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Research Article

Haematinic Effects of Ethanol Extract of *Ficus sur* Leaves on Diethylnitrosamine-induced Toxicity in Wistar Rats

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Abstract

Background and Objective: Many plant materials used in traditional medicine are readily available in rural areas and have made folklore medicine relatively cheaper than modern medicine. The significance of this study was to investigate the hematinic effect of the ethanol extract of *Ficus sur* leaves on diethyl nitrosamine induced haemolytic anaemia in Wistar rats. **Materials and Methods:** The leaves of *Ficus sur* plant were collected within the premises of Federal University Wukari, Taraba State. Twenty five Wistar rats of 100-150 g were acquired and transported to the animal house of the Department of Biochemistry, Federal University Wukari. The rats were grouped into 4 groups of 5 rats each. Group A served as the normal control and received no induction nor treatment, group B were induced with single dose 200 mg kg⁻¹/i.v diethyl nitrosamine but not treated while group C and D were induced with single dose 200 mg kg⁻¹/i.v body weight diethyl nitrosamine and subsequently treated with 200 mg kg⁻¹/p.o/daily of ethanolic extract of *Ficus sur* leaves and 50 mg kg⁻¹/p.o/daily silymarin (standard drug), respectively for a period of 21 days. **Results:** The results showed that administration of diethyl nitrosamine significantly decrease ($p < 0.05$) RBC, HGB, HCT, MCV, MCH, MCHC and PLT levels as compared to the normal control and significantly increase ($p < 0.05$) WBC, RDWc, MID, GRA and LYM. In the other hand, administration of ethanolic extract of *Ficus sur* leaves and silymarin ameliorated these haematological indices near normal levels. **Conclusion:** The results of this investigation was an indication of a possible ameliorative effect of ethanolic extract of *Ficus sur* leaves against diethyl nitrosamine induced haematotoxicity in wistar rats and may be a confirmation of the traditional use of this plant as blood enhancer.

Key words: *Ficus sur*, diethylnitrosamine, haematological damages, toxicity, haemolytic anaemia, haematinic effect

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anaemia is a pathological condition that results in the reduction of red cells in the blood below the normal range. It is one of the common red blood cells disorders arising from various conditions such as nutritional deficiencies, genetic disorder, parasitic infections, injuries as well as drug toxicity and several chemicals such as quinine, primaquine, acetylsalicylic acid, antipyrine and phenylhydrazine have been implicated in the pathology of anaemia¹. Consequently, several plants such as *Telfaria occidentalis*, *Waltheria indica*, *Eclipta alba*, *Carica candamarsnsis*, *Annona squamosa*, *Echinacea angustifolia*, *Hygrophila spinosa*, *Brillantasia nitens* and *Spondias mombin* have been shown to exhibit haematinic effects as well as anti-anaemia activity as reviewed by N'Guessan *et al.*².

Ficus sur belongs to the family Moraceae and is found mostly in the tropics and warm temperate regions³. It is a medium size tree that grows up to 30-35 m tall while the trunk diameter can reach 150 cm with foliage leaves of 2.5-15 cm long⁴. The fresh leaves of *Ficus sur* is traditionally used as food in the form of vegetable, blood enhancer and in folklore medicine where it is used to treat diarrhea, anaemia as well as sexually transmitted diseases⁵. The roots, barks, leaves and fruits have been previously used to treat various pathological conditions, consequently, the roots are braised and crushed with grilled corn cobs and then the filtrate is used to treat female infertility in Africa⁶. Similarly, the fluid obtained via decoction after mixing the roots and leafy twigs is used to treat eczema while decocted trunk bark is used to treat amenorrhea, dysentery, hepatic and cardiovascular pain^{7,8}. The assessment of the metabolites of this plant has indicated the presence of flavonoids, saponins, alkaloids, tannins, proteins, reducing sugar, fats and oil and carbohydrate⁹ whereas, the study by Imo *et al.*¹⁰ has demonstrated the suitable antioxidant activity of *Ficus sur* against free radicals. *Streptococcus faecalis* and *Pseudomonas mirabilis* were effectively inhibited by varying concentrations of ethanolic leaves extract of *Ficus sur* as reported by Yakubu *et al.*¹¹. Similarly, the leaf was also found to show antimicrobial activity against broad spectrum of bacteria such as *Enterobacter aerogens*, *Bacillus species*, *Pseudomonas pyocyania* and *Staphylococcus aureus* as well as antifungal activity against *Aspergillus niger* and *Aspergillus flavus*¹².

