African Scientist Vol. 24, No. 2 June 30, 2023
Printed in Nigeria

1595-6881/2023 $80.00 + 0.00

https://africanscientistjournal.org

AFS2023021/24206

Effects of *Senna alata Aqueous* Extract on the Hematological and Biochemical Alterations in Rats Exposed to N-Nitroso-N-Ethyl Urea

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(Received April 24, 2023; Accepted in revised form May 1, 2023)

ABSTRACT: The effect of carcinogen exposure has been established to alter biochemical and hematological profiles in humans. Secondary metabolites from plant sources like flavonoids, alkaloids, saponins, and others have been found to be potent anticancer agents and also investigated to protect against the effect of environmental carcinogens. Female albino rats were rats exposed to freshly prepared 3 fractionated doses of 50 mg/kg N-Nitroso-N-ethyl urea (carcinogen) dissolved in 1/15 M Phosphate buffer, given intraperitoneally and treated with varying concentrations of *Senna alata* plant aqueous extracts. At the end of the test period, hematological and biochemical parameters were determined in blood and serum samples. Compared to the control group, the carcinogen exposed rats treated group showed significances in several hematological parameters, including decreases in White blood cell (WBC), Red blood cell (RBC), and Platelet (PLT) counts. Furthermore, in comparison to the control group, the carcinogen exposed rats showed significantly increased blood glucose, serum total cholesterol, Low density lipoprotein (LDL-cholesterol), triacylglycerols levels and High density lipoprotein (HDL-cholesterol) level. The hematological and biochemical parameters in the carcinogen exposed rats treated group were approximately similar to control group. The *S. alata* extract significantly ($P<0.05$) restored the hematological and biochemical parameters in N-Nitroso-N-ethyl urea carcinogen exposed rats

Keywords: *Senna alata*; Carcinogen; N-Nitroso-N-ethyl urea; Natural product; Cancer

Introduction

A carcinogen is any chemical, physical or biological compound that has the potential to induce cancer in living tissues (Irigaray and Belpomme, 2010). Exposure to carcinogens can be through inhalation, ingestion, or absorption into the bodies of multicellular organisms (Genchi *et al*., 2020; Mulware, 2013). Alkylating agents are members of a structurally diverse group of DNA damaging compounds which form adducts at ring Nitrogen and extra-cyclic Oxygen atoms of DNA bases (Bodakuntla *et al*., 2014) N-Nitroso-N-ethyl urea (NEU), an ethylating agent has been described as a very potent transplacental teratogen and carcinogen in rodents (Bodakuntla *et al*., 2014)

Human carcinogens could be genotoxic, which means to directly alter the genetic material of target cells (Mulware, 2013). Other carcinogens are classified as non-genotoxic, in which case are capable of producing cancer by some secondary mechanism and not by direct gene damage (Hernández *et al*., 2009) (Lee *et al*., 2013). These secondary mechanisms include the abilities of an agent to alter DNA repair or cause genomic instability, epigenetic alterations, oxidative stress, chronic inflammation, immunosuppression, immortalization or alter cell proliferation, cell death, or nutrient supply (Smith *et al*., 2016). The effect of carcinogen exposure has also been established to alter biochemical and hematological profiles in humans (Shukla *et al*., 2019; Mohamed *et al*., 2018). Secondary metabolites from plant sources like flavonoids, alkaloids, saponins, and others have been found to be potent anticancer agents (Ramakrishna *et al*., 2021; Wink *et al*., 2012). The

anticancer effect of these natural products is mediated by different mechanisms, including apoptosis induction, immune system modulation, and angiogenesis inhibition (Talib *et al*., 2020). *Senna alata* is highly significant due to the abundant bioactive chemical compounds which have been reported to display numerous biological activities (Oladeji *et al*., 2020). These biological activities include antimicrobial, antioxidant, anti-cancerous, anti-mutagenic and anti-diabetic activities. Leaves of *S.alata* plant have been used in treatment of eczema, ringworm, asthma, bronchitis and in poisonous insect bites (Lahare *et al*., 2020). In Ayurvedic, Chinese, and African traditional medicine, various parts of *S. alata* have shown a wide range of therapeutic potentials in treatment and disease control (Oladeji *et al*., 2020). Therefore, this study was planned to investigate the effects of administration of *Senna alata* extract on hematological and biochemical alterations induced by Nitroso-N-ethyl urea exposure in rats.

