

Full Length Research Paper

Effect of consumption of *Cucurbita pepo* seeds on haematological and biochemical parameters

G. K. A. Adepoju* and A. A. Adebajo

Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, Olabisi Onabanjo – University, Sagamu, Ogun State, Nigeria.

Accepted 07-01-2011

The study investigated the effect of *Cucurbita pepo* seeds on haematological and biochemical parameters on Wistar albino rats. The rats were maintained on diets composed of different concentrations of pulverized seeds of *C. pepo*. Blood samples and organs investigations were carried out using standard laboratory tests. The result revealed that *C. pepo* seeds significantly affected these parameters with beneficial effects on the vital organs and blood. The concentrations of the platelets, white blood cells and eosinophil were increased, while it reduced the concentrations of the neutrophils, packed cell volume and lymphocytes. It also significantly increased mean weights of the liver and the kidneys as evidenced by the statistically significant increase in total protein values of these organs. The doses significantly reduced the plasma levels of AST and ALT in these organs ($p < 0.05$). The seeds showed a dose-dependent nephro- and hepato-protective ability. In conclusion, *C. pepo* seed has beneficial nutritional values when consumed adequately.

Key words: Haematological parameters, white blood cells, alanine aminotransferase, aspartate aminotransferase, *Cucurbita pepo*, nutritional values, laboratory investigation.

INTRODUCTION

Cucurbita pepo, Family Cucurbitaceae, (English name: Pumpkin; Mexico/Spanish: Calbaza; Yoruba: Elegede; Guatemala: Huiioy) is a medium sized plant grown for its fruits and edible seeds. Hence, it is known to be used as human food in Nigeria. Other members of the family are also available. It has been used locally in Eritrea to treat tapeworm and has also been used in other regions of the world to treat the early stages of prostate disorders (FAO, 1995; Matus et al., 1993; Winkler et al., 2005; Sicilia et al., 2003; Rodriguez et al., 1996; Murkovic et al., 1996). Its use in this prostate condition is due to its high zinc content (Chevallier, 1996; Dreikom et al., 2002). The seed of *C. pepo* contains 30 to 51% of oil; other constituents are fatty acids – linoleic and oleic acids (27 to 38%); proteins (31 to 51%), carbohydrate (6 to 10%); mineral elements (4 to 5%). This includes phosphorous, calcium, potassium, iron, selenium and zinc. Selenium is particularly important, as it ranges between 0.08 and 0.40

µg/g. Other substances in the seeds include tocopherols and sterols. The β -sitosterol constituent has been shown to be a strong inhibitor of prostaglandin biosynthesis in prostatic tissue of patients with Benign Prostatic Hyperplasia (BPH) and then to exert a marked anti-inflammatory action and it also exerts a marked anti-inflammatory action. Hence, its use as an alternative treatment for stages I and II BPH (Zdunczyk et al., 1999; Duke and Ayensu, 1985).

The seed has been used in traditional medicine as an antihelmintic agent and for supportive treatment in functional disorders of the bladder and for difficulties in urination (Srivastava et al., 1967); childhood enuresis nocturnal and irritable bladder had been treated successfully with pumpkin seed (Weiss, 1988). The seeds are mildly diuretic and vermifuge (Chiej, 1984). The diuretic action has been employed in the treatment of nephritis and other problems of the urinary system. The leaves are applied externally to burns (Chopra et al., 1986). The *in vitro* anti-oxidative activity of pumpkin seed protein isolate and its *in vivo* effect on alanine transaminase and aspartate transaminase in acetam-inophen-induced and CCL₄-induced liver injury in low protein fed rats had been

*Corresponding author. E-mail: monarch012002@yahoo.com.
Tel: 0803-474-0000.

investigated (Nkosi et al., 2005; Nkosi et al., 2006). Significantly reduced activity levels of the enzymes were reported. Tarhan et al. (2007) found out that the antioxidant capacities of the female flower extracts were significantly higher than the male flower extracts. The flowers were reported to be consumed in Turkey and were a great source of folic acid which contributes to its measurable effects on the blood components (Eggum, 1970; Church et al., 1984; Kerr et al., 1982; Emenalom et al., 2004; Tarhan et al., 2007; Adepoju and Odubena, 2009). The protein isolate of the seed has also been shown to increase the activity levels of certain plasma enzymes in CCL₄-induced liver injury in low-protein fed rats (Nkosi et al., 2005). It was concluded that the administration of pumpkin seed protein isolate was effective in alleviating the detrimental effects associated with malnutrition.

