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Research Article
Ethanol Extract of *Senna occidentalis* Leaves May Ameliorate Haematological Damages Induced by Diethylnitrosamine in Wistar Rats

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Abstract

Background and Objective: Blood disorders are frightful diseases because the patient suffers pain, disfigurement and loss of many physiological processes. In this study, the effects of ethanol extracts of *Senna occidentalis* leaves on haematological parameters in diethyl nitrosamine-induced toxicity was analyzed. **Materials and Methods:** In the experiment, 24 Wistar albino rats were divided into 4 groups of 6 rats each and toxicity was induced intraperitoneally by using diethyl nitrosamine (DEN) at 200 mg kg^{-1}. Group one served as the normal control, group two was administered DEN only. Group three was administered DEN and ethanolic extract at 200 mg kg^{-1}. Group four was administered DEN and silymarin (5 mg kg^{-1}). The rats were sacrificed after 3 weeks of treatment. Blood samples were collected through cardiac puncture and used for hematological analysis; Red Blood Cell (RBC), White Blood Cell (WBC), platelets (PLT), procalcitonin test (PCT), Mean Platelet Volume (MPV), Platelet Distribution Width count (PDWc), haematocrit (HCT), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Count (MCHC) and red blood distribution width count (RDWc). **Results:** The DEN administration caused a significant increase ($p<0.05$) in RBC and HGB compared to the normal control and a significant decrease in PLT levels, whereas the concentrations of WBC, MID and GRA and LYM decreased significantly ($p<0.05$). Administration of ethanolic extracts/silymarin significantly ($p<0.05$) moderated the hematological indices. **Conclusion:** Due to its ability to moderate levels of haematological parameters, it is therefore, recommended for household management of blood related anomalies.

Key words: Toxicity, diethylnitrosamine, *Senna occidentalis*, haematology, anaemia, bioactive ingredients

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

INTRODUCTION

Nature has served as a source of medicinal agents for thousands of years. An impressive number of modern drugs have been isolated from natural sources[1]. Awareness and general acceptability of the use of herbal drugs in today's medical practice is increasing. According to the World Health Organization, 80% of the world's population uses plant-based remedies as their primary form of healthcare[2]. Many pharmaceuticals currently available have a long history of use as herbal remedies. About 70% of the human population is dependent wholly or partially on plant-based medicines. Ethno-historical accounts showed that medicinal plants have been used as a remedy for various human ailments, the reason of using these plants is that they are reservoirs of potent chemical compounds which acts as a curative medicine with fewer side effects[3,4]. Over the past decade, healthcare system is all around the herbal medicine and gained global importance, making an impact on both world health and international trade. Due to the low economic rate of the majority of population in developing countries and high cost of Western medicine, herbal medicine has a long and uninterrupted history of continuous usage by a large proportion of the population in the developing countries[5]. The high cost of conventional drugs, particularly in resource poor communities of the African continent has led to the increased use of plants as an alternative for the treatment of diseases. Although, several synthetic drugs are available, attention is currently being focused on the use of plants and plant products in prevention or correction of various metabolic disorders or in the treatment of specific diseases because of several side effects associated with the use of synthetic drugs[6,7].

Senna occidentalis also known as *Cassia occidentalis* belongs to Caesalpiniaceae family and subfamily of Caesalpinioideae. The roots, leaves and seeds were the parts of the plant used. It is an erect herb, commonly found by road sides, ditches and waste dumping sites. It has been widely used as traditional medicine. *Senna occidentalis* L. is known as "ewe oriesi" in Yoruba, "Akidi agbara" in Igbo, "Dora rai" in Hausa and Coffee Senna in English[8]. The entire parts of the plant have medicinal values.

Medicinal plants relieve and treat cancer by making use of the compounds naturally present with antioxidant and anticancer activities that are known to inhibit or kill carcinogenic cells, some plants may contain properties that naturally have the ability to prevent the spread or risk of developing various forms of cancer. A potent hepatocarcinogen is produced from the metabolism of some drugs and found in tobacco smoke, processed meats, soybean, cheese and wide variety of foods[9]. The cytochrome P_{450}-dependent mono-oxidase systems biotransform DEN. The lethal effects of DEN are initiated by this metabolic activation. During metabolism, DEN induced oxidative stress, resulting in cytotoxicity, mutagenicity and carcinogenicity[10,11]. This nitrosamine targets the liver primarily, in which it is biotransformed by cytochrome P_{450}-dependent mechanisms that have the highest activity in the centrilobular hepatocytes in rats. This research work sought to investigate the effect of ethanol extract of *Senna occidentalis* on haematological parameter in diethylnitrosamine-induced toxicity in Wistar rat.