Materials and methods

Collection and authentication of plant: Fresh leaves of *Senna alata* (Candle plant) were collected from a private garden in Akure, Nigeria. The plants were identified and authenticated by Dr. Nodza George of the Herbarium, Department of Botany, University of Lagos, Nigeria.

Preparation of plant extract: The fresh leaves were dried under shade and then ground into fine powder,mixed with distilled water, and extracted for 24 h at 150 rpm at 25 °C in a shaker. The mixture was then centrifuged at 3000 rpm for 20 min. The supernatants were subsequently filtered through Whatman No. 1 filter paper and the filtrate was concentrated in rotary evaporator at 70 °C and was lyophilized. (Ramadan and Alshamrani, 2015). The extracts were fed to the animals on daily basis by gavage method (Zhang, 2011).

Chemicals used: N-Nitroso-N-ethyl urea were purchased from Sigma Aldrich, Germany. Freshly prepared 3 fractionated doses of 50 mg/kg N-Nitroso-N-ethyl urea dissolved in 1/15 M phosphate buffer was given intraperitoneally at average ages 10, 11.5 and 13 weeks according to a modified method of Barathidasan *et al*. (2013). 3.3 mg/kg of standard drug, Tamoxifen, used as control was obtained commercially and of analytical grade. All other chemicals and drugs were gotten of analytical grade and obtained commercially.

Animals

Ethical approval for animal studies: The use of animal has been approved by the Health Research Ethics Committee of the College of Medicine of the University of Lagos, CMULHREC Number: CMUL/ACUREC/04/21/835.

35 female albino rats (Sprague-Dawley) were brought in at 45-60 g weight at ages 2-3 weeks and fed with growers' mash of composition: protein 15.00 %min, fat 3.00 %min, fiber 6.00 %min, calcium 1.00 %min, phosphorus 0.38 %min, and energy 2450 kcal/kg. The animals were bred in the laboratory under ideal conditions of temperature, humidity and light and fed with right rations (Carter and Lipman, 2017). The animals' weights were monitored weekly until an average age of 10 weeks with an average weight of 170 g after which NEU was induced.

Table 1: Experimental design

Rats	Induction with NEU	Treatments and control
35 Sprague Dawley Rats (10weeks old)	3 Intraperitoneal injection with NEU 1. 50mg/kg (10weeks) 2. 50mg/kg (11.5weeks) 3. 50mg/kg (13weeks)	3 week treatment with aqueous Senna alata plant extracts dosages (15weeks-18weeks) A. 50mg/kg B. 100mg/kg, C. 200mg/kg, D. 300mg/kg E. Induced with NEU No Plant Extract Treatment F. Control drug Tamoxifen 3.3mg/kg G. Negative control (Not induced with NEU No Plant Extract)

Collection blood for analysis: Blood samples were collected by bleeding with the aid of heparinized capillary tube and kept in lithium heparinized bottles for hematology and biochemical analysis. Blood samples were collected from rats into heparinized tubes under light ether anesthesia. White blood cells (WBC), lymphocyte and monocyte ratio, red blood cells (RBC), hematocrit (Hct), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and platelet count (PLT) were measured on Hematology Analyzer (Abacus Junior Vet 5, Austria).

At the end of the experimental period, animals were fasted for 8-12 h before blood collection in order to cause no interference in the analysis of blood glucose and serum lipid profile. Blood samples were withdrawn by end

tail vein cutting method from overnight fasted animals and blood glucose was measured by one touch electronic glucometer ACU check. Other blood samples were collected and allowed to coagulate at room temperature for 30 min and were subsequently centrifuged at 3000 g for 10 min. Serum was removed and stored at −80 °C until analysis. Estimation of biochemical parameters were determined using standard procedures.

Statistical analysis: Data were obtained from three separate experiments and presented as the mean ± standard deviation of the mean. T test was employed for comparison among multiple groups using GraphPad Prism 9 software. A value of $p<0.05$ was considered as statistically significant.

