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Vernonia amygdalina Extract Reverts Hematological and Biochemical Alterations in Rats Exposed to N-Nitroso-N-Ethyl Urea

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ABSTRACT: Environmental carcinogens or exogenous carcinogens includes chemical, biological, and physical agents that have the potential to increase cancer risk because of prolonged high prevalence of exposure. Exposure can be through the respiratory, digestive, cutaneous or other possible contamination routes. N-Nitroso-N-ethyl urea (NEU), an ethylating agent has been described as a very potent transplacental teratogen and carcinogen in rodents. Plants have been investigated to protect against the effect of environmental carcinogens due to their arrays of phytochemicals. 3 5 Female albino rats of average ages 10weeks were rats exposed to freshly prepared 3 fractionated doses of 50 mg/kg N-Nitroso-N-ethyl urea dissolved in 1/15 M phosphate buffer, given intraperitoneally and treated with varying concentrations of *Vernonia amygdalina* leaf aqueous extracts. At the end of the test period, hematological and biochemical parameters were determined in blood samples. Compared to the control group, the NEU 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 NEU exposed rats showed significantly ($p<0.05$) increased blood glucose, serum total cholesterol, low density lipoprotein (LDL-cholesterol), triacylglycerols levels and igh density lipoprotein (HDL-cholesterol) level. The hematological and biochemical parameters in the NEU exposed rats treated group were similar to control group. *V. amygdalina* extract significantly ($P<0.05$) restored the hematological and biochemical parameters in N-Nitroso-N-ethyl urea exposed rats

Keywords: *Vernonia amygdalina*; Carcinogen; N-Nitroso-N-ethyl urea; Natural product; Cancer

Introduction

Cancer is a major cause of death globally and a leading cause of premature death in 112 out of 183 countries (Sung *et al.*, 2021). About one-fifth of people worldwide and one-third of people in developed will have cancer during their lifetimes as a result of exposure to environmental carcinogens (Gatto, 2021). Environmental carcinogens or exogenous carcinogens includes chemical, biological, and physical agents that have the potential to increase cancer risk because of prolonged high prevalence of exposure. Exposure can be through the respiratory, digestive, cutaneous or other possible contamination routes (Gatto, 2021; Irigaray and Belpomme, 2010). 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) For example, there is an indication that occupational exposure to benzene causes significant alterations in hematological and biochemical parameters and workers are at high risk of developing blood, hepatic or renal related disorder (Pizent *et al.*, 2022). Exposure to carcinogens is associated with different forms of DNA damage which includes single-strand breaks, double-strand breaks, covalently bound chemical DNA adducts, oxidative-induced lesions and DNA–DNA or DNA–protein cross-links (Barnes *et al*., 2018).

N-Nitroso-N-ethyl urea (NEU), an ethylating agent has been traditionally characterized as a severely potent transplacental teratogen and carcinogen in rodents (Bodakuntla *et al*., 2014).

Vernonia amygdalina is traditionally used to treat a variety of diseases including fungal and bacterial infections, inflammation, cancer, diabetes, and wounds (Ugbogu *et al*., 2021). *V. amygdalina* is rich in phytochemicals. Some of the identified phytochemicals of *V. amygdalina* include alkaloids, saponins, terpenes, lignans, flavonoids, phenolic acids, steroids, anthraquinone, coumarins, sesquiterpenes, xanthones and edotides (Adesanoye *et al*., 2012).

Therefore, this study was planned to investigate the effects of administration of *Vernonia amygdalina* aqueous extract on hematological and biochemical changes caused by NEU exposure in rats

Materials and methods

Collection and authentication of plant: Fresh leaves of *Vernonia amygdalina* (bitter leaf) were gotten from a private garden in Ogudu area of Lagos, Nigeria. The plants were identified and authenticated voucher number 8759 by Dr. Nodza George of the Herbarium, Department of Botany, University of Lagos, Nigeria

Preparation of plant extract: The fresh leaves (1 kg) 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 and concentrated in rotary evaporator at 70°C and was lyophilized (Ramadan and Alshamrani, 2015).

