PHYTOCHEMICAL CONSTITUENTS AND HAEMATOLOGICAL EFFECT OF HYDRO-ETHANOLIC EXTRACT OF *NEWBOULDIA LAEVIS* LEAVES ON GLYPHOSATE INDUCED TOXICITY IN RABBIT BUCKS

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ABSTRACT

This study analyzed some of the secondary metabolites present in hydro-ethanolic extract of Newbouldia laevis (NBL) leaves and its potential protective effects on the haematological indices of glyphosate herbicide exposed rabbit bucks. To determine the protective effects of NBL against herbicide toxicity, 36 rabbit bucks were randomized into four treatment groups designated as control, glyphosate-only, glyphosate + NBL 100 mg and glyphosate + NBL 200 mg in a completely randomized design experiment. Result of phytochemical screening of NBL showed high presence of flavonoid, terpenoid and phenol. Saponin was found to be moderately present with slight presence of alkaloid, steroid, tannin and cyanogenic glycosides. Acute toxicity evaluation indicated that the extract had a substantial safety margin with a tolerance of up to 1000 mg/kg in Albino rats. The result of glyphosate on haematological indices showed that the herbicide did not significantly (p>0.05) alter most of the measured blood parameters. However, significant (p<0.05) effects were observed on the concentration of lymphocyte and platelet counts. The administration of NBL leaves extract appeared to counteract the adverse effect of glyphosate, as evidenced by the enhanced platelet count in the treated groups. These findings support the traditional use of NBL in herbal remedies due to its phytochemical composition. It also suggests that glyphosate has minimal toxicity on haematological profile and that NBL leaves may have potential applications in regulating haematological parameters.

Keywords: Herbicide toxicity, Phytothreapy, Bioactive compounds, Blood indices

INTRODUCTION

Pollutants are materials or factors that introduce impurities into the environment, resulting in detrimental impacts on living organisms, ecosystems and the overall equilibrium of the natural world. These contaminants may have diverse origins, encompassing industrial operations, farming methods, transportation and human activities. Concerning herbicides, pesticides and other agro-chemicals, their application has played a significant role in causing environmental contamination through various means (Tudi *et al.*, 2021).

Glyphosate, a widely used herbicide, has been instrumental in modern agriculture due to its effectiveness in weed control. However, the increased use of glyphosate has raised concerns about its potential adverse effects on non-target organisms, including farm animals and wildlife. Glyphosate exposure in mammals has been associated with the disruption of various physiological and biochemical processes, including changes in red blood cell count (RBC), white blood cell count (WBC), haematocrit (HCT), haemoglobin (Hb) concentration and platelet count (Naz *et al*., 2019). These alterations can have detrimental effects and has become a subject of increasing research interest due to its potential impact on animal health and ecosystem balance.

In recent years, there has been growing interest in exploring the potential of natural extracts in ameliorating the adverse effects of chemical toxicants (Ansa *et al*., 2018; Ukwubile *et al*., 2023). While conventional treatments are available, they may come with side effects or limitations, making botanical therapies an appealing alternative or complementary approach. One such natural candidate is *Newbouldia laevis* (P.Beauv.) Seem. ex Bureau (Lamiales: Bignoniaceae) plant, which has exhibited promising bioactive properties, including antioxidant, cytoprotective properties and anti-inflammatory effects (Solomon *et al*., 2019; Salemcity *et al*., 2020; Ukwubile *et al*., 2023). These attributes suggest the potential of *NBL* leaf extract as a possible mitigating agent against the haematological disturbances induced by glyphosate toxicity. However, despite its traditional use in various folk medicines, its precise role in countering glyphosate-induced haematological alterations in animal remains largely unexplored. Therefore, this study aims to investigate the effect of *NBL* extract on the haematological parameters of rabbit bucks exposed to glyphosate toxicity. This research endeavours to provide valuable insights into the potential therapeutic role of *NBL* leaf extract in mitigating the haematological repercussions of glyphosate toxicity in animal population, using rabbit as a model by comprehensively assessing key haematological markers such as RBC count, WBC count, Hb concentration and HCT levels. This will ultimately contribute to the welfare and preservation of important ecological organisms.

MATERIALS AND METHODS

Location of Study: The research was conducted in the Department of Animal Science Teaching and Research Farm of the Nnamdi Azikiwe University, Anambra State, Nigeria.