Results

Effect of Senna alata on hematological parameters in control and rats exposed to NEU: The *S. alata* treated rats exhibited significant improvement in most of the hematological parameters and red blood cells indices compared to carcinogen exposed rats (Table 2). The data showed that NEU resulted in a significant decrease in RBC, Hgb, PCV, and PLT counts as compared to control group, and a significant increase in WBC. The hematological parameters in rats treated with 300 mg/kg of plant extract, the rats treated with standard cancer drug (Tamoxifen) and control group were similar. The *S. alata* extract alleviated the adverse effects on WBC, RBC, and PLT counts caused by N-Nitroso-N-ethyl urea.

Table 2: Effect of *Senna alata* extract on hematological parameters in control and rats exposed to NEU

Values are expressed as Mean ± Standard Error. Mean values with similar superscript across rows are not significantly different from each other ($p<0.05$) SaA = *Senna alata* 50 mg/kg, SaB = *Senna alata* 100 mg/kg, SaC = *Senna alata* 200 mg/kg, SaD = *Senna alata* 300 mg/kg

	Control (no carcinogen)	**Not Treated**	**SaA**	**SaB**	**SaC**	**SaD**	**Standard Drug (Tamoxifen 3.3 mg/kg)**
WBC $x10^9$/L	7.30±0.32^a	15.37±2.31^b	11.24±0.58^c	10.08±0.27^c	9.54±0.13^c	9.06±0.54^c	7.92±0.40^a
RBC$x10^{12}$/L	8.98±0.28^a	3.75±0.39^b	4.45±0.40^c	5.37±0.47^c	6.42±0.46^d	7.72±1.07^d	9.47±0.34^a
HGB g/dl	12.82±0.18^a	6.93±0.29^b	7.44±0.31^c	8.28±0.63^c	9.66±0.58^c	11.04±0.76^d	12.50±0.22^a
HCT(pcv)%	47.72±0.39^a	28.88±3.32^b	34.82±0.50^b	36.04±0.76^b	37.54±0.31^d	39.84±3.89^e	47.02±0.49^a
PLT$x10^9$/l	358.44±12^a	939.48±97^b	765.40±90^b	587.00±35^c	508.00±33^d	435.80±27^e	380.20c±15^a
MCV/l	53.22±1.97^a	77.17±0.82^b	78.64±5.30^b	67.40±4.00^c	58.68±3.40^c	51.80±1.80^a	49.70±2.46^a
MCH /g	14.33±0.61^a	18.64±1.04^b	16.78±0.72^c	15.44±0.13^d	15.12±0.18^e	14.42±0.85^a	13.24±0.76^a
MCHC g/dl	26.89±0.18^a	24.22±1.34^b	21.83±0.33^c	22.95±1.22^c	25.67±1.19^d	27.53±0.59^e	26.57±0.22^a

Effect of S. alata extract treatment on serum lipid profile of Control and carcinogen exposed rats: The effects of *S. alata* extract treatment on serum lipids profile of control and carcinogen exposed rats are listed in Table 3. N-Nitroso-N-ethyl urea elevated serum cholesterol, triacylglycerols (T.G), LDL-cholesterol and HDL-cholesterol levels in stressed carcinogen exposed rats. *S. alata* extract in a dose dependent manner significantly decreased the higher levels compared to the control (Table 3).

Table 3: Effect of *Senna alata* extract on lipid profile in control and rats exposed to NEU

	Control (no carcinogen)	**Not Treated**	**SaA**	**SaB**	**SaC**	**SaD**	**Standard Drug (Tamoxifen 3.3 mg/kg)**
HDL (mmol)	0.97±0.02^a	1.96±0.18^b	1.63±0.00^c	1.56±0.19^c	1.25±0.08^d	1.36±0.08^d	0.43±0.02^e
LDL (mmol)	0.45±0.05^a	0.65±0.08^b	0.49±0.05^a	0.40±0.02^c	0.43±0.01^c	0.42±0.01^c	0.39±0.05^c
CHOL (mmol)	1.44±0.03^a	2.19±0.27^b	1.72±0.01^c	1.67±0.15^c	1.42±0.04^a	1.48±0.04^a	1.49±0.03^a
T.G (mmol)	0.68±0.13^a	0.79±0.13^a	0.57±0.02^b	0.59±0.06^b	0.48±0.01^c	0.46±02^c	0.51±0.13^c