MATERIALS AND METHODS

Study area: The study was carried out in the Biochemistry Laboratory, Federal University Wukari, from October, 2018-March, 2019.

Sample collection and extraction: The healthy looking *Senna occidentalis* leaves were collected from a farm in Wapan-Ngakwu Wukari LGA, Taraba state. The specie was authenticated and the voucher number deposited. They were dried at room temperature on the laboratory bench.

Sample extraction: The extraction was carried out as described by Yakubu *et al.*[12]. Exactly 400 g of pulverized sample was soaked in absolute ethanol in the ratio 1:4 w/v (400 g: 1600 mL) for exactly 24 h. The extract was filtered out first by using a clean white sieving mesh and then using the Whatman No. 1 filter paper. The filtrate was concentrated by using a rotary evaporator under reduced pressure. The concentrated extract was then transferred into air-tight containers, corked and preserved in a refrigerator (4°C) before analysis.

Experimental animals: Adult albino Wistar rats weighing approximately 150-180 g were used as experimental model. The Wistar rats were acclimatized for 10 days before the commencement of the experiments. The Wistar rats were divided into four groups of six rats each.

Experimental design: The rats were randomly distributed into four groups (n = 6):

- Group 1 (Normal control) was fed normally without any treatment
- Group 2 (DEN control) was administered single intraperitoneal dose of diethylnitrosamine (DEN) at 200 mg kg^{-1} b.wt.

- Group 3 was administered DEN and subsequently treated with ethanolic extract of *Senna occidentalis* at 200 mg kg^{-1} b.wt. per day
- Group 4 was administered DEN and silymarin (5 mg kg^{-1} b.wt.)

Animal treatment: This research work was approved by the ethical committee of the Faculty of Pure and Applied Sciences, Federal University Wukari, with the approval number, FUW/FPAS/19/040. The treatment/induction was carried out as described by Yakubu *et al.*[13] with few modifications. Toxicity was induced in groups 2-4 through a single intraperitoneal dose of 200 mg kg^{-1} b.wt., diethylnitrosamine. Subsequently, group 3 was administered daily oral dose of 200 mg kg^{-1} b.wt., extract, while group 4 was administered daily oral dose of 5 mg kg^{-1} b.wt., silymarin (standard drug). However, group 2 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 under chloroform anaesthesia for samples collection.

Biochemical analysis: Full blood count, Red Blood Cell (RBC), White Blood Cell (WBC), platelets (PLT), procalcitonin test (PCT), Mean Platelet Volume (MPV), Platelet Distribution Width count (PDWc), Haematocrit (HCT), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Count (MCHC) and Red Blood Distribution Width count (RDWc) were analyzed using Abacus 380 haematological analyzer.

Statistical analysis: All the values estimations were expressed as mean±standard deviation and analyzed for ANOVA and *post hoc* Duncan by using SPSS version 25. Differences between groups were considered significant at $p<0.05$.

RESULTS

Haematological parameters: The results of hematological parameter such as; Red Blood Cell (RBC), Platelets (PLT), Haemoglobin level (HGB) and White Blood Count (WBC) were obtained and presented in Table 1. These parameters decreased significantly ($p<0.05$) in group 2, but moderated in group 3 and 4 to normal and near-normal (as in the case of MCH, RBC, MCHC and RDWc group 2). Although, there were differences in the mean HGB values, but not statistically significant ($p>0.05$) across the groups.

The results of platelets blood count (PLT), procalcitonin test (PCT), Mean Platelet Volume (MPV) and Platelet Distribution Width count (PDWc) are presented in Table 2. The PLT values significantly ($p<0.05$) differ across the groups with group 4 having the lowest followed by groups 2, 3 and 1 having the highest. Same pattern of observation was made in PCT although not statistically significant ($p>0.05$) across the groups except group 1. PWDc result was statistically $p<0.05$ across the groups with group 4 having the highest value and group 2 with the lowest value.