The global search for alternative treatment of anaemia and other illness associated with the blood using herbal medicine to replace the chemotherapeutic drugs is on the increase due to the toxicity of most chemotherapeutic drugs. This study was aimed at investigating the effect of ethanol

extract of *Ficus sur* leaves against haematotoxicity induced by diethyl nitrosamine to further increase the spectrum of the already existing plants with anti-anaemic activity.

MATERIALS AND METHODS

Animal management and care: Twenty five Wistar rats of 100-150 g were acquired and transported to the animal house of the Department of Biochemistry, Federal University Wukari and were then maintained under standard laboratory conditions, allowed free access to standard diet and water *ad libitum*. They were allowed to acclimatize for 2 weeks¹³. All experiments were conducted in compliance with ethical guide for care and use of laboratory animals of the Faculty of Pure and Applied Sciences (FUW/FPAS/19/027), Federal University Wukari, Nigeria. This research project was conducted from November, 2018 to August, 2019.

Collection of plant materials: The leaves of *Ficus sur* plant were collected within the premises of Federal University Wukari, Taraba State. The specimen was identified at the university herbarium. The leaves were air dried away from direct sunlight at room temperature for 3 weeks, pulverized using mortar and pestle and stored in air tight container until required.

Ethanol extraction of *Ficus sur* leaves: The extraction was carried out as described by Yakubu *et al.*¹³. The pulverized air-dried leaves were soaked in sufficient volume of ethanol for 24 h at room temperature in a ratio of 1:4 w/v (400 g: 1600 mL). It was continually stirred after each 5 h. After 24 h, the extract was filtered out using clean white sieving mesh and then using What man No. 1 Filter paper. The ethanol used for extraction was recovered from the extract using rotary evaporator. This helped to concentrate the extract which was dried using water bath. The concentrated extract was transferred to an airtight container, corked and preserved in a refrigerator (4°C) for further analysis.

Experimental design: The rats were divided into 4 groups of 5 rats each according to Yakubu *et al.*¹³ with little modification.

Group A : Normal Rats
Group B : Single dose 200 mg kg⁻¹/i.v diethyl nitrosamine+no treatment
Group C : Single dose 200 mg kg⁻¹/i.v diethyl nitrosamine+200 mg kg⁻¹/p.o/daily extract
Group D : Single dose 200 mg kg⁻¹/i.v diethyl nitrosamine+50 mg kg⁻¹/p.o/daily silymarin (Standard drug)

Induction/treatment plan: This animal experiment was approved by the Ethical committee of the Faculty of Pure and Applied Sciences, Federal University Wukari, with the approval number; FUW/FPAS/19/027. The treatment/induction was carried in accordance to Yakubu *et al.*¹³ with little modification. Toxicity was induced in Groups B-D through a single intraperitoneal dose of 200 mg kg⁻¹/body weight diethyl nitrosamine. Subsequently, group C was administered daily oral dose of 200 mg kg⁻¹/body weight extract while group D was administered daily oral dose of 5 mg kg⁻¹/body weight silymarin (standard drug). However, group B was left untreated after the induction throughout the experimental duration. The treatment was then carried out daily for a period of 21 days after which the animals were fasted for 24 h after the last administration of the extract/silymarin and consequently sacrificed for samples collection.

Collection and preparation of animal samples: Animals were sacrificed after the experimental duration and consequently, venous blood was collected by cardiac puncture. The blood samples collected in plane bottles were allowed to clot after which they were centrifuged at 3000 rpm for 5 min to obtain the sera for further analysis¹³.