Chemicals used: N-Nitroso-N-ethyl urea were purchased from Sigma Aldrich, Germany. Tamoxifen, drug used as control to be gotten 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.

Experimental design: 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, energy 2450 kcal/kg. The animals were bred in the laboratory under ideal conditions of temperature, humidity and light and fed with right rations. The animals' weights are to be 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 (10 weeks old) with average weight of 170 g	Freshly prepared 3 fractionated doses of 50 mg/kg N-Nitroso-N-ethyl urea dissolved in 1/15 M phosphate buffer were given intraperitoneally at average ages 10,11.5 and 13 weeks according to a modified method of (Barathidasan *et al*., 2013) 1. 50 mg/kg (10weeks) 2. 50 mg/kg (11.5weeks) 3. 50 mg/kg (13weeks)	3 week treatment with aqueous *Vernonia amygdalina* plant extracts dosages (15-18 weeks) A. 50 mg/kg B. 100 mg/kg, C. 200 mg/kg, D. 300 mg/kg E. Induced with NEU no plant extract treatment F. Control drug Tamoxifen 3.3 mg/kg G. Negative control (not induced with NEU, no plant extract) The extracts were fed to the animals on daily basis by gavage method (Zhang, 2011).

Collection of blood for analysis: Blood samples were collected from rats into heparinized tubes a. 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 Vernonia amygdalina on hematological parameters in control and rats exposed to NEU: Rats treated with *Vernonia amygdalina* exhibited significant improvement in hematological parameters compared to untreated NEU exposed rats (Table 1). The data showed that the NEU caused a significant ($P<0.05$) decrease in RBC (red blood cells), HGB (hemoglobin) and PCV (packed cell volume) counts as compared to control group, and a significant ($p<0.05$) increase in WBC (white blood cells), PLT (platelets), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration). The hematological parameters in rats treated with *V. amygdalina* plant extract improved the hematological parameters in a dose dependent manner.

Table 1: Effect of *V. amygdalina a*queous leaf extract on hematological parameters in control and rats exposed to NEU

	Control (no NEU)	**Not Treated**	**VaA**	**VaB**	**VaC**	**VaD**	**Standard Drug (Tamoxifen 3.3 mg/kg)**
WBCx10^9/L	7.30±0.32^a	15.37±2.31^b	11.63±0.55^c	10.55±0.50	9.75±0.10	9.64±0.08d	7.92±0.40^a
RBCx10^{12}/L	8.98±0.28^a	3.75±0.39^b	4.23±0.59	5.26±0.19^c	5.86±0.65	6.59±1.45	9.47±0.34^a
HGB g/dl	12.82±0.18^a	6.93±0.29^b	7.33±0.45	8.20±0.40^c	9.25±0.90	10.26±0.68^d	12.5±0.22^a
PCV%	47.72±0.39^a	28.88±3.32^b	33.23±1.65	35.83±0.25	36.70±1.00	37.83±0.75^c	47.02±0.49^a
PLTx10^9/l	358.44±12^a	939.48±97.00^b	762.75±106.00	587±32.00^c	521±35.00	482.13±26^d	380.2±15.00^a
MCV/l	53.22±1.97^a	77.17±0.82^b	78.95±6.50	68.22±2.00	62.95±4.70	57.66±5.53^a	49.70±2.46
MCH/g	14.33±0.61^a	18.64±1.04^b	17.23±1.15	15.63±0.25^c	15.85±0.30	15.51±0.23	13.24±0.76^a
MCHC g/dl	26.89±0.18^a	24.22±1.34^b	21.50±0.61^c	22.91±1.00	25.25±1.70	27.16±1.28^a	26.57±0.22

Values are expressed as Mean ± Standard Error. Mean values with similar superscript across rows are not significantly different from each other ($p<0.05$) VaA=*V. amygdalina* 50 mg/kg, VaB= *V. amygdalina* 100 mg/kg, VaC= *V. amygdalina* 200 mg/kg, VaD= *V. amygdalina* 300 mg/kg

Effect of aqueous V. amygdalina leaf extract on lipids profile of control and NEU exposed rats: *V. amygdalina* aqueous extract treatment on serum lipids profile of control and NEU-exposed rats are listed in Table 2. N-Nitroso-N-ethyl urea raised the serum cholesterol (CHOL), triacylglycerols (T.G), LDL-cholesterol and HDL-cholesterol levels in NEU-exposed rats compared to the control. *V. amygdalina* extract decreased these raised levels in a dose dependent manner.