However, allergy caused by ingestion of zucchini (*C. pepo*) has been reported (Reindl et al., 2000). The evaluation of the insecticidal effect of pumpkin (*C. pepo*) has been carried out (Sinary, 2006). The World Health Organization (1963) has recommended the use of blood biochemical and haematological parameters in medical nutritional assessment. Hence, this study aims at examining the effect of *C. pepo* on the haematological and biochemical parameters of experimental animals.

MATERIALS AND METHODS

Test material

C. pepo was obtained from the Central Market, Ijebu – Ode, Ogun State, Nigeria. The specimen was validated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The pumpkin seeds were shade-dried and then blended into a powder. The powdered drug was weighed into 100, 200, 300 and 400 mg proportions.

Chemicals

Distilled water, sulphuric acid, mercuric nitrite, hydrochloric acid, phenol red, sodium hydroxide, diacetylmonoxine, acid reagent, chloro – 2, 4 – dinitro benzene, (CDNB), phosphate buffer, Ellman's, reagent, EDTA.

Animals

Thirty (30) healthy albino Wistar rats of both sexes (weighing 190 to 330 g) were obtained from the Animals House, Pre-clinical Department, University of Ibadan, Nigeria. Feed and water were provided *ad libitum*. The rats have no history of drug consumption, that is, they have not been used for any investigation before. The animals were acclimatized for 30 days before the commencement of the study. The 'principles of laboratory animal care' (NIH Publication N85.23, 1985) were followed in this study.

Animal and experimental design

The rats were divided into six groups of five rats in each group.

They were fasted for 24 h before the commencement of the experiment and were administered with different doses of *C. pepo* powdered seeds for 21 days according to their groups in proportionate specifications as follows:

Group A: 100 mg/kg body weight.

Group B: 200 mg/kg body weight

Group C: 300 mg/kg body weight

Group D: 400 mg/kg body weight

Group E: Free seeds consumption (prn)

Group F: Control group (animal feed only)

Group E was included to eat the seed freely like the people eat it without measure. The result here might reveal any acute toxic effect pertaining to the seed.

Group F was allowed to eat the normal feed in order to compare the effect of the seed with the regular feed of the animals. The doses were administered to Groups A to D with an oral canula with the respective doses dissolved in distilled water.

The animals were stunned after 21 days of oral administration of the test material and control. They were sacrificed and blood was removed by cardiac puncture. The liver and kidneys were carefully removed, trimmed free of extraneous tissues and rinsed in ice-cold 1.15% w/v potassium chloride solution. The organs were blotted dry, weighed and homogenised in ice – cold 0.1 M phosphate buffer pH 7.4 in a mortar. The homogenate was cold – centrifuged for 5 mins at 4000 rpm to remove cellular debris and the crude homogenate was recovered.

Haematological and biochemical evaluations

This was performed to examine the effect of *C. pepo* seeds on these parameters. Haematological parameters were determined using the method of Baker and Silverton (1984) to determine the leukocyte counts. Ivy's method (1940) was used to assay the bleeding time. Standard spectrophotometry methods were used for the biochemical determination of serum glutamic oxaloacetate transaminase (SGOT) or aspartate aminotransferase (AST), serum glutamic – pyruvic transaminase (SGPT) or alanine aminotransferase (ALT) and the method of Beulter et al. (1963) was used for the determination of blood glutathione. The analytical method of Lowry et al. (1951) and Reitman and Frankel (1957) were used for the protein determination.

Statistical analysis

All the results were expressed using the mean + standard error of the mean. Significant difference between the means was assessed using the student's t – test at 95% level of significance (p<0.05).