Haematological analysis: Complete blood count (CBC) was carried out using automated counter (counter machine model: Siemens 1608).

Statistical analysis: The result was analyzed by one-way ANOVA using SPSS statistical package version 20. All data were expressed as Mean $\pm$ SD and difference between groups was considered significant at $p < 0.05$.

RESULTS

Effect of the ethanol leaves extract of *Ficus sur* on haematological parameters: Diethyl nitrosamine significantly ($p < 0.05$) decreased the concentrations of the red cell indices such as RBC, HGB, HCT, MCV, MCH, MCHC while RDWc was significantly increased by this agent in Table 1. These indices were significantly $p < 0.05$ lower in the negative control rats except for the RDWc that was higher as compared to the induced but treated groups that have their red blood cells significantly ($p < 0.05$) improved comparable to the positive control rats after treatment with extract and silymarin.

Table 2 shows the results of differential white blood cells count of diethyl nitrosamine induced-haemolytic anaemic rats. There was an indication of a significant ($p < 0.05$) rise in the white blood cell (WBC), lymphocyte (LYM), granulocyte (GRA), lymphocyte percentage (LYM (%)) maximum inhibition dilution percentage (MID (%)) and granulocyte percentage (GRA (%)) of the induced groups as compared to the positive control rats. This increase remained high in the induced but not treated rats as compared to the treated groups and the normal group whereas, all these indices significantly reversed near normal values in the treated groups.

On the contrary, the platelets differential parameters were significantly ($p < 0.05$) reduced in the induced but not treated group as shown in Table 3. The PLT, PCT, MVP and PDWc where all depleted significantly ($p < 0.05$) in the positive control rats but their levels were considerably elevated in group C and D after they were treated with extract and silymarin for 21 days, respectively.

Table 1: Effect of the ethanol extract of *Ficus sur* leaves on complete blood count of rats

Treatments	RBC ($10^6 \times \text{mm}^3$)	HGB (g dL ⁻¹)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g dL ⁻¹)	RDWc (%)
P. Control	6.92 $\pm$ 0.11 ^a	11.8 $\pm$ 0.31 ^a	37.07 $\pm$ 0.48 ^a	54.00 $\pm$ 1.00 ^a	17.00 $\pm$ 0.17 ^a	32.85 $\pm$ 0.91 ^c	11.05 $\pm$ 0.05 ^a
N. Control	3.03 $\pm$ 0.04 ^b	7.4 $\pm$ 0.40 ^b	28.66 $\pm$ 0.29 ^b	44.00 $\pm$ 0.14 ^b	10.27 $\pm$ 0.16 ^b	22.13 $\pm$ 0.10 ^a	24.83 $\pm$ 0.77 ^b
DEN+ <i>Ficus sur</i>	4.97 $\pm$ 0.20 ^a	10.4 $\pm$ 0.10 ^a	34.04 $\pm$ 0.04 ^a	55.67 $\pm$ 0.48 ^a	15.03 $\pm$ 0.04 ^a	27.1 $\pm$ 0.54 ^b	18.33 $\pm$ 0.47 ^c
DEN+silybon	5.91 $\pm$ 0.09 ^a	11.4 $\pm$ 0.37 ^a	35.75 $\pm$ 0.33 ^a	51.5 $\pm$ 1.04 ^a	16.45 $\pm$ 0.98 ^a	28.05 $\pm$ 1.03 ^b	16.85 $\pm$ 0.97 ^c

n = 5, Mean $\pm$ SD, values with different superscript down the column are significantly difference at $p < 0.05$, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RBC: Red blood cells, HGB: Haemoglobin, HCT: Haematocrit, RDW: Red cell distribution width, DEN: Diethyl nitrosamine

Table 2: Effect of the ethanol extract of *Ficus sur* leaves on white blood cells differential count of rats

