

The Effects of Crude Methanol Seed Extract of *Brassica juncea* on Haematological Parameters in Wistar Rats

¹S.A. Ufelle, ¹E.O. Ukaejiofo, ²E.E. Neboh, ¹P.U. Achukwu, ³S. Ghasi,
⁴J.E. Ikekpeazu and ⁵I.C. Maduka

¹Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology,
College of Medicine, University of Nigeria Enugu Campus (UNEC) Enugu State, Nigeria.

²Department of Chemical Pathology, College of Medicine, Enugu State University of Science and
Technology (ESUT) Enugu State, Nigeria Enugu Campus

³Department of Pharmacology and Therapeutics, College of Medicine University of Nigeria
Enugu Campus (UNEC) Enugu State Nigeria

⁴Department of Medical Biochemistry, College of Medicine, University of Nigeria Enugu
Campus (UNEC) Enugu State Nigeria

⁵Department of Chemical Pathology, University of Nigeria Teaching Hospital (UNTH) Ituku-
Ozalla, Enugu State, Nigeria

Abstract: *Brassica juncea*, (*B. juncea*) also known as Indian Oriental or Brown Mustard is widely used as a medicinal crop, oil seed, condiment and vegetable and much hematological effects have not been recorded on *B. juncea*. Thirty (30) male wistar rats aged 2-3 months, weighing 100-200 g were obtained and housed at the Animal House of College of Medicine, University of Nigeria Enugu Campus for this study. The rats were divided into five (5) groups of six (6) rats per group, labeled A to E and were acclimatized for two (2) weeks and were fed with commercially available rat feed. They had access to water and feed *ad libitum*. Groups A to D were orally administered with graded doses of the crude seed extract (50, 100, 200 and 300mg/kg body weight respectively) daily for 30 days while group E which served as control received no extract. Blood samples (2.0 mL) were collected from each rat through the retro orbital plexus of the median canthus into K₃-EDTA containers for analysis using standard operative procedures. The results obtained, revealed that the mean Hemoglobin (Hb) values of groups C and D (C = 12.6±0.57 and D = 11.1±1.4 g/dL) were significantly decreased (p<0.05) when compared with control (E = 14.2±0.74 g/dL). The mean Hematocrit (PCV) values of groups C and D (C = 0.38±0.02, and D = 0.34±0.04 L/L) were also significantly decreased (p<0.05) compared with control (E = 0.43±0.02 L/L). The mean Total WBC values of all the treated groups were significantly increased (A = 6.45±0.3, B = 6.23±0.83, C = 6.0±0.34, and D = 6.05±0.41 x 10⁹/L) when compared with control E = 4.28±0.17×10⁹/L (p<0.05). The mean platelet values were significantly decreased in all the treated groups (A = 30.00±4.1, B = 30.00±5.8, C = 46.25±7.5 and D = 33.75±2.5 x 10⁹/L) when compared with control E = 86.25±4.8×10⁹/L. This study revealed that crude methanol seed extract of *Brassica juncea* affects some hematological parameters when orally administered at different concentrations.

Key words: Animal models, *Brassica juncea*, graded dosing, hematocrit, treated groups

INTRODUCTION

Medicinal plants are plant parts or seeds that can be used for therapeutic purposes (Ayoola *et al.*, 2006). More than half of prescribed medicine in most African countries come from indigenous plants/seeds directly or indirectly

and is predominant in Nigeria where modern medicine is often insufficient and exorbitant (Usfoelda *et al.*, 2010).

Brassica juncea (*B. juncea*) also known as Indian Oriental or Brown Mustard is widely believed to be one of the earliest domesticated plants as well as a condiment since early times. It can be traced to different areas of

Corresponding Author: Silas A. Ufelle, Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria Enugu Campus (UNEC) Enugu State, Nigeria. Tel: +2348033456095

Europe, Asia, with white variety originating from the Eastern Mediterranean regions and the foothills of the Himalayan Mountains and the black from the Middle East and the areas that produce the greatest amount of *B. juncea* currently include Hungary, Great Britain, India, Canada and United State (Usfoelda *et al.*, 2010). The leaves, seeds and stem of this mustard variety are edible (Anutmidaiair *et al.*, 2009) and has been shown to reduce the severity of asthma, to lower high blood pressure, to restore normal sleep pattern in women having difficulty with the symptoms of menopause, to reduce the frequency of migraine attacks and to prevent heart attack in patients suffering from atherosclerosis or diabetic heart disease.

B. juncea whole plant has been reported to increase the concentration of hepatic glycogen and glycogenesis and suppress the activity of glycogen phosphorylase and gluconeogenic enzymes, leading to reduced glycolysis and gluconeogenesis, when administered orally (10% w/w) in normal rats for 60 days (Khan *et al.*, 1995) whereas the ancient Greeks and Romans used this seed to treat arthritis pains (Buhler and Miranda, 2000).

