



Effects of hexane extract of *Ricinus communis* L. (var. *minor*) on blood parameters in rats

Rosaleen McNeil^{1*}, Ekwere Ekwere¹, Sylvanus Dafur¹ and Francis Okwuasaba²

¹Department of Anatomy; ²Department of Pharmacology, University of Jos, Jos, Nigeria.

Received 24th May 2005; Accepted 15th August 2005

Abstract

Ricinus communis L. (var. *minor*), the castor plant, has been proven to possess anti fertility properties in adult cyclic rats. The purpose of this work was to examine the effect of the extract on blood parameters in adult male Wistar rats. Ten male rats weighing between 150 and 200g were divided into two groups (n=5). Group 1 (test group) was given 5mg /kg body weight of the extract. Group two (control) had equal volume of physiological saline. All treatments were given subcutaneously on the abdominal wall after ascertaining the baseline blood parameters of the animals. The rats were monitored closely and weighed weekly for six weeks. At the end of six weeks all the animals were sacrificed under anaesthesia using ether and their full blood count (FBC) carried out. Results showed that the drug significantly improved white blood cells, neutrophils, lymphocytes and monocytes but reduced haemoglobin values as compared to those of the control animals. The rise in the total white blood cells was progressively steady. This effect may be antimicrobial. The reduction in haemoglobin values was attributed to generalized toxicity from the extract or maybe a result of an allergic reaction to the castor bean extract.

Keywords: *Ricinus communis*; Haemoglobin; White blood cell count; Wistar rats.

Introduction

Castor plant belongs to the family Euphorbiaceae and is popular for its yield of castor oil. It is commonly a shrub of 3-4 metres but can be 7-10 metres high (Hutchinson and Dalziel, 1958). It is tap-rooted and has an erect dark red stem (Chiej, 1984). Each tree bears both male and female flowers, which occur in clusters, upper (male) and lower (female) flowers. The seed has 3 lobes, each lobe containing a seed which is smooth and elliptical in shape (Chiej, 1984). They exhibit extreme variation in size and colour (Evans, 1996). They may be grey,

brown, black or brown/black mottled with a size of 0.8-1.8cm in length, 0.4-1.2 cm in width and oval or compressed in shape (Evans, 1996). Each seed is elliptical, convex on one side and flat on the other. They are usually gathered in November (Chiej, 1984). The seed contains about 20% shell and 80% kernel. Both consist of about 58-66% oil (Hutchinson and Dalziel, 1958). Evans (1996) recorded a fixed oil yield from castor seed, of 55-60%. Other constituents include 20% proteins; ricin (toxabulmin ;) albumin; enzymes (e.g. lipase, chymase); nucleo-albumin; amino acids; globulins, glycol proteins and alkaloids, steroids and elements

* Corresponding author. E-mail address: mcneils5p@yahoo.com

– magnesium, calcium, and manganese (Evans, 1996). These elements are present in high concentrations and contribute to the toxicity of the seeds.

Many advances have been made in the molecular biology of castor seeds. Seedlings of the plant have been found to contain a characteristic set of more than 100 different polypeptides against which a complex of antiserum has been raised (Irwin and Lord, 1990). An mRNA (messenger ribonucleic acid) for an albumin called, the 2s albumen has been isolated from maturing castor bean endosperm. The mRNA was isolated by hybridization and then used as a template for the synthesis of a generation of the proteins. It is a storage protein, a heterodimer of glutamine rich polypeptides; Ric 1 and Ric 11, which consist of 258 amino acid residues. Their nucleotide sequences have been established (Irwin and Lord, 1990; Bashir *et al.*, 1998).

The current interest in castor seeds however, lies in its contraceptive property in the female; a relatively new research area. It is interesting to know that among the Rukuba tribe in Bassa Local Government Area of Plateau State (Nigeria) the use of castor seed for contraception is widespread (Okwuasaba *et al.*, 1991 1997 and Osunkwo *et al.*, 1988). The diethyl ether fraction of the methanolic extract of the seed has significant antifertility property in cyclic rats. (Okwuasaba *et al.*, 1991, 1997, Das *et al.*, 2000, Isichei *et al.*, 2000). It is antioviulatory in adult cyclic Sprague-Dawley rats (McNeil *et al.*, 2003).