Result on the effect of ethanol extract of *Senna occidentalis* on White Blood Cell (WBC), lymphocyte (LYM), Maximum Inhibition Dilution (MID), granulocyte (GRA), lymphocyte percentage (LYM%), Maximum Inhibition Dilution percentage (MID %) and granulocyte percentage (GRA %). The

Table 1: Result on effect of ethanol extract of *Senna occidentalis* RBC, HGB, HCT, MCV, MCH, MCHC and RDW

Treatments	RBC	HGB	HCT	MCV	MCH	MCHC	RDWc
N. Control	6.92 ± 0.11^{b}	11.8 ± 0.31^{a}	37.07 ± 0.48^{b}	54.3 ± 1.00^{b}	17.21 ± 0.17^{b}	31.85 ± 0.91^{b}	19.05 ± 0.05^{b}
P. Control	5.03 ± 0.04^{a}	10.3 ± 0.40^{a}	30.66 ± 0.29^{a}	48.3 ± 0.14^{a}	14.27 ± 0.16^{a}	22.13 ± 0.10^{a}	16.83 ± 0.77^{a}
DEN+SENNA	6.21 ± 0.20^{ab}	10.4 ± 0.10^{a}	40.04 ± 0.04^{c}	55.6 ± 0.48^{b}	15.03 ± 0.04^{a}	27.1 ± 0.54^{ab}	18.33 ± 0.47^{ab}
DEN+SILYMARIN	6.97 ± 0.09^{b}	11.4 ± 0.37^{a}	35.75 ± 0.33^{b}	51.5 ± 1.04^{ab}	16.45 ± 0.98^{b}	32.05 ± 1.03^{b}	19.45 ± 0.65^{b}

Results represent mean±standard deviation of group obtained (n = 6), mean in the column, having different superscripts are statistically significant ($p<0.05$), RBC: Red blood cell, HGB: Haemoglobin level, HCT: Haematocrit, MCV: Mean cell volume, MCH: Mean cell haemoglobin, MCHC: Mean cell haemoglobin count, RDWc: Red blood distribution width count

Table 2: Result on the effect of ethanol extract of *Senna occidentalis* on PLT, PCT, MPV and PDWc

Treatments	PLT	PCT	MPV	PDWc
N. Control	287.00 ± 7.40^{d}	0.47 ± 0.02^{b}	8.5 ± 0.08^{a}	38.6 ± 0.63^{bc}
P. Control	239.67 ± 0.51^{b}	0.38 ± 0.04^{a}	6.9 ± 0.21^{a}	35.6 ± 0.73^{a}
DEN+SENNA	248.67 ± 0.29^{c}	0.41 ± 0.09^{a}	8.3 ± 0.29^{b}	37.9 ± 0.13^{b}
DEN+SILYMARIN	209.00 ± 1.00^{a}	0.38 ± 0.04^{a}	9.6 ± 0.21^{b}	39.9 ± 0.80^{c}

Results represent mean±standard deviation of group obtained (n = 6), mean in the column having different superscripts are statistically significant ($p<0.05$), PLT: Platelets, PCT: Procalcitonin test, MPV: Mean platelet volume, PDWc: Platelet distribution width count

Table 3: Result of WBC, LYM, MID, GRA, LYM, MID and GRA (%)

Treatments	WBC	LYM	MID	GRA	LYM (%)	MID (%)	GRA (%)
N. control	5.48±0.23^{a}	4.05±0.36^{a}	0.56±0.04^{a}	0.87±0.06^{a}	74.65±0.75^{c}	10.25±0.58^{a}	15.19±0.05^{a}
P. control	7.87±0.55^{c}	3.64±0.16^{a}	1.22±0.08^{b}	3.01±0.20^{b}	48.47±0.27^{a}	20.34±0.11^{c}	31.52±0.71^{c}
DEN+SENNA	6.46±0.48^{b}	4.81±0.14^{b}	0.66±0.05^{a}	0.98±0.08^{a}	74.3±0.30^{c}	10.33±0.01^{a}	15.39±0.06^{a}
DEN+SILYMARIN	11.72±0.14^{d}	6.61±0.30^{c}	1.50±0.06^{c}	3.59±0.09^{c}	58.04±0.17^{b}	12.70±0.31^{b}	29.25±0.78^{b}

