria; it is given to women during labour to ease pain (Iwu,

2015). In spite of the above literatures, none is known about the toxic effects of the widely traditionally used decoction of the *E.senegalensis* leaf extract on the liver. Hence, the present study is aimed to evaluate the effect of ethanolic leaf extract of *E.Senegalensis* on the biochemical functions and histology of liver of albino wistar rats.

MATERIALS AND METHOD

Ethical Approval

Necessary approval was obtained from the Faculty of Basic Medical Science Ethics Committee, Nnamdi Azikiwe University, Nnewi Campus.

Collection and Authentication of the Plant Material

Fresh mature leaf samples of *E. senegalensis* were collected in Nnewi, Anambra State, and botanical identification of the plant was done by Mr. Egboka Tochukwu of the Department of Botany, Nnamdi Azikiwe University, Awka, with reference no NAU/BOT/232.

Plant Preparation

All the samples of E. senegalensis were thoroughly rinsed with running tap water and distilled water before being air-dried at room temperature for 30 days. Then, the plant sample was pulverized to dry powder using an electric grinder into minute pieces and the extract was soaked in absolute ethanol for 4 days with frequent agitation at room temperature. The extract was filtered with Whatman paper No.1 and the residue of fine powder was then resoaked with a fresh portion of ethanol twice for four days each time at room temperature. The filtrate was concentrated under reduced pressure in vacuum at 45°C and evaporated to dryness on a rotary evaporator (Model 342/7, Corning Ltd). The yield of the extract was 17.1% based on dry weight.

Care of the Animals and Experimental Design

All animal experiments in this study followed the principles of laboratory Animal Care (National research council publication 2011). Daily, the animals were fed with the normal diet of feed grower's mash produced by Premier feed mills Co. limited (a subsidiary of Flour mills Nigeria PLC). The feed was provided in plastic plates while 20cl distilled water was provided in plastic bottles with stainless steel nozzles which were placed each at the top of the cages to allow the animals suck the water comfortably the animals were allowed access to feed and deionized water ad-libitum before the commencement of the experiment.

Twenty-five (25) apparently healthy female wistar rats (2-3 months old) weighing between 100g to 200g were used for this study. The rats were purchased from Animal Farm in Nnewi, Nnewi-North, Anambra State Nigeria and were transferred to the Animal House of the Department of Anatomy, College of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi. These animals were fed with the normal diet of feed grower's mash produced by Premier feed mills Co. limited (a subsidiary of Flour mills Nigeria PLC) and distilled water adlibitum for the period of two weeks to help them acclimatize to the new environment under standard conditions (23 \pm 2C, humidity 60-70%, 12h light and dark cycles) before commencement of the actual experiment.

The rats after acclimatization were randomly grouped into five groups for experimentation. The rats were weighed and the weight recorded. The rats were grouped and oral administration of the leaf extract of *Erytherina senegalensis* based on the acute toxicity study which was found to be greater than 5000mg/kg body weight (Nnama *et al.*, 2016). The rats were grouped and oral administration was done as below;

GROUP 5(control) received only water and feed for 8 weeks, weighed once per week

GROUP 4(high dose) received 8000mg/kg body weight, weighed once per week

GROUP 3(medium dose) received 6000mg/kg body weight, weighed once per week

GROUP 2(moderate dose) received 4000mg/kg body weight, weighed once per week.

GROUP 1(low dose) received 2000mg/kg body weight, weighed once per week.

Observation of Behavioural Changes in the Animals

Visual observations for mortality, behavioural pattern changes such as weakness, aggressiveness, food or water refusal, diarrhoea, salivation, discharge from eyes and ears, noisy breathing, changes in locomotor activity, injury, pain or any signs of illness in each treated group were monitored carefully on daily basis throughout the experiment period.

Monitor of Fluid, Feed Intake and Body Weight of Animals

The fluid and feed intake of the animal were monitored and recorded daily while the weights of the animals were taken weekly.

Necropsy

After 60 days of exposure the final body weights of the Wistar albino rats were taken, and thereafter, sacrificed by painlessly anesthetization with chloroform using chloroform in a closed jar. After anesthetization blood samples were collected directly from the heart (at the thoracic region) using 5ml syringes and indirectly from the eyes (ocular puncture) using cannula with catheter and placed in a specific sterilized plastic containers required collected for hormonal level evaluation. Thereafter the animals were sacrificed by cervical dislocation and the ovaries were harvested and weighed immediately. Thereafter, the organs were fixed in 10% buffered formal saline and processed for routine histopathological studies, and the slides analyzed.