Treatments	WBC ($\times 10^9 \text{ cell L}^{-1}$)	LYM (Cell mL ⁻¹)	GRA (10^9 L^{-1})	LYM (%)	MID (%)	GRA (%)
P. Control	5.48 $\pm$ 0.23 ^a	3.64 $\pm$ 0.16 ^a	0.87 $\pm$ 0.06 ^a	54.65 $\pm$ 0.75 ^a	10.25 $\pm$ 0.58 ^a	15.19 $\pm$ 0.05 ^a
N. Control	11.72 $\pm$ 0.14 ^c	6.61 $\pm$ 0.30 ^b	3.01 $\pm$ 0.20 ^b	88.47 $\pm$ 0.27 ^b	20.00 $\pm$ 0.11 ^b	31.52 $\pm$ 0.71 ^c
DEN+ <i>Ficus sur</i>	7.87 $\pm$ 0.55 ^a	4.05 $\pm$ 0.36 ^a	0.98 $\pm$ 0.08 ^a	74.3 $\pm$ 0.30 ^c	10.33 $\pm$ 0.01 ^a	15.39 $\pm$ 0.06 ^a
DEN+silybon	6.46 $\pm$ 0.48 ^a	3.98 $\pm$ 0.64 ^a	1.01 $\pm$ 0.09 ^a	68.04 $\pm$ 0.17 ^c	12.70 $\pm$ 0.31 ^a	29.25 $\pm$ 0.78 ^b

n = 5, Mean $\pm$ SD, values with different superscript down the column are significantly difference at $p < 0.05$, WBC: White blood cells, LYM: Lymphocyte, GRA: Granulocyte, LYM (%): Lymphocyte percentage, MID (%): Maximum inhibition dilution percentage, GRA (%): Granulocyte percentage, DEN: Diethyl nitrosamine

Table 3: Effect of the ethanol extract of *Ficus sur* leaves on platelets differential count of rats

Treatments	PLT (10^9 L^{-1})	PCT (%)	MPV (fl)	PDWc (%)
P. Control	587.00 ± 7.40^d	0.47 ± 0.02^b	8.00 ± 0.08^b	39.9 ± 0.80^b
N. Control	209.00 ± 1.00^a	0.18 ± 0.04^a	6.90 ± 0.21^a	35.6 ± 0.73^a
DEN+ <i>Ficus sur</i>	248.67 ± 0.29^b	0.21 ± 0.09^{ac}	8.37 ± 0.29^b	37.93 ± 0.13^b
DEN+silybon	439.67 ± 0.51^c	0.38 ± 0.04^{cb}	9.00 ± 0.21^b	38.6 ± 0.63^b

n = 5, Mean $\pm$ SD, values with different superscript down the column are significantly difference at $p < 0.05$, PLT: Platelets blood count, PCT: Procalcitonin test, MPV: Mean platelet volume, PDWc: Platelet distribution width count, DEN: Diethyl nitrosamine

DISCUSSION

The results showed that administration of diethyl nitrosamine significantly decrease ($p < 0.05$) RBC, HGB, HCT, MCV, MCH, MCHC and PLT levels as compared to the normal control and significantly increase ($p < 0.05$) WBC, RDWc, MID, GRA and LYM. In the other hand, administration of ethanolic extract of *Ficus sur* leaves and silymarin ameliorated these haematological indices near normal levels. The results of this investigation showed possible ameliorative effect of ethanolic extract of *Ficus sur* leaves against diethyl nitrosamine induced haematotoxicity in wistar rats and may be a confirmation of the traditional use of this plant as blood enhancer.

Deleterious effects of toxic chemicals substances in the body are usually expressed via blood¹⁴, hence, alterations in haematological indices are often utilized to determine several status of the animal biological system and to determine stresses caused by environmental, nutritional and pathological factors¹⁵.