Results represent mean±standard deviation of group obtained (n = 6), mean in the column, having different superscripts are statistically significant ($p<0.05$), WBC: White blood cell, LYM: Lymphocyte, MID: Maximum inhibition dilution, GRA: Granulocyte, LYM (%): Lymphocyte percentage, MID (%): Maximum inhibition dilution percentage, GRA (%): Granulocyte percentage

WBC, LYM, MID and GRA increased significantly ($p<0.05$) in all the test groups compared to the control. The mean of the percentage LYM in normal control (group 1) decreased compared with that of positive control (group 2). The value is moderated for the third group and decrease steeply in the fourth group. The percentage MID (%) and GRA (%) level increased down the group having a high increase in the group 2 followed by the group 4 (Table 3).

DISCUSSION

The results of hematological parameter decreased significantly ($p<0.05$) in group 2, but moderated in group 3 and 4 to normal and near-normal (as in the case of MCH, RBC, MCHC and RDWc group 2). Although, there were differences in the mean HGB values, but not statistically significant ($p>0.05$) across the groups.

Haematological parameters provide valuable information on the health status of an animal. The DEN will have to bind to the RBC which will hinder the binding of haemoglobin (Hb) for adequate transportation, accounting for the disparities in the RBC and haemoglobin levels in the blood. Some medicinal plants are known to cause destruction of red blood cells leading to anaemia[14]. There were significant increases on RBC and Hb implied that the extract positively have effects on the oxygen carrying capacity of the blood to the tissues which is in tandem with the report of Effiong and Akpan[15] and Adedapo *et al*.[16]. If the value of RBC is high then the level of MCV will be low because the high value reticulocyte showed replacement of RBC in circulation, while drastic decrease in RBC consequently results in anaemia.

It was observed that there was significant decrease in the concentrations of RBC, WBC, HGB and PLT of the negative control (group 2) compared with the normal control (group). However, extract administration was able to significantly ($p<0.05$) revert the levels of these parameters compared to the normal indicated that the ethanol extract of *Senna occidentalis* has the ability to mitigate DEN effects on blood and blood forming tissue in Wistar rats. This result is in tandem with the experiment of Atawodi *et al*.[17]. It has some appreciable effect on white blood count compared to silymarin. High effect was also recorded in LYM, MID and GRA (%). No significant effect was observed in MID (%) and GRA (%). These levels are significantly ($p<0.05$) increased compared to the normal control. This significant increase is an evidence of hepatotoxicity which may be as a result of leakage from the cells through peroxidative damage of its membrane[18].

Health is the subject of priority as far as life is concerned, but despite effort to maintain good health, man and animals alike still confront disease conditions which are due to exposure to chemical agents[19]. Though, the body system is made in such a way that it tackles invading foreign substance in most cases, the body system needs to be protected, enhanced and activated[20]. The observed increase in WBC count administered ethanol extract of *Senna occidentalis* indicated an enhanced phagocytic function of the leucocytes[21], the modulation of the platelet count followed oral administration of *Senna occidentalis* leaves extract indicated that extract may not cause any coagulation problem, but has the potential to enhance clotting and prevent haemorrhages[22]. This ability to activate the body defense mechanism or to protect the body system has been found to be present in some natural herbal sources. So, it has become expedient to examine scientifically the protective effects of these herbal plants.

CONCLUSION

The results of this research work revealed that the ethanolic leaves extract of *Senna occidentalis* tends to ameliorate effects of DEN on blood and blood-forming tissues and enhancement of haematopoiesis in Wistar rats. The research as a whole further supported the fact that cheap, easy available and relative safe herbal medicine could be developed from this tropical plant. Hence, *Senna occidentalis* is a promising plant for the treatment/management of these toxicities especially as it results from oxidative stress and enhancement of haematopoiesis.

SIGNIFICANCE STATEMENT

This study discovers the effect of *Senna occidentalis* leaves extract against diethylnitrosamine induced toxicity and blood formation. This study will serve as a sina quenon to researchers in uncovering salient areas in this research to be addressed that many researchers have not been able to explore. Thus, a new theory on the mechanism of effect of *Senna occidentalis* leaves may be arrived at for the enhancement of life.

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