Source of *Newbouldia laevis* Leaf: The plant material for this study was sourced from Awka, in Anambra State, Nigeria, identified (CABI, 2017) and authenticated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State. Voucher specimen (AST/NAU/22/004) of the leaf was kept in the Departmental herbarium for reference purposes. The leaves were air dried, finely ground using an electric blender and stored in an air-tight container.

Preparation of *Newbouldia laevis* leaf Extract: The ground leaves was weighed into a glass jar and extraction was carried out with aqueous-ethanolic solution in a ratio of 30:70 % using maceration method. After 72 hours, a muslin cloth was used for filtration and the resulting hydro-alcoholic extract was further filtered with Whatman No. 1 filter paper. The obtained extract was concentrated using rotary vacuum evaporator and subsequently lyophilized.

Acute Toxicity of *Newbouldia laevis* Leaf Hydro-Alcoholic Extract: Acute toxicity of hydro-ethanolic leaf extract of NBL was studied using the method outlined by Lorke (1983). Twenty (20) matured males' albino rats with body weight range of 27 to 33 g were distributed into five groups of four rats per group. Each group received a single dose of NBL extract at 0, 10, 200, 500 and 1000 mg/kg body weight, respectively, through oral routes, while the control group (0) received normal saline at 10 ml/kg body weight. The rats were closely observed for signs of toxicity and mortality for 72 hours.

Phytochemical Screening: Qualitative phytochemical assessment of NBL leaf extract was carried out using standard procedures as described by Harborne (1973) to identify the presence of some classes of secondary metabolites (flavonoids, saponins, tannins, alkaloids, steroids, terpenoids, phenol and cyanogenic glycosides).

Experimental Animals and Management: A total of 36 rabbit bucks, aged 26 to 30 weeks and weighing 1.56 – 1.70 kg were used for this study. The rabbits were housed individually in units in a hutch. Each hutch unit was provided with a feeder and drinker. The animals were quarantined for two weeks before the commencement of the research work. Throughout the research period, the rabbits were allowed *ad-libitum* access to water, Vital Feed grower mash (15.1% crude protein and 2663.3 kcal/kg metabolizable energy) ration and forage (*Panicum maximum, Tridax procumbens* and *Pueraria phaseoloides*).

Presentation of the Glyphosate Herbicide: In preparing the forage for the rabbits, 5 ml of Force-Up herbicide was thoroughly mixed in 0.5 litre of water (manufacturer's recommendation for weed control). The mixture was applied to the forage by spraying and the contaminated forage was allowed to dry before being introduced to the rabbits.

Experimental Design: Thirty-six rabbit bucks were randomly assigned to four treatment groups designated as: Control, Glyphosate only, Glyphosate + 100 mg NBL and Glyphosate + 200 mg NBL. The sub-lethal dosages used were 1/10 (100 mg/kg) and 2/10 (200 mg/kg) fractions of NBL LD_{50} (1000 mg/kg). Each treatment group had three replications with three rabbits per replicate in a completely randomized design (CRD). The administration of the extract preceded the introduction of the contaminated forage and the study continued for 56 days duration.

Blood Collection and Evaluation: The test ingredients were administered for 56 days before the aspiration of 3 ml of blood through the ear vein using syringe and needle from each buck. The collected blood samples were emptied into Ethylenediamine tetraacetic acid (EDTA) bottles and taken for the assessment of haematological parameters using haematology analyzer (Mindray Auto Haematology Analyzer, BC-5200, USA) following the manufacturer's instructions. The parameters analyzed were RBC counts, PCV, Hb concentration, platelets, Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), WBC counts and its differentials.

Statistical Analysis: The data generated from this study were subjected to analysis of variance (ANOVA) at 5% level of significance. Significant means was separated using Duncan Multiple Range Test (DMRT). All analyses were done using SPSS Statistics Base 17.0 (SPSS, 2011).