Values are expressed as Mean ± Standard Error. Mean values with similar superscript across rows are not significantly different from each other ($p<0.05$) SaA = *Senna alata* 50 mg/kg, SaB = *Senna alata* 100 mg/kg, SaC = *Senna alata* 200 mg/kg, SaD = *Senna alata* 300 mg/kg

Effect of S. alata extract treatment on liver function parameters of control and carcinogen NEU rats: The effects of *S. alata* extract treatment liver function parameters of control and carcinogen exposed rats are listed in

Table 4. N-Nitroso-N-ethyl urea elevated liver enzymes level in carcinogen exposed rats. *S. alata* extract in a dose dependent manner significantly ($p<0.05$) decreased the higher levels compared to the control (Table 4).

Table 4: Effect of *Senna alata* extract on liver function parameters in control and rats exposed to NEU

	Control (no carcinogen)	**Not Treated**	**SaA**	**SaB**	**SaC**	**SaD**	**Standard Drug (Tamoxifen 3.3 mg/kg)**
AST (u/l)	80.04±3.44^{a}	181.54±22.05^{b}	138.38±8.32^{c}	120.38±6.98^{d}	105.24±5.95^{e}	92.92±3.76^{f}	84.66±3.44^{a}
BIL-T (µmol/l)	0.98±0.18^{a}	4.04±0.80^{b}	2.54±0.13^{c}	2.28±0.04^{d}	2.12±0.18^{e}	1.70±0.22^{f}	1.22±0.18^{a}
ALB (g/l)	40.42±0.27^{a}	47.76±2.55^{b}	43.18±0.04^{c}	42.96±0.31^{c}	42.38±0.04^{c}	42.02±0.63^{c}	40.78±0.27^{a}
T.P (g/l)	64.84±0.24^{a}	76.07±2.92^{b}	70.42±0.93^{c}	68.43±0.75^{d}	66.95±0.32e	66.15±0.50^{e}	68.43±0.75^{d}
ALT (u/l)	39.16±1.25^{a}	83.90±11.85^{b}	62.48±0.49^{c}	60.18±3.18c	53.32±2.64^{d}	47.16±3.22^{e}	40.84±1.25^{a}
ALP (u/l)	95.82±13.91^{a}	179.02±10.46^{b}	156.18±9.21^{b}	138.1±3.58^{c}	130.62±2.41^{a}	125.18±2.50^{a}	114.48±13.91^{a}

Values are expressed as Mean ± Standard Error. Mean values with similar superscript across rows are not significantly different from each other ($p<0.05$) SaA = *Senna alata* 50 mg/kg, SaB = *Senna alata* 100 mg/kg, SaC = *Senna alata* 200 mg/kg, SaD = *Senna alata* 300 mg/kg

Effects of Senna alata extract on blood glucose level in control and rats exposed to NEU: Exposure of rats to carcinogen resulted in an increased blood glucose level (Figure 1), which was significantly decreased($p<0.05$) by *S.alata* extract in a dose dependent manner when compared to control group.