According to the World Health Organization (WHO), more than one million people rely on herbal medicines to some extent and the WHO has listed 21,000 plants with reported medicinal uses around the world (Singh *et al.*, 2010) with little or no attention being paid to the possible adverse effects or otherwise of these herbs in the body. The haematological effect of the crude extract of *B. juncea* has not been isolated and this forms the basis for this present study, designed to investigate the effect of crude methanol seed extract of *B. juncea* on Hematological parameters in Wistar rats.

Plant materials: The seeds of *Brassica juncea* were obtained and authenticated by the Botany Department of University of Nigeria Nsukka and a voucher specimen were kept in the herbarum for future reference.

MATERIALS AND METHODS

Animal housing: Thirty (30) male Wistar rats aged 2 to 3 months, weighing 100-200 g were obtained and housed at the Animal House of College of Medicine University of Nigeria Enugu Campus. They were kept to acclimatize for 2 weeks and fed with commercially available

rat feed and had access to the feed and water *ad libitum*

Extraction of plant material: One hundred gram (100 g) of the powder from the grounded dried seeds of *Brassica juncea* were soaked in 250 mL of methanol for 48 hours with constant vigorous shaking and mixture sieved. The remaining methanol in the extract was evaporated to get the concentrated crude extract which was reconstituted with 3% dimethylsulphoxide (DMSO) and stored in the refrigerator until needed.

Experimental Design: Thirty (30) male Wistar rats were divided into five groups of six rats per group labeled A to E. Groups A to D were orally administered with graded doses of the crude methanol seed extract of *Brassica juncea* (50, 100, 200 and 300 mg/kg body weight respectively) for 30 days while group E which served as control received no extract. Blood samples (2.0 mL) were collected from each rat through the median canthus into K₃-EDTA containers for haemoglobin (Hb) estimation, haematocrit (PCV), Total White Blood Cell count (TWBC) and platelet count using standard operative procedures according to Dacie and Lewis (2006).

Statistical analysis: The Statistical Package for Social Sciences (SPSS) computer software version 15 was used for data analysis. The results of the tests were analyzed using student's t-test at 95% confidence interval with $p \leq 0.05$ being considered as significant.

RESULTS

Table 1 shows the mean \pm standard deviation (\pm SD) of the haemoglobin (Hb) values. The results of Hb in groups C and D (200 and 300 mg/kg body weight extract) were significantly decreased ($p < 0.05$) when compared with the control group.

Table 2 shows the mean \pm SD of the haematocrit (PCV) values. Also, the values of groups C and D were decreased significantly ($p < 0.05$) when compared with the control.

The Total White Blood Cell values (TWBC), are shown in Table 3, with the values of all the treated groups significantly increased ($p < 0.05$) when compared with the control group.

Table 1: The mean \pm standard deviation of Haemoglobin values in the test and control animals

Groups	Extract Dose (mg/kg) bodyweight	Haemoglobin (g/dL)	p-value
A	50	14.2 \pm 1.7	0.9375
B	100	12.9 \pm 1.3	0.1305
C	200	12.6 \pm 0.6	0.015*
D	300	11.1 \pm 1.4	0.011*
E (Control)	DMSO (5 mg/mL)	14.2 \pm 0.7	

*: Statistically significant compared with the control

Table 2: The mean±standard deviation of Haematocrit (PCV) values in the test and control animals

Groups	Extract Dose (mg/kg) bodyweight	Haematocrit (L/L)	p-value
A	50	0.43±0.05	0.93
B	100	0.39±0.04	0.21
C	200	0.38±0.02	0.025*
D	300	0.34±0.04	0.0135*
E (Control)	DMSO (5 mg/mL)	0.43±0.02	

*: Statistically significant compared with the control

Table 3: The mean±standard deviation of Total White Blood Cells (WBC) values in the test and control animals

Groups	Extract Dose (mg/kg) bodyweight	Total WBC ×10 ⁹ /L	p-value
A	50	6.45±0.30	0.000*
B	100	6.23±0.83	0.01*
C	200	6.0±0.34	0.000*
D	300	6.05±0.41	0.0005*
E (Control)	DMSO (5 mg/mL)	4.28±0.17	

*: Statistically significant compared with the control

Table 4: The mean±standard deviation of Platelet values in the test and control animals

Groups	Extract Dose (mg/kg) bodyweight	Platelet ×10 ⁹ /L	p-value
A	50	30.00±4.1	0.000*
B	100	30.00±5.8	0.000*
C	200	46.25±7.5	0.000*
D	300	33.75±2.5	0.000*
E (Control)	DMSO (5 mg/mL)	86.25±4.8	

*: Statistically significant compared with the control

The mean±SD of the platelet counts of all the treated groups were decreased significantly ($p < 0.05$), when compared with the control (Table 4).

