Qualitative Phytochemical Analysis and Effect of Aqueous Extract of *Moringa oleifera* Seed on Haemoglobin Concentration in Albino Rats

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ABSTRACT

The chemical constituents of the crude aqueous extract of Moringa oleifera seed were analyzed and its effect on haemoglobin concentration in albino rats determined during three weeks of treatment and one week of withdrawal. The phytochemical analysis was carried out using standard laboratory methods. The mean haemoglobin concentration was determined by using the colourimetric method. The seed extract was found to contain carbohydrate, saponins, cardiac glycoside, terpenes and steroids, and alkaloids. There were significant increases in mean haemoglobin concentration of the extract compared to the control during the experimental period. The values indicated significant increases in haemoglobin concentration one week after withdrawal of the extract. Therefore, this study concludes that the aqueous extract of Moringa oleifera seeds contained Haematinic components that must have enhanced the significant increase in haemoglobin concentration, and pharmacologically, it contains useful phytochemical constituents.

Keywords: *Moringa oleifera, extract, haemoglobin concentration, phytochemical, rats*

INTRODUCTION

Moringa oleifera is the most widely known, cultivated and utilized of the *moringaceae;* a single family with 14 known species (Ram, 1994). It is widely distributed in the sub-Himalayan ranges of India, SriLanka, north eastern and south western Africa, Madagascar and Arabia (Fahey, 2005). The *Moringa* tree is a multi-functional plant. It is being cultivated in the tropics because of its medicinal and nutritional values. Besides, it has been reported to have various biological

activities; including cholesterol regulation, regulation of thyroid hormone level, gastric ulcers amelioration, anti-tumour activity and hypotensive effects, treatment of various disease conditions such as inflammation and liver diseases (Ram, 1994). Phytochemically, this plant family is rich in compounds containing the simple sugar rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates. These components of *Moringa* have been reported to have hypotensive, anti-cancer and antibacterial activities. It is also rich in a number of vitamins and minerals as well as other phytochemicals such as the carotenoids including B-carotene (Caceres, Cabrera, Morales, Mollinedo and Mendia, 1991; Caceres *et al*., 1992; Akhtar and Ahmad, 1995; Bharah, Tabassum and Azad, 2003; Fahey, 2005). The crushed seeds of *Moringa oleifera* have been used traditionally as natural flocculants to purify drinking water. Studies show that one of the seed peptides mediates both sedimentations of suspended particles such as bacteria cells and has direct bactericidal activity. Low concentrations effectively kill bacteria such as *Pseudomonas aeruginosa* and *Streptococcus pyogenes* without any toxic effect on human red cells (Fahey, 2005).

The elemental screening of the seed by Ojo, Oghojafor and Mahre (2010) reveals the presence of potassium, calcium, sodium, phosphorous, zinc, iron, copper and manganese. Haemoglobin is a respiratory pigment found in red blood cells which helps in carrying oxygen and carbon dioxide in the blood. It gives the red colour to blood. There are six million red blood cells per mm^3 of the whole blood in man, and each one of these cells contains about 250 million haemoglobin molecules (Ojo, Oghojafor and Mahre, 2010). If there is an insufficient amount of haemoglobin in the cell, the individual may suffer from anaemia and may have a tired, rundown feeling. Decrease of haemoglobin with or without an absolute decrease of red blood cells leads to symptoms of anaemia (Ojo, Oghojafor and Mahre, 2010). Therefore, this study seeks to qualitatively screen *Moringa oleifera* seed and evaluate the effect of its aqueous extract on haemoglobin concentration in albino rats.

MATERIALS AND METHOD

Seeds of *Moringa oleifera* were collected from Biu local Government Council Area of Borno State, Nigeria. One kilogram of the seed was air-dried in the shade within the laboratory, made into powder using an electric blender and then stored in a plastic container. Five hundred grams of the dried powder plant material was extracted using Soxhlet extractor and water was used as solvent (Trease and Evans, 1989). The crude extract was concentrated in vacuo, cooled, put in an airtight plastic container, properly labeled and stored in refrigerator at 4^oC until required. The crude aqueous extract of the plant was subjected to qualitative chemical screening for the identification of various classes of chemical constituent using the method described by Trease and Evans (1989), Clarke (1975) and Odebiyi and Sofowora (1978). Twenty five albino rats were used in this study. They were

maintained at the experimental animal house of the Faculty of Veterinary Medicine, University of Maiduguri. They were kept in rat cage and fed on commercial rat cubes (Vital Feeds, Bukuru, Jos, Nigeria) and allowed free access to clean fresh water in bottles with nipples *ad libitum*. All experimental procedures were in compliance with internationally accepted principle for laboratory animal use and care (C.I.O.M.S., 1985). The haemoglobin concentration was determined by the colourimetric method. Zero point two ml (0.2ml) of blood sample was pipetted into a test tube containing 5ml of Drabsken's solution and was vigorously shaken.