Table 2: Effect of aqueous leaf extract of *V. amygdalina* on lipid profile in control and rats exposed to NEU

	Control (no NEU)	**Not Treated**	**VaA**	**VaB**	**VaC**	**VaD**	**Standard Drug (Tamoxifen 3.3mg/kg)**
HDL (mmol)	0.97±0.02^a	1.96±0.18^b	1.82±0.08	1.62±0.18^c	1.52±0.09	1.08±0.09^d	0.43±0.02
LDL (mmol)	0.45±0.05^a	0.65±0.08^b	0.79±0.06	0.47±0.04^a	0.42±0.03^a	0.31±0.02	0.39±0.05
CHOL (mmol)	1.44±0.03^a	2.19±0.27^b	2.22±0.02	1.69±0.23	1.69±0.20^c	1.61±0.06	1.49±0.03^a
T.G (mmol)	0.68±0.13	0.79±0.13	0.41±0.12	0.81±0.09	0.45±0.02	1.84±0.03	0.51±0.13

Values are expressed as Mean ± Standard Error. Mean values with similar superscript across rows are not significantly different from each other ($p<0.05$). VaA=*V. amygdalina* 50 mg/kg, VaB= *V. amygdalina* 100 mg/kg, VaC= *V. amygdalina* 200 mg/kg, VaD= *V. amygdalina* 300 mg/kg

Effect of aqueous leaf extract of V. amygdalina on liver function parameters of control and rats exposed to NEU: *V. amygdalina* extract treatment effect on liver function parameters of control and NEU exposed rats are listed in

Table 3. N-Nitroso-N-ethyl urea elevated liver enzymes level in NEU exposed rats compared to the control. *V. amygdalina* extract gradually restores elevated levels in a dose dependent manner (Table 3).

Table 3: Effect of aqueous leaf extract of *V. amygdalina* on liver function parameters in control and rats exposed to NEU

	Control (no NEU)	Not Treated	VaA	VaB	VaC	VaD	Standard Drug (Tamoxifen 3.3 mg/kg)
AST (u/l)	80.04±3.44^{a}	181.54±22.05^{b}	148.90±8.50	128.70±7.00	112.80±6.00	98.2±4.00^{c}	84.66±3.44^{a}
BIL-T (µmol/l)	0.98±0.18^{a}	4.04±0.80^{b}	2.80±0.26	2.40±0.06^{c}	2.3±0.28	1.90±0.45	1.22±0.18
ALB (g/l)	40.42±0.27^{a}	47.76±2.55^{b}	43.70±0.15	42.80±0.50	42.60±0.05	42.60±0.70^{c}	40.78±0.27^{a}
T.P (g/l)	64.84±0.24^{a}	76.07±2.92^{b}	70.46±1.05	69.73±0.80	67.28±0.34	66.93±1.00^{a}	68.43±0.75
ALT (u/l)	39.16±1.25^{a}	83.90±11.85^{b}	63.8±0.50	61.9±3.20	55.90±3.00	51.7±5.00^{c}	40.84±1.25
ALP (u/l)	95.82±13.91^{a}	179.02±10.46^{b}	161.80±11.00	141.9±4.00	133.50±3.50	127.60±2.49^{c}	114.48±13.91

Values are expressed as Mean ± Standard Error. Mean values with similar superscript across rows are not significantly different from each other ($p<0.05$). VaA=*V. amygdalina* 50 mg/kg, VaB= *V. amygdalina* 100 mg/kg, VaC= *V. amygdalina* 200 mg/kg, VaD= *V. amygdalina* 300 mg/kg, AST=aspartate aminotransferase, BIL-T=bilirubin, ALB=albumin, T.P=total protein, ALT=alanine transaminase, ALP=alkaline phosphatase

Effects of aqueous leaf extract of V. amygdalina on blood glucose level in control and rats exposed to NEU: Exposure of rats to carcinogen (NEU) resulted in an increased blood glucose level when compared to control group and rats treated with control cancer drug (Figure 1), this was gradually normalized by *V. amygdalina* aqueous extract in a dose dependent manner.