DISCUSSION

There is evidence of a tradition of use of medicinal plants that is more than 3000 years old (Singh *et al.*, 2010), hence the need to assess the effect of plants like *B. juncea* on the haematological parameters, since they have previously been in use as medicinal plants.

This study revealed dose-dependent significant decrease in Haemoglobin (Hb) concentration and haematocrit (PCV) values of the treated groups when compared with the control group ($p < 0.05$). The study also revealed significant increase in Total White Blood Cell count (TWBC) and significant decrease in Platelet count when compared with the control group ($p < 0.05$). These observed changes might probably be due to some of the phytochemical constituents of the *Brassica juncea* seed extract like isothiocyanate and flavonoids which have been reported to have antiviral, anti-allergic, anti-platelet, anti-inflammatory, anti-tumor, and antioxidant activities (Buhler and Miranda, 2000). Other phytochemical constituents of this seed extract include nitric oxide, linoleic acids, oleic acids, palmitic acids, sinapic acids and thiamine (Ayoola *et al.*, 2006). The Hb concentration

and PCV values were decreasing as the dosage of the seed extract increases thereby indicating anaemia which might probably be as a result of the isothiocyanate content of the seed extract. Anaemia, which is the decrease in Hb and PCV, usually occurs in relation to age, sex, weight, location and physiological status of the individual considered (Dacie and Lewis, 2006). The observed leucocytosis in this study might probably be due to the nitric oxide content of the seed extract which have leucopoietic effect directly on the bone marrow (Ahad *et al.*, 2010). The observed low platelet count (thrombocytopenia) in this study agrees with the findings of earlier research work which states that the flavonoid content of the seed extract of *Brassica juncea* might have anti-platelet activity (Buhler and Miranda, 2000).

CONCLUSION

This present study has demonstrated that ingestion of the crude methanol seed extract of *Brassica juncea* possibly affects the bone marrow, thereby leading to anaemia, and most probably to leucocytosis and thrombocytopenia, as shown by the significant decrease in Hb, PCV, Platelets and also increase in TWBC in the treated animals compared with the control.

Adequate care should be taken in administering the seed extracts of *B. juncea*, since it has been shown to also

harbor some haematological disturbances in addition to all other medically beneficial effect that might have been previously observed during its use as a medicinal plant. Further studies, however, needs to be carried out in order to characterize the seed extracts and hence, find out the active components.

ACKNOWLEDGMENT

The authors wish to acknowledge the staff of the Botany Department of University of Nigeria Nsukka, and the Animal House of College of Medicine University of Nigeria Enugu Campus for their valuable contribution to the success of the study.

REFERENCES

- Ahad, H.A., C.S. Kumar, P.S.M. Nanda, T. Udaya Bhanu, B.V. Ravindra and V.G. Mohan, 2010. Traditional indian herbs used for diabetes. *JITPS*, 1(2): 69-78.
- Anutmidaiar, K., C. bBeDti, A. Mitra and M. Manjunatha, 2009. *In vitro* and *in vivo* studies of Indian Medicinal plants: A review. *J. Herbal Med. Toxi.*, 3(2): 9-14.
- Ayoola, G.A., O. Odukoya, H.A.B. Coker and T. Sofidiya, 2006. Phytochemical Screening and free radical scavenging activity of some Nigeria medical plants. *J. Pharmacol. Sci.*, 8: 133-136.
- Buhler, D.R. and C. Miranda, 2000. Antioxidant activities of flavonoids. Department of Environmental and Molecular Toxicology, Oregon State University. Linus Pauling Institute.
- Dacie, J.V. and S.M. Lewis, 2006. Investigation of haematological disorders. *Practical Haematology*, Churchill Livingstone Edinburgh, United Kingdom, pp: 177-180.
- Khan, B.A., A. Abraham and S. Leelamma, 1995. Hypoglycemic action of *Murraya koeingii* (curry leaf) and *Brassica juncea* (mustard): mechanism of action. *Ind. J. Biochem. Biophys.*, 32: 106-108.
- Singh, A., K. Singh and A. Saxena, 2010. Hypoglycaemic activity of different extracts of various herbal plants. *Int. J. Res. Ayurveda Pharm.*, 1(1): 212-224.
- Usfoelda, A., N. Yina and T.O. Sunmonu, 2010. *In vivo* studies on antidiabetic plants South African herbal medicine. *J. Clin. Biochem. Nutr.*, 47: 98-106.