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Original Research Article

Effect of Aqueous Leaf Extract of *Justicia carnea* on Hematological Parameters of Male Wistar Rats Exposed to Thioacetamide

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ABSRTACT

This study evaluated the haematological potential of aqueous leaf extract of *Justicia carnea* against thioacetamide (TAA) induced toxicity in male Wistar rats. Thirty (30) male Wistar rats were randomly assigned into six (6) groups of five (5) animals each. Group A served as control. Group B received a single dose of TAA (300 mg/kg b.w.t) intraperitoneally, group C received TAA and 50 mg/kg b.w.t of silymarin while groups D, E, and F rats received TAA prior to administration of 200, 400, and 600 mg/kg b.w.t of the extract. The values of haemoglobin concentration (Hb), packed cell volume (PCV), and red blood cell count (RBC) were significantly ($p < 0.05$) decreased, while a significant ($p < 0.05$) increase in white blood cells (WBC) was observed in rats exposed to TAA. Treatment with the extract ameliorated the haematological alterations caused by TAA toxicity. These findings show the extract's efficacy in ameliorating haematological alterations induced by TAA exposure and also validate its use in the folkloric management of haemolytic anemia.

Keywords: Hematology, *Justicia carnea*, Silymarin, Thioacetamide, Wistar rats

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Introduction

Life has embraced its existence with chemicals as an essential habitat. Chemicals play a significant role in sustaining the activities of a living organism and preventing and controlling the pathogenesis of many terminal diseases.[1] Consequently, chemicals endanger human health, reduce lifespan, destroy wildlife, and challenge the ecosystem.[2-5] Thioacetamide is a centrilobular hepatotoxicant, widely used as a model compound to induce acute and chronic liver disease.[6] Study by[7] suggests that the relatively short half-life of thioacetamide could be the mechanism of its induced hepatic necrosis. The rapid elimination of thioacetamide results in gross injury to the hepatocyte. Thioacetamide goes through a two-step bioactivation interceded by microsomal CYP2E1 to sulfine, and afterward to sulfene, a reactive metabolite.[8,9] This mechanism of induced toxicity has been shown to include induction of oxidative stress leading to oxidative damage of biomolecules, interference with the essential trace elements of metabolism, and mediation of cell apoptosis.[10] The hematopoietic systems represent a sensitive target of toxic compounds and an essential index of physiological and pathological status in experimental animals.[11] White blood cell counts usually increase following foreign invaders (pathogens) resulting in a normal body physiological response that boosts the body's defense mechanisms.[12,13]

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The possible anti-anaemic property of any plant could be linked to the improved hemoglobin, PCV and RBC upon administration of such plant to the model.[14] *Justicia carnea* (*J. carnea*) is a flowering plant in the family Acanthaceae native to the Atlantic Forest ecoregions of eastern Brazil.[15] It is a folklore plant used conventionally as a blood tonic, for the management of inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes, diarrhea, liver diseases, rheumatism, and arthritis[16]. Considering the safety and financial friendliness of medicinal plants, research has intensified into exploring more therapeutic advantages of this plant. Silymarin a naturally occurring compound is extracted from the dried seeds of 'milk thistle' (*Silybum marianum*). The seeds have a higher concentration of silymarin than their other plant parts (Luper, 1998).[17] Silymarin contains a complex mixture of four flavonolignan isomers, namely silybin, isosilybin, sildianin and silychristin. Silymarin has been found to possess cytoprotective and hepatoprotective properties.[18] In many cases, the antioxidant mechanism of silymarin is considered to be responsible for its protective action

Materials and Method

Chemicals and reagent: Thioacetamide salt, silymarin, absolute ethanol, chloroform were products of Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

Collection and extraction of plant material

Fresh leaves of *Justicia carnea* were Obtained from Umuariaga, Umudike in May 2018. The leaves were identified and authenticated by a Botanist Mr. Nwoko Magnus of the Department of Plant Science and Biotechnology, College of Natural Sciences in Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, and a voucher specimen number MOUAU – 0623 was obtained.