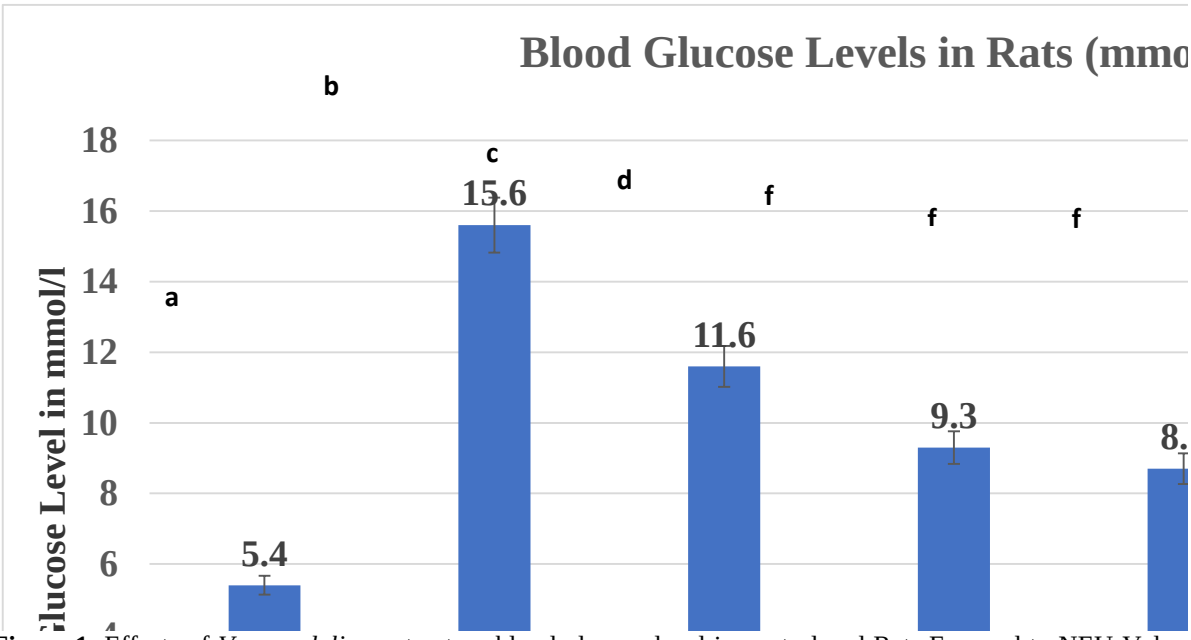

Figure 1: Effects of *V. amygdalina* extract on blood glucose level in control and Rats Exposed to NEU Values are expressed as Mean ± Standard Error. Mean values with similar superscript are not significantly different from each other ($p<0.05$). VaA=*V. amygdalina* 50 mg/kg, VaB= *V. amygdalina* 100 mg/kg, VaC= *V. amygdalina* 200 mg/kg, VaD= *V. amygdalina* 300 mg/kg

V. amygdalina aqueous leaf extract treatment on kidney function parameters of control and NEU exposed rats: The effects of *V. amygdalina* extract treatment on kidney function parameters of control and carcinogen exposed rats are listed in Figure 2. N-Nitroso-N-ethyl urea elevated kidney enzymes levels in carcinogen exposed rat compared to the control. *V. amygdalina aqueous* extract in a dose dependent manner significantly decreased the higher Creatinine and Urea levels.