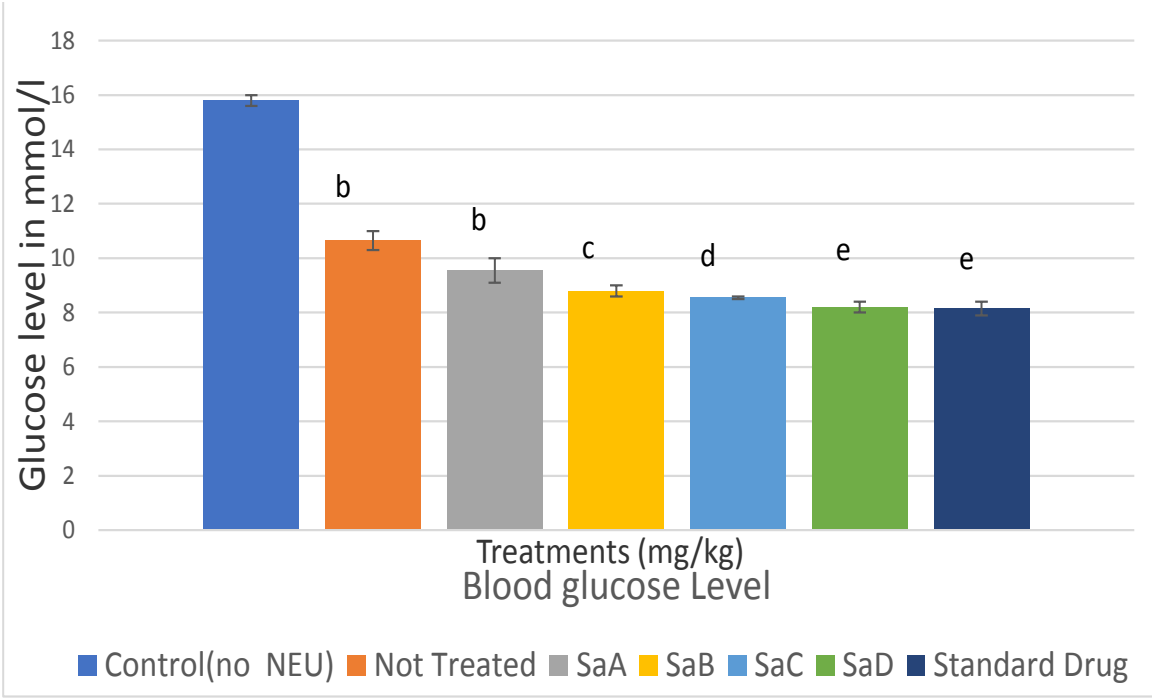

Figure 1: Effects of *Senna alata* extract on blood glucose level in control and rats exposed to NEU. The results are expressed as the mean ± SD. Mean values with similar superscript are not significantly different from each other ($p<0.05$) SaA = *Senna alata* 50 mg/kg, SaB = *Senna alata* 100 mg/kg, SaC = *Senna alata* 200 mg/kg, SaD = *Senna alata* 300 mg/kg

Effect of S. alata extract treatment on kidney function parameters of control and NEU exposed rats: The effects of *S. alata* extract treatment Kidney function parameters of control and carcinogen exposed rats are listed in Figure 2. Carcinogen N-Nitroso-N-ethyl urea elevated kidney enzymes level in NEU exposed rats. *S. alata* extract in a dose dependent manner significantly decreased ($p>0.05$) the higher levels compared to the control (Figure 2).