RESULTS AND DISCUSSION

Haematological examination

From Table 1, the effect of *C. pepo* on bleeding time showed that, it reduced the bleeding time in all the dose ranges but the reduction became statistically significant from 300 mg/kg body weight and upwards in a dose – dependent manner (Group C to E) when compared to the control (Group F). As bleeding time is used to evaluate the vascular and platelet factors associated with haemostasis, hence, *C. pepo* seed has a considerable

Table 1. Showing the weight of experimental rats, liver and kidneys.

Group	Total weight	Weight kidney	AK %	Weight of liver	AL %
A (100)	236.94	1.72	0.73	7.60	3.20
B (200)	196.3	1.34	0.68	6.58	3.35
C (300)	215.7	1.36	0.63	7.82	3.62
D (400)	281.1	1.78	0.63	9.74	3.46
E (Seed only)	308.4	1.98	0.64	10.40	3.37
F (Control)	246.46	1.62	0.66	8.44	3.42

All doses were in mg/kg body weight; A = organ: body weight ratio; A% = organ: body weight ratio (percent). AL = organ (liver): body weight ratio; AK = organ (kidney): body weight ratio.

haemostatic effect in a dose – dependent manner. The reduction in bleeding time is an indication of the enhanced clotting action thus preventing excessive blood loss. The packed cell volume (PCV) values were reduced at all the dose levels. These reductions were statistically significant when compared with the control ($p < 0.05$). The reduction was dose – dependent such that, as the dose increased, the PCV reduced in like manner.

The values of the white blood cells (WBC) showed that, there was an increase in the WBC count in Groups A and B and this was statistically significant when compared with the control (Group F). At higher doses (Groups C to E), there were reductions in the WBC values, that were statistically significant when compared to the control. Hence, its effect on WBC was dose-dependent. The neutrophil levels were reduced at all the dose levels tested and were significantly different from the control except at 100 mg/kg body weight (Group A). The same dose – dependent reduction was observed with the lymphocyte count except that statistically significant values were observed from Groups D and E. The counts for Groups A to C were not statistically significant. The eosinophil count was increased at 100 mg/kg body weight (Group A) but insignificant reductions were observed at higher doses in a dose – dependent manner. This meant that *C. pepo* has a tendency to reduce the eosinophil count at higher doses though the reductions were not statistically significant when compared to the control (Group F).

The entire WBC contributes to the host defence mechanism (Lloyd et al., 1999) hence, overall, this reduction in white blood cells at higher doses, in particular, could compromise immunity and predispose to opportunistic and supra - infections in spite of the nutritional benefits of the seeds of *C. pepo*. Hence, in addition to its effects on the PCV, the seeds should not be consumed heavily, in order to harness its beneficial effects.

Biochemical evaluation

The mean weight of the kidney showed increasing values with increasing doses of *C. pepo* except Group A (Table

1). However, the organ: body weight ratio of the animals showed that, there was a maximum dose intake at which there will be no significant changes in this ratio concerning the kidney even with increasing doses of the seed of *C. pepo*. Hence at low doses, the *C. pepo* increased the kidney weight and the organ: body weight ratio. The mean weight of the liver showed increasing values with increasing doses of the *C. pepo* seeds except with Group B. The increase in mean weight was very significant when compared to the control ($p < 0.05$). The organ; body weight ratio also showed that, there was an optimum dose beyond which there would be no increase in this ratio with increasing doses of the *C. pepo*. This optimum dose appeared to be at 300 mg/kg body weight of the seed.

The total protein levels in the kidney and liver showed increasing values to increasing doses of the *C. pepo* seed. This indicated an increase in the kidney and liver total protein levels when compared to the control group (Group F). These levels were statistically significant ($p < 0.05$). There were increasing values of total body weight with increasing doses of *C. pepo*. This was statistically significant (Groups D and E). This correlated with the kidney and liver weight values that also increased with increasing doses of *C. pepo*.