RESULTS AND DISCUSSION

The acute toxicity evaluation indicated that the hydro-ethanolic leaf extract of NBL did not result in any mortality or noticeable health issues in the experimental rats, even when administered at the highest dosage of 1000 mg/kg during the 72-hour observation period. The rats behaved normally, maintained regular feeding habit, and the consistency of their droppings did not differ from those of the control group. In earlier studies, varied LD_{50} of NBL extract have been reported, all above the 1000 mg/kg reported in this study, depending on the solvent used for extraction, animal model used and route of administration. The LD_{50} of the aqueous leaf extract of NBL when administered intraperitoneally in mice was calculated to be 1264.9 mg/kg (Tsado *et al*., 2020). Similarly, the intraperitoneal LD_{50} value of the ethanolic flower extract of NBL was found to be 1264.9 mg/kg body weight in mice (Tanko *et al*., 2008). Higher LD_{50} have been reported, for example, the LD_{50} of the aqueous leaf extract of NBL when administered orally to albino mice was calculated to be up to 5000 mg/kg (Ene *et al*., 2023) and the LD_{50} of the ethanol leaf extract of NBL when administered orally to albino rats was 6000 mg/kg (Owolabi *et al*., 2011). These results indicate that the extract is safe (non-toxic) even at high dose when taken through either oral or intraperitoneal route.

The result of phytochemical screening of hydro-ethanolic extract of NBL in this study is presented in Table 1.

Table 1: Qualitative phytochemical analysis of hydro-ethanolic extract of *Newbouldia laevis* leaves

Phytochemical	Hydro-ethanolic extract
Flavonoid	+++
Saponin	++
Tannin	+
Alkaloid	+
Steroid	+
Terpenoid	+++
Phenol	+++
Cyanogenic glycosides	+

+ slightly present, ++ moderately present, +++ highly present, - absent

Phytochemicals are naturally occurring compounds found in plants and have gained attention for their potential health-promoting effects. While not considered essential nutrients, they offer significant health benefits through their activities as antioxidant, anti-inflammatory, anticancer properties among many others (Kumar *et al*., 2023). The result of this study showed high presence of flavonoid, terpenoid and phenol. Saponin was found to be moderately present with slight presence of alkaloid, steroid, tannin and cyanogenic glycosides. Methanolic phytochemical screening of NBL leaves by Ushie *et al*. (2021) revealed the presence of alkaloid, flavonoid, phenol, terpenoid and saponin and the absence of phlobatannin, tannin and steroid. Salemcity *et al*. (2017) and Iteze *et al*. (2020) found methanolic extract of NBL leaves to contain alkaloid, cardiac glycoside, phenol, saponin, tannins, terpenoids, quinine and oxalate.

Given the phytochemical properties of NBL extract and their known pharmacological activities, the implication is that this plant can contribute significantly to botanical remedies in ameliorating some physiological dysfunction in animals. Oxidative stress occurs when there is an imbalance between the levels of free radicals and the antioxidants required to neutralize them. Flavonoids have been demonstrated to control inflammatory response and mitigate oxidative stress (Li *et al*., 2020). Ullah *et al*. (2020) documented that flavonoids possess a number of substances of medicinal value that offers antioxidant, anti-inflammatory, antiviral properties, neuroprotective and cardio-protective effects. In a study conducted by Salemcity *et al*. (2020), they examined the antioxidant profile of extracts derived from the leaves of NBL. Their research yielded valuable insights, demonstrating that *NBL* possesses the ability to inhibit lipid peroxidation, exhibits notable antioxidant properties, high phenolic content and contains substantial amount of vitamin C. Furthermore, the leaves were also observed to effectively scavenge free radicals. Also, Solomon *et al*. (2019) reported that the methanol extract of *NBL* contains a substantial quantity of phenols, which could serve as a valuable source of antioxidants. This suggests that it may have the potential to be used effectively in the treatment of diseases caused by reactive oxygen species, as antioxidants are known to act as scavengers for harmful radicals. Similar conclusions were earlier drawn by Habu and Ibeh (2015), Dermane *et al*. (2020) as well as Ujam *et al*. (2021). Phenolic compounds (Aksoy *et al*., 2013) and alkaloids (Macáková *et al*., 2019; Dolanbay *et al*., 2021) can effectively neutralize free radicals because they contain hydroxyl groups. These groups can donate hydrogen atoms to radicals, leading to the creation of stable phenoxyl radicals, which demonstrate strong antioxidant properties. Therefore, the abundance of phenols and flavonoids in the result of this study may imply strong antioxidant capability of the plant extract.