The leaves were rinsed in distilled water and allowed to air dry at room temperature. The dried leaves were pulverized into a coarse powder using a waring blender. A known quantity (500 g) of the powdered

leaves was macerated in an extraction glass jar containing 2000 ml of distilled water for 48 hours with occasional shaking to increase extraction. The macerated leaves were strained through a muslin cloth and then filtered using No 1 Whatman filter paper. The filtrate was collected and stored in a glass beaker and then put in a freeze dryer. The filtrate was allowed to dry at ≤ -40ºC for 24 hours. The freeze-dried extract was stored at 4ºC in an air-tight glass container until required for biochemical assays.

Qualitative phytochemical analyses of Justicia carnea
Phytochemical screening of the aqueous sample was carried out to identify secondary metabolites- alkaloids, flavonoids, saponins, tannins, and phenol using standard phytochemical methods.[19-23]

Determination of lethal dose (LD_{50})
An acute toxicity study was carried out on the aqueous extract according to the method described by Lorke.[24]

TAA preparation and administration
TAA (thioacetamide) was prepared by suspending it in normal saline and administered to the rats at 300 mg/kg body weight intraperitoneally(*i.p.)*.

Animal care and experimental design
Thirty healthy male Wistar rats were purchased from the Department of Zoology and Environmental Sciences, University of Nigeria, Nsukka. The rats were acclimatized for two weeks at the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State. The animals were housed in well-ventilated cages (stainless steel bottom and wire mesh top) and kept under controlled environmental conditions with twelve hours of light and dark cycles. The rats weighed 100 – 120 g at the commencement of this study and were randomly grouped into six groups of five animals each. Group A served as normal control and received standard diets and clean water *ad libitum* throughout the experiment. Groups B, C, D, E, and F received a single dose of thioacetamide (300 mg/kg b.w.t) intraperitoneally. However, group B served as negative control while rats in group C were treated with 50 mg silymarin/kg body weight of rats. Animals in groups D, E, and F were treated with 200, 400, and 600 mg extract/kg body weight of rats respectively for 14 days.

Ethical statement
All the experimental handling procedures were performed in strict accordance with protocols approved by the Animal Care and Ethical Committee of Michael Okpara University of Agriculture, Umudike with the approval number COLNAS18098.

Sacrificing of experimental animals and collection of blood samples
At the end of the treatment, the animals were euthanized in a chloroform-saturated chamber and the abdominal cavity opened up after an overnight fast. The blood sample was collected in an EDTA bottle via cardiac puncture. [25]

Hematological analysis
The hematological indices of the blood samples were determined using an automated URIT – 2900 Plus 3 Differential Hematological Analyzer. Parameters that were determined include hemoglobin concentration (HB), packed cell volume (PCV), red blood cell count (RBC), and white blood cell count (WBC).[26]

Statistical analysis
Data obtained from this study were presented as mean ± standard error of mean (SEM) and analyzed by one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences) statistics version 20. Groups were compared using Duncan's multiple test range and values of $p < 0.05$ were considered statistically significant.