Effect on hepatic and renal functional indices

From Table 2, there was a reduction in the levels of reduced glutathione in the liver when compared to the control. The reduction was not statistically significant (Groups A to C): however, there were statistically significant increases in the glutathione levels for Groups D and E. Hence, the observations in the levels of the enzyme were dose – dependent. There were statistically significant reductions at low concentrations but statistically significant increased levels were observed at higher doses of the seeds. There were increases in the enzyme level in the kidneys and this was also dose – dependent. This was statistically significant for Groups C to D and E. Reduced glutathione is the body's key antioxidant and protective agent. It is involved in detoxification of xenobiotics that cause toxicity and carcinogenicity. GSH

Table 2. Effect of *C. pepo*. on haematological and biochemical parameter.

Mg/kg	Packed cell volume (%)	Bleeding time (min ⁻¹)	White blood cells (per mm ³)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Reduced glutathione (µg)	Total protein (g/100 ml)		Aspartate aminotransferase (U/L)		Alanine aminotransferase (U/L)		
	Blood	Blood	Blood	Blood	Blood	Blood	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver
A 100	34.0+1.05	46.8+1.02	*6120+101.99	19.4+1.44	77.6+2.48	2.0+1.05	32.56+0.65	24.0+2.29	*6.2+0.24	*5.76+0.15	*107+1.84	*118.1+3.41	*118.8+5.15	*120+6.94
B 200	*32+0.55	45.2+1.43	*6080+111.35	*17.4+2.02	75.8+2.29	1.8+0.49	33.76+1.00	24.8+2.72	*6.7+0.15	*6.5+0.26	*99.0+5.62	*111.5+3.41	*115.2+1.99	*115.2+1.99
C 300	*31.6+0.93	*44.0+1.41	*4480+315.29	*15.6+1.47	74.6+2.23	1.6+0.24	*35.6+0.97	24.88+1.3	*7.5+0.23	*8.7+0.48	*98.0+11.82	*88.2+19.98	*96.6+2.18	*112.8+4.97
D 400	*29.6+0.93	*43.8+1.46	*5250+339.72	*14.8+1.36	*70.8+1.39	1.6+0.68	*35.68+0.46	*30.16+2.06	*8.1+0.35	*9.2+0.29	*82.5+10.67	*111.5+3.41	*90.4+5.73	*100.6+5.23
Seed only E	*29.6+1.03	*42+0.55	*5240+265.7	*13.2+0.58	*70.6+0.51	1.4+0.24	*13.28+1.20	*32.48+0.75	*8.6+0.55	*9.5+1.37	*85.5+10.94	*102.5+0.79	*71.6+1.60	*103.6+1.29
Control	37.0+1.30	46.8+1.07	5500+491.95	21+2.47	75.6+3.93	1.4+0.51	32.48+0.75	25.04+3.42	2.20+0.06	4.14+0.08	120.0+3.47	109.1+0.66	122.8+1.56	130.6+1.29

Results are expressed as mean values + standard error mean. *P<0.05 when compared with the control group.

has a variety of functions in the prevention of diseases and in the detoxification of chemicals and drugs while its depletion is associated with increased risk of toxicity and diseases.

Under oxidative conditions, the concentration of the enzyme can be considerably reduced through conjugation of xenobiotics and by secretion of the enzyme conjugates in the affected cells (Janis, 2002; Videla and Valenzuela, 1982). When the concentration falls, cellular defence against toxic compounds is impaired and cell death results. Its increased level as observed, would therefore, be beneficial to the compromised body especially. Considering the effect of *C. pepo* on AST levels in the kidney, it was observed that there was a statistically significant reduction in the enzyme levels in the kidney when compared to the control. In the liver, there were statistically significant increases in the levels of AST for Groups A, B and D. However, there were statistically significant reductions in its levels as observed in Groups C and E. *C. pepo* significantly decreased the levels of ALT both in the kidney and the liver. This was dose – dependent when compared with the control (p<0.05). When disease or injury affects the body tissues, the cells are destroyed and

these enzymes (AST and ALT) are released into the blood stream. Their levels in the blood stream become higher than normal. AST (unlike ALT) is found in parts of the body other than the liver – including the heart, kidneys, muscles and the brain. When cells in any of these parts of the body are damaged, AST level is elevated. Thus, a high level of AST often means that there is liver damage. If its level is high but with a normal ALT level, it means that AST is coming from a different part of the body. A high ALT level (alone) often means that, there is some liver damage as the enzyme is located in the liver (Young et al., 1999; Mario et al., 2004; Hubscher, 2006). Therefore, the enzyme is considered a more sensitive marker of hepatocellular damage than AST (Aniagu et al., 2005).