A moderate presence of saponin was observed in this study (Table 1). Earlier studies have suggested the health promoting effect of saponin in regulation of blood glucose response (Shi *et al*., 2004) and lowering blood cholesterol levels (Nath *et al*., 2015). The role of saponins as modulators of blood coagulation system and its use in the prevention of venous thromboembolic incidents is well documented by Olas *et al*. (2020). Naturally, saponins appear to act as antibiotics that protect plants from microbes (Opara *et al*., 2019). Terpenoids was also shown to be highly present in this study and have been reported by Masyita *et al*. (2022)

to possess antifungal and antibacterial properties. These properties are associated with their ability to disrupt cell membranes and inhibit the growth of bacteria and fungi (Mahizan *et al.*, 2019; Masyita *et al.*, 2022), indicating that NBL leaves can serve as effective agents against both fungal and bacterial infections. Tannin is recognised for its effectiveness in the treatment of inflammation and ulcer in tissues and also has potential activity in the prevention and expelling of cancer (Olajide *et al.*, 2004). Thus, NBL leaves containing high levels of tannin may serve as a source of bioactive compound in the control of helminths (antihelminths).

Results of haematological indices of glyphosate exposed rabbit bucks treated with hydro-ethanolic extract of NBL leaves is shown in Table 2. Blood related measurements can serve as a valuable tool for assessing the degree of harm caused by external substances. Apart from the results of platelets and lymphocytes counts, the mean values of all the measured haematological indices in the glyphosate-only treated group were found not to significantly differ ($p>0.05$) from the mean values obtained in the control group. These insignificant differences ($p >0.05$) in the level of haematological parameters suggests that glyphosate herbicide did not substantially alter most of the evaluated blood parameters. The elevated concentration of WBC observed in this study aligns with the findings made by Modesto and Martinez (2010), as well as the findings of Tizhe *et al*. (2013). Both studies documented an increase in WBC counts in animals exposed to glyphosate-roundup toxicity. This change may be attributed to the activation of the immune system in the presence of harmful substances, possibly indicating an adaptive reaction by the animals to enhance their immune defenses. Similarly, Tizhe *et al*. (2013) evaluated the haematogical changes induced by subchronic glyphosate exposure in Wistar rats. They accessed haematocrit, haemoglobin, leucocytes and some of its differentials (neutrophils and lymphocytes) and reported no significant ($p>0.05$) changes in all the measured haematological indices. They also indicated relatively insignificant lower and higher mean concentrations of evaluated parameters in treatment groups that received higher (375 mg/kg) and lower (14.4 mg/kg) doses of glyphosates respectively. Notably, Tizhe *et al*. (2013) further made an intriguing observation in their study where they found that rats subjected to a lower dose of glyphosate exhibited comparatively elevated levels of both haematocrit and haemoglobin concentrations when compared with the control group that received distilled water. These results are at variance with results of studies with mice by Jasper *et al*. (2012) and with rabbits by Naz *et al*. (2019) who indicated that glyphosate-roundup can trigger significant haematological alterations, even at low doses and for a relatively short period of time.

The result of lymphocytes and platelet counts showed that glyphosate-only treated group had significantly lower ($p<0.05$) mean values. However, the NBL extract treated groups were not significantly different ($p>0.05$) from the control group. This result showed that glyphosate significantly affected the lymphocytes (42.50 ± 1.44 %) and platelet counts (141.70 ± 26.85 × 10^3/μL) in this study. When compared with results in the glyphosate-only treated group, the NBL treated groups showed significantly enhanced platelets mean value, especially in the 200 mg NBL treated group. The platelets mean values observed in this study were within the normal range (135.0 – 1005.0 ×10^3/μL) for rabbit reported by Özkan *et al*. (2012). However, the mean values of 235.00 ± 10.39 and 248.50 ± 13.38 ×10^3/μL recorded for the NBL extract treated groups were slightly lower than the range of 250 – 650 ×10^3/μL reported by Weiss *et al.* (2010). Platelets, also known as thrombocytes, are cellular fragments involved in the process of blood clotting. They play a vital role in initiating the restoration of blood vessel walls and are recognized as components that respond to acute infections or inflammatory conditions (Periayah *et al*., 2017). Invariably, the higher significant increase in platelet levels observed in the groups that received both glyphosate and NBL extract, as opposed to the group treated solely with glyphosate, may be attributed to the extract's

Table 2: Haematological indices of glyphosate exposed rabbit bucks treated with hydro-ethanolic extract of *Newbouldia laevis* leaves