Histopathological Assessment

Histopathological examinations were carried out on ovary of the rats. They were fixed in 10% formalin, dehydrated in gradual ethanol concentrations (50-100%), cleared in xylene and embedded in paraffin. Sections (4-6 μ M thick) were prepared and then stained with hematoxylin and Eosin (H-E) dye for photomicroscopic observation under light

microscope	at	high	power	magnifications	(x400
objective)					

Statistical Data Analysis

The ovary weights (relative) and oestrogen level were evaluated using the statistical package of social sciences (SPSS) software version 21.0 (SPSS) Inc. Chicago and Microsoft. Statistical analysis of variance was carried out using student T-test and one way ANOVA (SPSS 21.0). A value of p < 0.05 was used as the level of significance.

RESULTS

Physical Observation/Clinical Signs

Daily oral administration of *Erytherina senegalensis* extract for 8weeks did not induce any obvious symptom of toxicity in rats, including the highest dose tested at 8000 mg/kg body weight, deaths was not recorded and no obvious clinical signs were found in any groups throughout the experimental period.

Physical observation of the treated rats throughout the study indicated that none of them showed signs of toxicity in their skin, fur, eyes, mucus membrane, or behavioural changes, diarrhoea, tremors, salivation, sleep, and coma. Although rats in group 4(high dose) showed relative weakness immediately after administration.

Gross observation of the organs showed no change in colour, hypertrophy and abnormal fat deposition.

GROUPS	WEEK 1(g)	WEEK 2(g)	WEEK 3(g)	WEEK 4(g)
Ι	87.71±26.77	112.57±20.59	77.86±17.82	75.71±12.12
II	79.86±14.04	85.14±29.05	72.29±12.93	65.00±15.62
III	87.29±20.13	102.00 ± 24.47	67.29 ± 18.17	$61.00{\pm}18.81$
IV	92.43±23.17	99.43±26.68	73.00±16.46	60.71±19.41
V(Control)	82.43±13.55	105.14 ± 4.98	73.57±12.49	76.43±33.24
F-Ratio	0.414	1.364	0.401	0.949
Sig	0.797	0.270	0.807	0.450

Fable1.	Food	intake	of the	Rats
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Table showed the mean and standard deviation of the volume of weekly food intake in grams per group, in all the groups the food intake increased in the second week thereafter a progressive decline was observed. Analysis of variance (ANOVA) showed that there was no significant difference (p>0.05) in food intake between the test and control group during the 4 weeks of administration.

Table2.	Fluid	intake	of the	rats
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GROUP	WEEK 1(mls)	WEEK 2(mls)	WEEK 3(mls)	WEEK 4(mls)
Ι	81.43±36.71	103.43±43.52	82.29±22.54	79.00±43.82
II	85.83±19.60	104.29±47.12	86.71±39.74	70.86±37.61
II	93.00±12.23	86.00±42.97	79.43±41.32	82.14±38.06
IV	108.33±21.13	106.29 ± 45.48	98.43±14.11	64.86±45.21
V(Control)	101.67±9.83	123.71±26.39	89.71±22.37	105.14 ± 24.60
F-Ratio	1.533	0.717	0.423	1.118
Sig	0.221	0.587	0.791	0.366

Table showed the mean and standard deviation of weekly fluid intake in millilitres, groups I, II, IV, increased in the second week thereafter a progressive decline was observed. Data showed, Analysis of variance (ANOVA) showed that there was no significant difference (p>0.05) in fluid intake between the test and control group during the 4 weeks of administration.