The mixture was allowed to stand for about 3 minutes to allow the blood to react with the cyanide solution properly. The colorimeter was switched on for ten minutes for stabilization before use. The mixture was transferred into a clean cuvette and then placed in a colorimeter using a fitter of 520 wave length to determine the optical density of the sample. The haemoglobin concentration corresponding to the optical density was read using a standard chart. The results were expressed as mean ± standard deviation (S.D). One way analysis of variance (ANOVA) was used to analyse the difference between the means. P d" 0.05 was considered significant (Armitage, 1980). Graphpad Instat Version 3.0 (2003) Statistica Computer Software was also used for data analysis.

RESULTS AND DISCUSSION

Phytochemical analysis of aqueous extract of *Moringa oleifera* seed showed the presence of carbohydrate, saponins, cardiac glycoside, terpenes and steroids, and alkaloids. There was an increase in the mean haemoglobin concentration during the first week of administration of this extract in comparison with the control. The increase was statistically significant. With the extract dose of 100 mg/kg, the mean haemoglobin concentration was 11.22±1.0 mg/dl ($p<0.01$), 10.08±0.8 mg/dl ($p<0.01$) at 200 mg/kg, 11.02±0.5 mg/dl ($p<0.01$) at 300 mg/kg,10.58±0.6 mg/dl ($p<0.05$) at 400 mg/kg compared to 9.04±0.2 mg/dl mean haemoglobin concentration for the control. During the second week of administration of the extract, the mean haemoglobin concentration with 100 mg/kg dose was 11.48±0.9 mg/dl ($P<0.01$), 11.12±0.9 mg/dl ($P<0.01$) at 200 mg/kg, 11.44±0.4 mg/dl ($P<0.01$) at 300 mg/kg, 10.48±0.5 mg/dl ($P<0.01$) at 400 mg/kg, compared to 9.30±0.4 mg/dl mean haemoglobin concentration for the control (Table 2).

There was also a very significant increase in haemoglobin concentration during the third week of extract administration. With the extract dose of 100 mg/kg, the mean haemoglobin concentration was 11.88±0.4 mg/dl ($P<0.01$), 11.44±0.4 mg/dl ($P<0.01$) at 200 mg/kg,11.82±0.4 mg/dl ($P<0.01$) at 400 mg/kg compared to 9.46±0.3 mg/dl mean haemoglobin concentration for the control. One week after withdrawal of the extract, the mean haemoglobin concentration of the rats remained significantly higher than that of the control at doses 100,300 and 400 mg/kg (Table 2). The phytochemical screening of aqueous extract of *Moringa oleifera*

seed indicated the presence of useful compounds such as carbohydrate, saponins, cardiac glycoside, terpenes and steroids, and alkaloids. These chemical constituents have many known therapeutic values, for instance saponins and alkaloids had been linked or suggested to be involved with antibacterial and anti-viral activity (Enzo, 2007). Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, develop resistance against diseases and endurance against stress (Gupta, 1994). Sofowora (1982) also reported antihypertensive actions of alkaloids. Carbohydrates are the main components of the cell wall, protoplasm and cell-sap. Saponin, one of the phytochemical components of *Moringa oleifera* seeds has haemolytic properties and produces frothing in aqueous solution (Umo, Itah, Akpan and Akpanekon, 2006).