Parameters	Control	Glyphosate	Glyphosate + (NBL 100 mg)	Glyphosate + NBL (200 mg)
RBC (x10⁶/µL)	5.56 ± 0.24	5.14 ± 0.44	5.03 ± 0.08	5.58 ± 0.13
PCV (%)	39.50 ± 0.29^{ab}	36.00 ± 1.73^{a}	36.50 ± 0.87^{ab}	40.00 ± 1.15^{b}
Hb (g/dL)	13.05 ± 0.03^{ab}	12.00^{a} ± 0.58^{a}	12.20 ± 0.29^{a}	13.35 ± 0.38^{b}
MCV (fL)	71.25 ± 2.57	70.25 ± 2.63	72.20 ± 0.98	72.10 ± 0.29
MCH (pg)	20.15 ± 0.95	19.35 ± 0.26	20.10 ± 0.23	20.15 ± 0.09
MCHC (%)	28.30 ± 0.35	27.75 ± 0.66	27.90 ± 0.06	27.95 ± 0.03
WBC (x10³/µL)	7.38 ± 0.56^{b}	8.97 ± 0.15^{c}	5.30 ± 0.64^{a}	5.60 ± 0.64^{a}
Neutrophils (%)	65.10 ± 1.42^{b}	68.83 ± 1.17^{b}	62.97 ± 2.01^{b}	49.00 ± 3.46^{a}
Lymphocytes (%)	25.50 ± 0.29^{a}	42.50 ± 1.44^{c}	30.25 ± 0.14^{b}	26.15 ± 4.53^{a}
Monocytes (%)	4.67 ± 2.19	4.83 ± 1.30	3.67 ± 2.19	4.00 ± 1.53
Eosinophils (%)	2.33 ± 1.86	1.83 ± 1.09	1.50 ± 0.87	2.17 ± 1.01
Basophils (%)	0.33 ± 0.33	0.25 ± 0.14	0.20 ± 0.15	0.33 ± 0.33
Platelets (x10³/µL)	269.00 ± 5.77^{d}	141.7 ± 26.85^{a}	235.00 ± 10.39^{b}	248.50 ± 13.38^{c}

^{a,b,c,d} *Means bearing different letter superscript within the same row differ significantly (p<0.05)*

potential to stimulate the generation of haematopoietic regulatory components, like thrombopoietin, erythropoietin, and colony-stimulating factors, by the bone marrow's stromal cells and macrophages (Esom-Ibe *et al.*, 2018). On the other hand, Nwaehujor *et al.* (2015) revealed that methanol leaves extract of NBL extended *in vitro* blood clotting time, led to increase bleeding times *in vivo*, and enhanced the thrombin-induced clotting time. This implies that the leaves of NBL have the ability to markedly lower blood platelet levels, prompting the idea that the extract from these leaves contains active anticoagulant components with potential clinical and physiological applications.

Although the erythrocyte mean values did not show significant distinction (p>0.05), but it did indicate a slight, greater numerical increase in the group treated with both glyphosate and 200 mg extract. The erythrocyte mean values obtained in all the treatment groups were within the normal range of 4 – 7 ×10⁶/µL reported by Weiss *et al.* (2010). Kolawole *et al*. (2013) and Ogbonnia *et al*. (2014) reported increase in RBC in NBL leaves fed rats. They attributed the increase to the extract's capacity to stimulate the generation of haematopoietic control factors, such as erythropoietin and colony-stimulating factors, by the macrophages within the bone marrow and the stromal cells. The observed slight numerical higher result in the glyphosate + NBL 200 mg group can be attributed to the presence of phytochemicals like flavonoids, as indicated by Usman and Osuji (2007) and particularly the presence of apigenin, as observed by Osigwe *et al*. (2017), within the leaves of NBL.

Conclusion: This study contributes significantly to the field of toxicology and natural remedies. The result revealed the presence of flavonoid, terpenoid, phenol, saponin, alkaloid, steroid, tannin and cyanogenic glycosides in NBL which supports the use of this plant parts in traditional treatment. Minimal toxicity of glyphosate to haematological profile was observed and also the potential of NBL leaves as a novel approach to boost or regulate haematological parameters. The implications of this research extend beyond the scope of rabbit bucks to potential applications in other species, including humans, who may be exposed to contaminants in various ways. However, it is important to note that additional studies, including those involving different animal models and long-term exposure, are needed to confirm the safety and efficacy of NBL extract.

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