Hence, the significant reduction in ALT levels in the kidneys and the liver was an indicative parameter of the hepato- and nephro – protective effects of *C. pepo* on these organs. The observed increases in the levels of AST in the liver at low doses (Group A and B) could be artefactual. Thus, *C. pepo*, at higher doses, protects against hepatocellular damage as observed with Groups C and E. Conclusively, if the seeds are moderately

consumed, the beneficial nutritional effects will be fully harnessed mainly because of the protein effects and the detoxification abilities. The nephro- and hepato-protective ability of the seeds make the seeds favourably disposed to consumption.

REFERENCES

- Adepoju GKA, Odubena OO (2009). EFFECT OF *Mucuna pruriens* on some haematological and biochemical parameters. *J. Med.Plants. Res.*, 3(2): 073-076
- Aniagu SO, Nwinyi FC, Akunka DD, Ajoku GA, Dzarma S, Izeba KS, Ditse M, Nwaneri PEE, Wanbebe C, Gamaniel K (2005). Toxicity Studies in Rats fed Nature Cure Bitters. ISSN 1684-5315. *Afr. J. Biotechnol.*, 4(1): 72-78
- Baker FJ, Silverton RE (1984). Introduction to Medical Laboratory Technology, 5th edition, Publisher Butterworth Scientific. (London), pp. 576-584.
- Beulter DV, Drum O, Kelly BM (1963). Improved method for the determination of blood glutathione. *J. Lab. Chem. Med.*, 61(5): 882-888.
- Chevallier A (1996). The Encyclopedia of Medicinal Plants Dorling Kindersley. London ISBN 9-780751-303148
- Chiej R (1984). Encyclopaedia of Medicinal Plants. MacDonald ISBN 0-356: 10541-1055
- Chopra RN, Nayar SL, Chopra IC(1986). Glossary of Indian Medicinal Plants (Including the Supplement). Council of Scientific and Industrial Research, New Delhi.
- Christiana W, Barbara W, Katharina S, Harald S, 2Dietmar F (2005). Extracts of Pumpkin (*Cucurbita pepo* L.) Seeds