Table3. Body weight changes throughout period of administration

	GROUP	GROUP	GROUP	GROUP	V(CONTROL)(G)	SIG
	I(G)	II(G)	III(G)	IV(G)		
WEEK1	170.00 ± 4.47	180.40 ± 4.56	182.80±9.96	167.00±10.95	$181.40{\pm}18.81$	0.112
WEEK2	175.60 ± 5.18	184.80 ± 3.03	187.00 ± 10.27	$171.80{\pm}10.40$	$186.40{\pm}16.82$	0.098
WEEK3	180.60 ± 4.45	190.40±3.85	192.40±7.37	180.00 ± 12.08	193.20±17.98	0.152
WEEK4	191.25±9.67	194.80 ± 2.28	192.60±6.07	179.20±9.83*	193.60±17.90	0.171
WEEK5	191.50±9.57	196.60±1.95	194.25±6.13	183.00±9.17*	197.80±19.03	0.267
WEEK6	191.75±9.18	197.00±1.73	199.25±2.99	185.40±10.11*	200.60±18.99	0.224
WEEK7	197.33±4.62*	203.25 ± 5.85	202.50 ± 4.65	194.00±4.90*	209.00±11.27	0.070
WEEK8	203.33 ± 5.51	208.75 ± 5.06	204.00 ± 4.62	196.50±4.43*	211.20±12.46	0.102

*Significant difference compared to control (p<0.05)

Table showed the mean and standard deviation, Analysis of variance (ANOVA) showed that there was no significant difference in body weight between groups (p>0.05) during the 8 weeks of administration. Post-hoc analysis however, showed significant differences in body weight between group I and control at week 7 (p<0.05), and also between group IV and the control at weeks 4 (p<0.05), 5 (p<0.05), 6 (p<0.05), 7 (p<0.05) and 8 (p<0.05).

Table4. Showing organ weight

Organ weight (G)	Group I(g)	Group II(g)	Group III(g)	Group IV(g)	Group V(Control)	Sig
Liver	6.55±0.62*	6.82±0.97*	5.93±0.98*	6.38±0.34*	4.55±0.64	0.042

* Significant increase (p<0.05) when compared to the control

Table 5: Showed organ weight for test and control groups, data showed mean and standard deviation. Analysis of variance (ANOVA) between groups showed significant difference in weights of the liver (p < 0.05).

Table5: Percentage of Organ Weight Relative to Body Weight of the Female albino rats

% of Organ Weight in Relation to Body Weight	Group I	Group II	Group III	Group IV	Control Group	Sig
Liver	3.33±0.32*	3.28±0.55*	2.91±0.49*	3.25±0.19*	2.09±0.29	0.033

* Significant increase (p<0.05) when compared to the control

Table 6 showed relative organ weight for test and control group, data showed means and standard deviation. Analysis of variance (ANOVA) between groups showed significant difference in % relative weight of the liver (p<0.05). Post-hoc analysis further depicted significant increase in % relative weight between certain test groups and the control group.

Parameter	Group I	Group II	Group III	Group IV	Group V(control)	Sig
D.Bil((UMOL/L)	$5.70 \pm .46^{\#}$	$5.37 \pm 1.12^{\#}$	$5.23 \pm .45^{\#}$	$6.47 \pm .65^{\#}$	$11.35 \pm .64$	0.000
T.Bil(UMOL/L)	19.37±4.23	23.03±.15*	20.93±2.58*	24.93±.15*	$14.65 \pm .78$	0.009
ALT((UL)	44.87±3.22*	39.97±.49*	35.40±3.78*	46.67±2.55*	29.00±.57	0.000
ALP (UL)	95.43±5.75*	66.10±3.29*	87.13±5.11*	22.27±.91	23.00±.14	0.000
AST(UL)	84.37±11.46*	48.43±1.64	52.87±1.81	56.37±1.12*	44.00±.14	0.000

 Table6: Liver and kidney function test for all groups

* Significant increase (p<0.05) when compared to the control

[#] Significant decrease (p<0.05) when compared to the control

Table: Liver and kidney function test for all groups data showed, mean and standard deviation. Analysis of variance (ANOVA) showed significant difference in D.bil (p<0.05), T.bil (p<0.05), ALT (p<0.05), ALP (p<0.05), and

AST (p < 0.05) between the groups. There was a significant increase in the level of liver enzymes, total bilirubin increased between groups 2, 3 and 4. And the level of conjugated bilirubin decreased significantly across the group when compared with the control.

Histological Findings.



Plate1. Showed a photomicrograph of the liver of albino rat from group V(control) with a normal typical liver histo-architectural features with no visible lesion, showing Hepatocytes (H) and Sinusoids (S) of normal histology [H&E×400].