Results and Discussion

In recent times, the use of natural herbs against poisoning caused by toxic substances has been explored owning to their protective effects against toxicity of various pollutants and pathogenic factors.[27,28] Phytochemical screening of *Justicia carnea* leaf indicated the presence of secondary plant metabolites such as alkaloids, flavonoids, saponins, phenols, tannins, and steroids as shown in Table 1. This result is similar to a study earlier reported by Onyeabo *et al*.[15] These secondary metabolites could be responsible for its therapeutic potential which include antioxidant, anti-cancerous, anti-inflammatory, and anti-anaemic properties.
Acute toxicity test (LD_{50}) of aqueous leaf extract of *Justicia carnea* in the experimental animals didn't record any death. The LD_{50} of the extract was greater than 5000 mg/kg. This suggests that the extract could be relatively safe for consumption.
Hemoglobin (Hb) concentration was significantly ($p < 0.05$) decreased in the negative control group compared to the normal control and test groups as shown in Table 2. Hb concentration was however increased ($p < 0.05$) in the groups treated with silymarin and extract. The effect of *J. carnea* on Packed Cell Volume (PCV) is also shown in Table 3. The PCV of the negative control group was shown to be significantly ($p < 0.05$) lower than that of the control. Administration of silymarin and the varying doses of the extract significantly improved PCV when compared to the negative control ($p < 0.05$). Amongst the varying doses of the extract, 400 mg/kg b.w.t had the most protective effect by improving the values of PCV when compared to that of 200 and 600 mg/kg. Results from Table 4 revealed that thioacetamide-induced stress significantly ($p < 0.05$) reduced red blood cell count relative to normal control. However, administration of silymarin and the varying doses of the extract improved ($p < 0.05$) red blood cell count. Administration of 400 mg/kg b.w.t of the extract, significantly improved red blood cell count when compared to 200 and 600 mg/kg of the extract.
Thioacetamide (TAA) causes harmful effects on cellular and metabolic systems. The present study demonstrated that TAA causes serious changes in haematological parameters. The haematopoietic system is one of the most sensitive targets of toxic compounds.[29] Haematological indices have been widely used in the diagnosis of a variety of diseases and pathologies induced by different toxicants, environmental pollutants, and drugs in humans and animals.[30]

Table 1: Phytochemical Constituents of Aqueous Leaf Extract of *Justicia carnea*

Phytochemical Constituents	*Justicia carnea* leaf
Alkaloids	+
Flavonoids	+
Saponins	+
Phenols	+
Tannins	+
Steriods	+

Key: + = detected

Table 2: Hemoglobin concentration in different groups treated with *J. carnea* aqueous leaf extract.

Groups	Hb (gdL^{-1})
A (Normal Control)	12.300 ± 0.42^{a}
B (TAA: Negative Control)	9.050 ± 0.07^{c}
C (TAA + silymarin 50 mg/kg b.w.t)	11.250 ± 0.35^{b}
D (TAA + *J. carnea* 200 mg/kg b.w.t)	11.500 ± 0.71^{b}
E (TAA + *J. carnea* 400 mg/kg b.w.t)	11.500 ± 0.71^{b}
F (TAA + *J. carnea* 600 mg/kg b.w.t)	12.000 ± 0.00^{b}

Values are represented as mean ± S.D. Means with different superscripts are significantly different ($p < 0.05$). Hb = Hemoglobin.

The results obtained show that exposure to TAA significantly ($p < 0.05$) reduced the values of hemoglobin concentration, packed cell volume, and red blood cells. These findings are similar to the effect of curcumin and thymoquinone in male mice exposed to TAA[31]. Reduction in haemoglobin concentration (Hb) and packed cell volume may be due to an increased rate of haemolysis and /or reduction in the rate of erythropoiesis. The decrease in red blood cell values may be due to the susceptibility of the haematopoietic system to being damaged by exposure to TAA. However, administration of the extract increased hemoglobin concentration, packed cell volume, and red blood cell count. These findings are similar to previous works done by Onyeabo *et al*[15] and Anthonia *et al*[32] The possible anti-anaemic property of the leaf could be linked to the improved hemoglobin concentration, PCV, and RBC as observed in studies by Akintimehin *et al*.[14] Therapeutic agents of plant origin such as *Xylopia aethiopica*,[33] *Tectona grandis*,[34] and studies on extracts of *M. indica*, *A. hybridus* and, *T. occidentalis*[35] have been revealed to increase RBC, hemoglobin concentration, and packed cell volume. The anti-anaemic potential of the extract could be credited to the presence of dietary bioactive constituents that stimulate the activities of hematopoietic cells and the stabilization of blood in circulation.[32]