- Suppress Stimulated Peripheral Blood Mononuclear Cells *in vitro* Am. J. Immunol., 1(1): 6-11, ISSN 1553-1619.
- Church JP, Young JT, Kebay CW, Kebay JL, Kin WW (1984). 'Relationship among dietary constituent and specific serum clinical components of subjects eating self – selected diet'. Am. J. Clin. Nutr., 40: 338-1344.
- Dreikorn K, Berges R, Pientka L, Jonas U (2002). Phytotherapy of benign prostatic hyperplasia. Current evidence-based evaluation. Urologe A., 41: 447-451.
- Duke JA, Ayensu ES (1985): Medicinal Plants of China Reference Publications, Inc. ISBN 0-917256: 20-24
- Eggum BO (1970). Blood urea measurements as a technique for assessing protein quality'. Br. J. Nutr., 24:983-988.
- El-Sinary NH (2006). Evaluation of the Insecticidal Effect of Pumpkin *Cucurbita pepo* Against Potato Tubermoth *Phthorimaea operculella* (Zeller) Combined with Gamma Irradiation. ISSN 1818-6785 . ISSN 1818-6785 American-Eurasian J. Sci. Res., 1(1): 18-22,
- Emenalom OO, Okoli IC, Udedibe ABI (2004). Observations on the Pathophysiology of Weaner Pigs Fed Raw and Preheated Nigerian *Mucuna pruriens* (Velvet Bean) seeds. Pakistan J. Nut., 3(2):112-117.
- Food and Agricultural Organisation Document Repository (1995). 'FAO Plant Production and Protection Series; T0646/E. (1995). *Cucurbita pepo*.
- Hubscher SG (2006). Histological Assessment of non-alcoholic fatty liver disease'. Histopathol., 49(5): 450 – 465
- Janis OF (2002). Gale Encyclopedia of Medicine.
- Ivy AC, Nelson D, Butcher G (1940). The Standardization of Certain Factors in the Cutaneous 'Venostasin' Bleeding Time Technique. J. Laborat. Clin. Med., 26: 1812-1822.
- Kerr GR, Lee ES, Lam MKM, Lorimor RJ, Raudal E (1982). Relationship between dietary and biochemical measures of nutritional status'. Am. J. Clin. Nutr., 35: 294-308.
- Lloyd YY, Mary AKK (Editor) (1999). The clinical use of drugs. 6th Edition. Publisher: Applied Therapeutics Inc. Vancouver WA. Chapter 4: 11-19.
- Lowry OH, Rosebrough NJ, Farr AR, Randall RJ (1951). Protein Measurement with Folin Phenol Reagent. J. Biol. Chem., 193: 265-275.
- Mario AT, Venancio AF, Cesar AN, Helena SF Edna S (2004). 'Clinical significance of elevated alanine aminotransferase in blood donors: a follow-up study'. Pakistan J. Nut., 2(2): 112-117.
- Matus Z, Molnar P, Szabo LG (1993). Main carotenoids in pressed seeds (*Cucurbitae semen*) of oil pumpkin (*Cucurbita pepo* convar. *pepo* var. *styriaca*). Acta. Pharm. Hung., 63: 247-256
- Murkovic M, Hillebrand A, Winkler J, Leitner EPfannhauser W (1996). Variability of fatty acid content in pumpkin seeds (*Cucurbita pepo* L.). Z. Lebensm Unters Forsch, 203: 216-219.
- Nkosi CZ, Opoku AR, Terblanche SE (2005). Effect of pumpkin seed (*Cucurbita pepo*) protein isolate on the activity levels of certain plasma enzymes in CCl4-induced liver injury in low-protein fed rats. Phytother. Res. Apr., 19(4): 341-345.
- Nkosi CZ, Opoku AR, Terblanche SE (2006). *In Vitro* antioxidative activity of pumpkin seed (*Cucurbita pepo*) protein isolate and its *In Vivo* effect on alanine transaminase and aspartate transaminase in acetaminophen-induced liver injury in low protein fed rats. Phytother. Res., 20 (9): 780-783.
- Reindl J, Aniker MD, Karanloo F, Vieths S, Wuthrich B (2000). Allergy caused by ingestion of zucchini (*Cucurbita pepo*): characterization of allergens and cross-reactivity to pollen and other foods. J. Allergy. Clin. Immunol., 106(2): 379-385.
- Rodriguez JB, Gros EC, Bertoni MH, Cattaneo P (1996). The sterols of *Cucurbita Moschata* "calabacita" seed oil. Lipids., 31: 1205-1208.
- Schmidit E, Schmidit FW (1963). Enzym. Biol. Clin. 3: p.1
- Sicilia T, Niemeyer HB, Honig DM, Metzler M (2003). Identification and Stereochemical characterization of lignans in flaxseed and pumpkin seeds. J. Agric. Food Chem., 26: (51): 1181-1188.
- Srivastava MC, Singh SW (1967). Anthelmintic activity of *Cucurbita maxima* (kaddu) seeds. Indian J. Med. Res., 55: 629-632.
- Tarhan L, Kayali H, Raziye OU (2007). *In Vitro* Antioxidant Properties of *Cucurbita pepo* L. Male and Female Flowers Extracts Plant Foods for Human Nutrition DOI: 10.1007/s11130-006-0038-0, 62: 49–51,
- Videla LA, Valenzuela A (1982). Alcohol ingestion, liver glutathione and lipid peroxidation, metabolic interrelation and pathological implications. Life. Sci., 31: 2395-2407.
- Weiss RF (1988). HerbalMedicine. Beaconsfield, England: Beaconsfield Publishers.
- WHO (1963). Technical Report Series. (Expert Committee on Medical Assessment of Nutritional Status). WHO, Geneva. p. 258.
- Zdunczyk Z, Minakowski D, Frejnagel S, Flis M (1999): Comparative study of the chemical composition and nutritional value of pumpkin seed cake, soybean meal and casein. Nahrung, 43: 392-395.