Plate2. Showed the photomicrograph of the liver of group I (low dose group) of albino rat, showing Hepatocytes (H) and Sinusoids (S) appears normal with normal liver histo-architecture, no visible lesion seen.[H&E×400].



Plate3. Showed the photomicrograph of group II (moderated dose) of the liver of an albino rat, showing Sinusoids (S) appearing prominent and congested. [H&E×400].



Plate4. Showed the photomicrograph of liver of group III (medium dose) of albino rat, showing Hepatic portal vein (H) with mild peri-portal cellular infiltration. [H&E×400].



Plate5. Showed the Photomicrograph of liver of group IV (high dose) of albino rat, showing portal vein (H) with a mild portal congestion and periportal cellular infiltration by mononuclear cells and hepatocytes (A) diffusely vacuolated histology [H&E ×400].

DISCUSSION

General toxicity can be accessed through organ weight measurements, in which changes in the body weight and organ weight is a sensitive indicator of toxicity (Thanabhorn et al., 2006; Norazmir and Ayub, 2010). The results of organ weight showed that liver has a significant increase across the group and the organ weight relative to body weight of the test groups as shown in table above reveals significant difference in weights of liver (p<0.05) when compared with the control group, which signifies there may be sign of toxicity in the liver. Also, significant increase was observed in relative organ weights of the liver of some treated groups when compared to the control group, which partly agree with Atsamo et al., 2011, that there were no significant changes in the relative weights of liver between the control and treated rats.

Fat accumulation in hepatocytes usually takes place in liver injury and leads to increase in the relative liver weight as well as liver enzymes such as Alkaline Phosphatase (ALP) and Serum Aspartate Aminotransferase (AST) (Chin et al., 2008). Serum Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) enzymes present in the liver are used as a marker to detect chronic liver disease (Hor et al., 2012). Alkaline Phosphatase (ALP) is mainly present in bile, liver, kidney, bone and placenta and there will be a significant elevation of this enzyme in liver injury (Betti et al., 2012). Serum ALP is a useful indicator to diagnose intra hepatic and extra hepatic bile obstruction in the liver. (Shonia et al., 2013). Also, Emerson et al., 1993) have reported that enhancement in the level of serum proteins is an indication of tissue injury and reflection of hepatic toxicity.

Serums ALT and AST considered in this study are important and play significant role in the diagnosis of liver cytolysis (Peters and Boyd, 1966). The estimation of the levels of protein and bilirubin is used to examine the synthetic and excretory function of the liver, respectively (Onu *et al.*,2013). Tissue enzyme assay can also indicate tissue cellular damage long before structural damage can be picked up by conventional histological techniques. Such measurement can also give an insight to the site of cellular tissue damage as a result of assault by the plant extract (Adebayo *et al.*, 2003).

Biochemical analysis in table 7, reveals the significant increase in the serum activity level of Serum Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and Total Bilirubin, at p<0.05 and significant decrease in Direct Bilirubin at p<0.05. Also, a significant rise in total and decrease Conjugated Bilirubin was also observed and it is obviously known that increase in bilirubin levels suggests increase in haemolysis (Orish et al., 2003, Atsamo et al., 2011). This may be easily understood and indicating toxic effect, which may be due to the high doses administered to the experimental rat for long period of time, although the result agreed with Atsamo et al., 2011. Who reported in a sub-chronic administration of Erytherina senegalensis given by oral route once a day for 28 days caused significant increase in the levels aspartate of total protein. serum (AST), aminotransferase alanine aminotransferase (ALT) and alkaline phosphatase (ALP), this may be an indication that the leaf extract would be toxic on the liver when administered orally for a long duration of time.

The result of the Histopathological analysis of the liver as shown in plate 2 showed no alteration and no lesion in group 2 but showed mild portal congestion in group 3 and 4 which signifies that the disruption of the architecture of the liver is dose dependent and this disagrees with previous studies which showed hepatoprotective effects of the extract and components isolated from the stem bark of *Erythrina senegalensis* (Njayou *et al.*, 2004; Donfack *et al.*, 2008).

In conclusion, the leaf extract of *Erytherina* senegalensis use in treatment of malaria, gastrointestinal disorders, fever, dizziness, secondary sterility, diarrhoea, jaundice, nose bleeding, pain and administered for venereal

diseases may not be consider safe at high dose level and for a longer period of time.

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