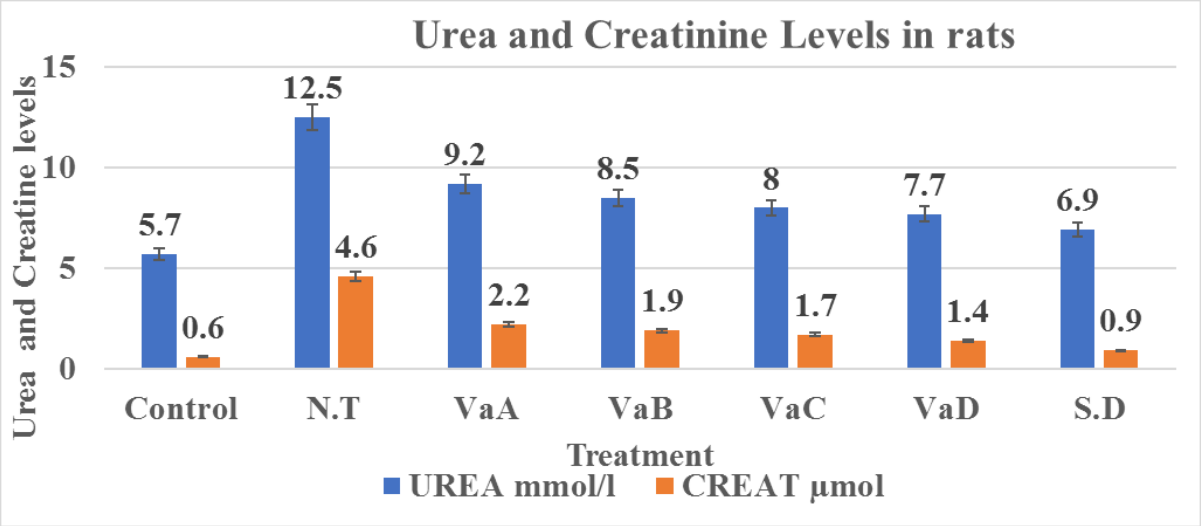

Figure 2: Effects of *V. amygdalina* leaf extract on kidney function in control and rats exposed to NEU. Values are expressed as Mean ± Standard Error. Mean values with similar superscript are not significantly different from each other ($p<0.05$) VaA=*V. amygdalina* 50 mg/kg, VaB= *V. amygdalina* 100 mg/kg, VaC= *V. amygdalina* 200 mg/kg, VaD= *V. amygdalina* 300 mg/kg

Discussion

The data showed that the NEU caused a significant ($p<0.05$) decrease in RBC, HGB and PCV as compared to control, and a significant increase in WBC. Anemia is the most common hematological manifestation of cancer (Gaspar *et al*., 2015; Busti *et al*., 2018; Azoulay *et al*., 2013). Anemia can develop as a result of malnutrition and malabsorption, bleeding inflammation or other factors (Busti *et al*., 2018). Total white blood cell (WBC) count is often elevated during infections, it is one of the nonspecific markers of inflammation and can be associated with certain kinds of cancers and subsequent cancer mortality (Wang *et al*., 2018; Shankar *et al*., 2006). Elevated platelet count has been associated with increased risk of cancer and could potentially serve as a marker for the presence of some cancer types (Giannakeas *et al*., 2022).
Glucose is required to meet the metabolic needs of cancer cells and it is a primary driving force for the growth of tumor cells and metastasis (Duan *et al*., 2014). In this research, rats exposed to NEU had increased blood glucose level, which was significantly reversed in a dose dependent manner when treated with *V. amygdalina* extract. Alterations in biochemical changes including liver enzymes kidney parameters have been reported in cancer patients (Abramczyk *et al*., 2021). The ratio of AST/ALT is a potential biomarker to assess healthy conditions, long-term mortality and also predicts the future development of cancer (Abramczyk *et al*., 2021; Chen *et al*., 2022). Treatment with *V. amygdalina* gradually reversed biochemical alterations in a dose dependent manner. N-Nitroso-N-ethyl urea exposure also elevated serum cholesterol, triacylglycerols, LDL-cholesterol and HDL-cholesterol levels. *V. amygdalina* extract in a dose dependent manner significantly decreased the higher levels compared to the control.

Conclusion

This study therefore concluded that the aqueous extract of *V. amygdalina* has potential to protect against the effect of N-Nitroso N-ethyl urea in rats. *V. amygdalina* significantly ($p<0.05$) reversed the alterations in hematological and biochemical parameters, indicating its protective effect against NEU.

Any Conflict of Interest

The authors have no conflict of interests to disclose.

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