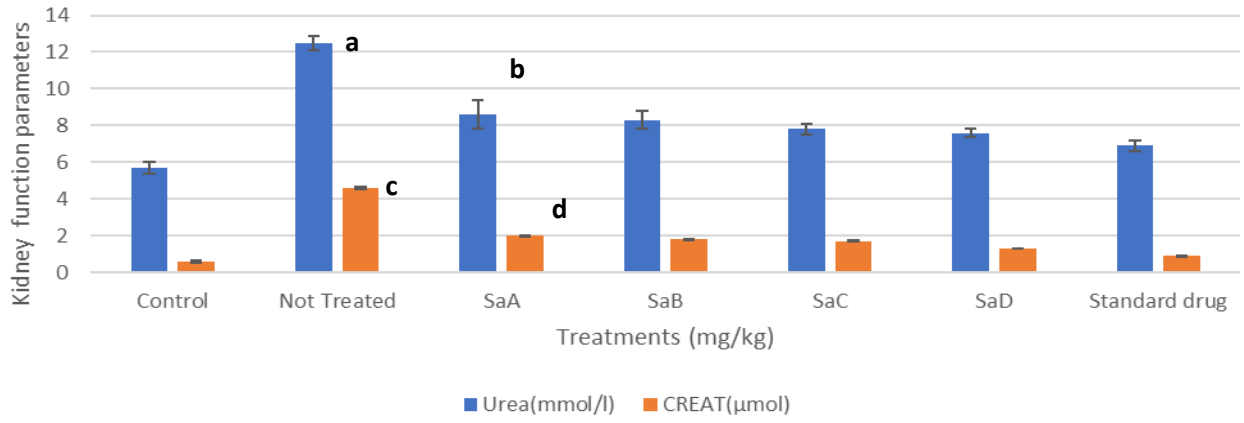

Figure 2: Effects of *Senna alata* extract on kidney function in control and rats exposed to NEU. Values are expressed as Mean ± Standard Error. Mean values with similar superscript are not significantly different from each other ($p<0.05$) SaA = *Senna alata* 50 mg/kg, SaB = *Senna alata* 100 mg/kg, SaC = *Senna alata* 200 mg/kg, SaD = *Senna alata* 300 mg/kg

Discussion

Cachexia is a syndrome of unintentional or uncontrolled body weight loss and muscle wasting usually experienced by cancer patients (Yeom & Yu, 2022). Rats in the control group had a normal weight gain, whereas the weight gain in the carcinogen exposed groups were significantly lower than the control group. However, the administration of *S. alata* extract significantly slowed the carcinogen-induced body weight loss in a dose dependent manner. Patients presenting to primary care with weight loss are at higher risk of having cancer than patients without recorded weight loss (Nicholson *et al*., 2018). The negative energy balance in cachectic patients is usually as a result of increased energy expenditure and decreased energy intake (Law, 2022).

Anemia has been recognized as an independent predictor of poor prognosis in cancer patients 22 (Azoulay *et al*., 2013). The *S. alata* treated rats exhibited significant improvement in most of the hematological parameters and red blood cells indices compared to carcinogen exposed rats. The data showed that the carcinogen caused a significant decrease in RBC, Hgb, PCV, and PLT counts as compared to control group, and a significant increase in WBC. *S. alata* extract alleviated the adverse effects on WBC, RBC, and PLT counts caused by N-Nitroso-N-ethyl urea. Anemia can develop as a consequence of malnutrition and malabsorption (leading to iron and other nutritional deficiency, e.g., folates or vitamin B12), acute and/or chronic bleeding, systemic inflammation, metastatic infiltration of bone marrow, and therapy-related myelosuppression (Busti *et al*., 2018).

Increased blood glucose level known as hyperglycemia can promote the proliferation, invasion and migration, induce the apoptotic resistance and enhance the chemoresistance of tumor cells (Li *et al*., 2019). In this study, exposure of rats to carcinogen resulted in an increased blood glucose level, which was significantly decreased by *S. alata* extract in a dose dependent manner when compared to control group and rats treated with standard cancer drug.

Cholesterol is transported by low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c), abnormal serum level of LDL-c and HDL-c is associated with breast cancer and its normal serum level is considered as prognostic marker for breast tumor(Kumie *et al*., 2020). In this study, exposure of rats to carcinogen elevated serum cholesterol, triacylglycerols, LDL-cholesterol and HDL-cholesterol levels. *S. alata* extract in a dose dependent manner significantly decreased the higher levels compared to the control.

Biochemical changes which include liver enzymes imbalance and alteration in kidney parameters occur in cancer patients (Abramczyk *et al*., 2021). In this study, N-Nitroso-N-ethyl urea elevated liver and kidney enzymes levels in carcinogen exposed rats. *S. alata* extract in a dose dependent manner significantly decreased the higher levels compared to the control. The careful monitoring of serum biochemical changes is very important in prognosis of cancer (Abramczyk *et al*., 2021).

Conclusion

The study therefore concluded that the aqueous extract of *Senna alata* has potential to protect against the effect of carcinogen N-Nitroso N-ethyl urea. *S. alata* significantly reversed the alterations in hematological and biochemical parameters, indicating the protective effect against carcinogen. Further study needs to be done with regard to isolation, and characterization to explore active constituents responsible for such activity and to determine the possible mechanism involved.

Abbreviations

AST: Aspartate Aminotransferase
BIL-T: Total Bilirubin
ALB: Albumin
T.P: Total Protein
ALT: Alanine Transaminase
ALP: Alkaline Phosphatase
CREAT: Creatinine
Bwt: Body Weight

Conflict of Interest

The authors have no conflict of interests to disclose.

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