Table 3: Packed cell volume in different groups treated with *J. carnea* aqueous leaf extract

Groups	PCV (%)
A (Normal Control)	37.000 ± 0.00^{a}
B (TAA: Negative Control)	30.500 ± 0.71^{b}
C (TAA + silymarin 50 mg/kg b.w.t)	36.000 ± 1.41^{a}
D (TAA + *J. carnea* 200 mg/kg b.w.t)	$32.500 \pm 0.71^{c,d}$
E (TAA + *J. carnea* 400 mg/kg b.w.t)	34.500 ± 0.71^{d}
F (TAA + *J. carnea* 600 mg/kg b.w.t)	32.500 ± 0.71^{c}

Values are represented as mean ± S.D. Means with different superscripts are significantly different ($p < 0.05$). PCV = Packed Cell Volume.

Table 4: Red Blood Cell Count in different groups treated with *J. carnea* aqueous leaf extract

Groups	RBC (x 106µL-1)
A (Normal Control)	3.700 ± 0.00^{a}
B (TAA: Negative Control)	2.850 ± 0.71^{b}
C (TAA + silymarin 50 mg/kg b.w.t)	$3.050 \pm 0.71^{b,c}$
D (TAA + *J. carnea* 200 mg/kg b.w.t)	3.200 ± 0.14^{c}
E (TAA + *J. carnea* 400 mg/kg b.w.t)	3.700 ± 0.28^{a}
F (TAA + *J. carnea* 600 mg/kg b.w.t)	3.200 ± 0.14^{c}

Values are represented as mean ± S.D. Means with different superscripts are significantly different ($p < 0.05$). RBC = Red Blood Cell.

Table 5: Total White Blood Cell count in different groups treated with *J. carnea* leaves extract

Groups	TWBC (x 10^{6}µL^{-1})
A (Normal Control)	3200.000 ± 0.00^{a}
B (TAA: Negative Control)	5535.000 ± 4.49^{b}
C (TAA + silymarin 50 mg/kg b.w.t)	3050.000 ± 7.71^{c}
D (TAA + *J. carnea* 200 mg/kg b.w.t)	3505.000 ± 7.07^{d}
E (TAA + *J. carnea* 400 mg/kg b.w.t)	3750.000 ± 7.71^{e}
F (TAA + *J. carnea* 600 mg/kg b.w.t)	3950.000 ± 7.71^{c}

Values are represented as mean ± S.D. Means with different superscripts are significantly different ($P < 0.05$). TWBC = Total White Blood Cell.

Results from Table 5 show that thioacetamide-induced stress significantly ($p < 0.05$) increased total white blood cell count (TWBC) relative to control. This finding is similar to the effect of curcumin and thymoquinone in male mice exposed to TAA.[31] However, the administration of silymarin and the varying doses of the extract significantly ($p < 0.05$) caused a reduction in total white blood cells when compared to the negative control. Administration of 200 mg/kg of the extract significantly ($p < 0.05$) restored total white blood cell count to near normal level when compared to control. The results obtained show that exposure to TAA significantly increased the values of total white blood cells. The increase in white blood cells may be due to an immune system activation in response to TAA exposure. The stress-induced via intraperitoneal administration of TAA may have led to an increase in the total white blood cell count. This result agrees with the finding of Onyeabo *et al.*[15] The presence of toxins or pathogens also increases the number of white blood cells which elicit a physiological response and concomitantly boost the body's defense mechanisms.[36,37] However, the elevated level of white blood cells was restored to near-normal levels after the rats were administered the extract. A reduction in white blood cell count was also observed after the administration of silymarin and graded doses of the extract. This might be due to the ameliorative effect of the extract against thioacetamide (TAA) induced stress.[38,39]

Conclusion

In this study, the results confirmed the safety, anti-anaemic potential of aqueous leaf extract of *J. carnea*, and its usefulness against TAA toxicity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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