The mechanism by which it haemolyses blood cells may also be the same as that used to haemolyse cell membrane of micro-organisms hence the antimicrobial properties often associated with the plant (Itah, 1999). Glycosides are known to exert pronounced physiological action in animals and man (Frantisek, 1991). Cardiac glycosides are still the choice drugs for the treatment of congestive heart failure. Additionally, glycosides with laxative, diuretic and antiseptic properties are used in diverse therapies (Frantisek, 1991). Terpenes and steroids are mainly present in essential oils whose antibacterial activities have been recognized for many years (Hammer, Carson and Riley, 1999). This could explain the role of *Moringa oleifera* seed as an anti-microbial agent (Pithayanukul, Tubprasert and Wuthi-Udomlert, 2007). This also suggests that *Moringa oleifera* seed may possess anti-microbial activity. The essential oils which contain terpenes and steroids with other compounds have been known to have diverse therapeutic uses such as the management of upper respiratory tract infections (Frantisek, 1991).

Steroids in modern clinical studies have supported their role as anti-inflammatory and analgesic agents (Singh, 2006). Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, develop resistance against diseases and endurance against stress (Gupta, 1994). Sofowora (1982) also reports antihypertensive actions of alkaloids. Alkaloids are linked or suggested to be involved with antibacterial and anti-viral activity (Enzo, 2007). Although, some research findings showed that the aqueous extract of *Moringa oleifera* leaves could precipitate anaemia if administered for a long period of time (Adedapo, Mogbojuri and Emikpe, 2009).

This study shows that the aqueous seed extract of *Moringa oleifera* caused an increase in the mean haemoglobin concentration during weeks of extract administration and even one week after stoppage of extract administration. This finding is in concurrence with an earlier finding by Ojo, Oghojafor and Mahre, (2010) ascribing the seed's ability to correct anaemic conditions to elemental constituents of iron and copper. The presence of phosphorus, calcium, manganese, iron and zinc are very important for red blood cell production and maturation. The

above elements are constituents of *Moringa oleifera* seeds, including potassium, sodium and copper (Ojo, Oghojafor and Mahre, 2010).

CONCLUSION

This experiment seeks to qualitatively screen *Moringa oleifera* seed and evaluate the effect of its aqueous extract on haemoglobin concentration in albino rats. The crude aqueous extract of the plant was subjected to qualitative chemical screening for the identification of various classes of chemical constituents. The haemoglobin concentration was determined by the colourimetric method. Phytochemical analysis of aqueous extract of *Moringa oleifera* seed showed the presence of carbohydrate, saponins, cardiac glycoside, terpenes and steroids, and alkaloids. The study therefore concludes that the aqueous extract of *Moringa oleifera* seeds possess pharmacologically useful phytochemical constituents and haematinic components that must have enhanced the significant increase in haemoglobin concentration.

Table 1: Qualitative presence of phytochemical constituents of aqueous extract of *M. oleifera* seed Phytochmical

Constituents	Tests	Results
Carbohydrate	Molisch's (General)	+
	Barfoed's (monosaccharide)	-
	Fehling's (Free reducing sugar)	+
	Fehling's (Free reducing sugar)	+
	Standard test for combine	+
	reducing sugar	+
	' ' ' Ketones	+
	Pentoses	+
Tannins	Ferric Chloride	-
	Formaldehyde	-
	Chlorogenic acid test	-
Phlobatannins	Hydrochloric acid	-
	Lime water	-
Antraquinone	Borntrager's (free anthraquinone	-
	Combined anthraquinone	-
	Reduced anthraquinone	-
Saponins	Fronthing	+
	Haemolysis	+
Cardiac Glycoside	General Test	+
Terpens and Steroids	Lieberman's	-
	Salkowski's	-
Flavonoids	Lead acetate	-
	Sodium hydroxide	-
	Ferric Chloride	-
	Pew's	-
Alkaloids	With dragendoff's reagent	+
	With Mayer's reagent	+

Key: - = Not present + = Present

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Table 2: Effect of prolonged administration of aqueous extract of M. oleifera leaves on mean haemoglobin (Hb) concentrations in Rats

Extract Dose (mg/kg)	Weeks of Treatment Mean ± SD (g/dl)			Week Post Withdrawal
	1	2	3	1
100	11.22±1.0**	11.48±0.9**	11.88±0.4**	10.28±0.5**
200	10.08±0.8**	11.12±0.9**	11.44±0.4**	10.04±0.4
300	11.02±0.5**	11.44±0.4**	11.82±0.4**	10.36±0.4**
400	10.58±0.6*	10.48±0.5**	11.82±0.4**	10.60±0.3**
Control (Distilled Water)	9.04±0.2	9.30±0.4	9.46±0.3	9.44±0.3

* P<0.05 signficant increase compared to control** P<0.01 signficant increase compared to control

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