From this study, the result of the complete blood count obtained was in agreement with the findings of Rajendran and Krishnaswamy¹⁶, where RBC, Hb, MCV, MCHC and PCV were distorted by the administration of diethyl nitrosamine in rats. This significant ($p < 0.05$) reduction in these haematological indices could be as a result of selective destruction of mature red blood cells marked by poikilocytosis and anisocytosis in rats as recorded by Brian¹⁷. Also, this decrease in the red cells indices levels may have arisen from the oxidative haemolysis possibly caused by the oxidative stress generated via diethyl nitrosamine in the rats. However, the administration of ethanolic extract of *Ficus sur* leaves significantly ($p < 0.05$) increased the red cells parameters near normal levels in rats which may be attributed to the presence of phytochemicals in the extract and its antioxidant capacity¹⁸. This relative increase in the red cells parameters is a clear case of positive erythropoiesis¹⁸. Similarly, this kind of ameliorative effect exhibited by *Ficus sur* leaves extract was also recorded by Li *et al.*¹⁹, who used ethanolic leaves extract of *Ficus sur* to improve the haematological profile of some diabetic rats.

White blood cells are defensive mechanisms used by the body to fight against cell infiltrations by foreign agents or infections; hence, their increased proliferation could be an indication of immunologic response arising from acute infections, cellular damage or inflammation^{20,21} which might have been caused by the introduction of toxic substance such as diethyl nitrosamine. In the other hand, the significant leukocytosis noticed in the positive control rats was significantly $p < 0.05$ reduced upon administration of extract and silymarin. The groups that were treated greatly reduced their white blood cells (WBC), lymphocyte (LYM), granulocyte (GRA), lymphocyte percentage (LYM (%)) maximum inhibition dilution percentage (MID (%)) and granulocyte percentage (GRA (%)) levels comparable to the normal group which signals a potential anti-haematotoxicity capacity of the extract exerted by its possible positive effect on the haematopoietic micro-environment and its free radical scavenging activity. This evident depreciation in WBC in rats after treatment, has being previously reported on calyx extract of *Hibiscus sabdariffa* by Famurewa *et al.*²², who ascribed such effect to the presence of secondary metabolites that are capable of reducing the oxidative generated by chemical compounds such as carbon tetrachloride and diethyl nitrosamine. Similarly, studies by Yakubu *et al.*²³ also implicated therapeutic effect of medicinal plants to the presence of metabolites which further substantiate the effect exerted by this extract.

Platelets are responsible for blood clotting and hence prevent excessive bleeding or loss of blood during cellular injuries. This evident reduction in PLT count could be the result of infusion of chemical agents which possibly interfered with the signalling pathways and maturation of cells²⁴ and as well may have arisen from the oxidative destruction of the tissues. However, *Ficus sur* leaves extract and silymarin increased the platelets blood count (PLT), procalcitonin test (PCT), mean platelet volume (MPV) and platelet distribution width count (PDWc) significantly ($p < 0.05$) comparable to normal levels. This result was in concomitance with the findings of Yakubu *et al.*¹³ were the administration of *Solanum villosum* extract and its silver nanoparticles greatly reversed haematological parameters including PLT count near normalcy against hepatocellular carcinoma induced by diethyl nitrosamine in rats.

This study significantly justified the use of this plant in the management/treatment of blood related challenges especially as it results from toxicity. There are scanty literatures on the use of DEN in the induction of haematological toxicities. Hence, the use of this plant to manage/treat DEN-induced haematological toxicity is of significance and novelty. This study was limited to the use of laboratory animals, more researches are on-going to decipher the active compound responsible for these effects.

CONCLUSION

The findings from this study has demonstrated the ameliorative effect of *Ficus sur* leaves extract against haematotoxicity via its haematinic properties which might have been exerted possibly owed to its previous reported abundance in phytochemicals and antioxidant capacity. However, mechanistic study can be carried out to really understand how this effect is brought about in the biological system.

SIGNIFICANCE STATEMENT

This study discovers the haematinic effect of *Ficus sur* leaves extract against diethyl nitrosamine induced toxicity. This study will help researchers to uncover the critical area of mechanistic study of this effect that many researchers have not been able to explore. Thus, a new theory on the mechanism of haematinic effect of *Ficus sur* leaves may be arrived at.

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