Experimental

Ten adult male rats were acclimatized for 2 weeks in the experimental animal house of the University of Jos, Jos-Nigeria. They were then divided into 2 groups (n=5) and treated as follows: Test group received 5mg/kg body weight of N-hexane extract of *Ricinus*

communis. Control group had equal volume of physiological saline.

All injections were given subcutaneously on the anterior abdominal wall. Blood was taken directly from the heart after sacrificing the animal. Baseline blood parameters were taken from the tail. The animals were weighed at onset and then weekly after treatment. Blood parameters measured were to include Haemoglobin, Total WBC, Differential WBC [Neutrophils, (N), Lymphocytes (L), Monocytes (M), Eosinophils (E) and Basophils(B)].

The Laboratory technique used for assessing blood parameters were as follows:

Haemoglobin (Hb). This is a measure of blood iron content in mg/dl or g/l. Hb can be estimated using: The stripe method; Chemical evaluation and Packed Cell Volume (PCV). In our experiment, HB was estimated using the stripe method; the strip is dipped in blood and the blood allowed to ascend the strip. The value is then calculated.

White blood cells (WBC). Total WBC count is done through the identification of the cells on basis of their properties in the stained film or using the Neuber counting chamber under X10 objective of the light microscope. The normal range is 4000-11000. Differential WBC count consists of the enumeration of the relative number of the component types of white blood cells and expressed in percentage. Statistical significance was done using ANOVA.

Results and Discussion

In this experiment the toxicity on blood cells was not high or fatal. Nevertheless, it was significant and worthy of note. *Haemoglobin (Hb)*. As can be seen, the Hb values increased steadily for control subjects. This finding differs in the test animals where Hb values increased for the first three weeks after treatment and began to fall. The fall may be attributed to generalized toxicity from the extract.

Table 1: RBC: Mean haemoglobin level

WEEK	CONTROL	TEST
0	11.6	9.9
1	11.6	10.2
2	11.3	10.1
3	11.3	10.3
4	11.2	9.5
5	11.1	10.1
6	11.0	9.4

Table 3: Mean neutrophil count

WEEK	CONTROL	TEST
0	55.0	53.3
1	53.3	55.0
2	56.7	57.7
3	58.3	59.7
4	56.7	58.3
5	56.7	58.3
6	63.3	56.7

Table 5: Mean monocyte count

WEEK	CONTROL	TEST
0	5.0	15.0
1	11.7	13.3
2	5	8.3
3	5	3.3
4	10	3.3
5	8.3	6.7
6	0	6.7

Table 7: Mean eosinophil count

WEEK	CONTROL	TEST
0	-	1.0
1	-	-
2	-	1.0
3	-	-
4	-	-
5	-	-
6	-	-

Table 2: Mean total WBC count

WEEK	CONTROL	TEST
0	5700.0	5700.0
1	5133.3	6100.0
2	5333.3	5900.0
3	4833.3	6033.3
4	5466.7	6233.3
5	5133.3	6200.0
6	5000.0	6066.7

Table 4: Mean lymphocyte count

WEEK	CONTROL	TEST
0	36.7	30.0
1	35.0	30.0
2	38.3	33.0
3	36.7	36.7
4	33.3	38.3
5	35.0	35.0
6	36.7	36.7

Table 6: Mean basophil count

WEEK	CONTROL	TEST
0	-	0.6
1	-	-
2	-	-
3	-	0.3
4	-	-
5	-	-
6	-	-

Total WBC count. This parameter was fairly constant in control animals but improved with time in test subjects. The extract seems to cause a steady rise in the total white blood cells. One would have thought this to be as a result of an allergic reaction to the castor bean extract but there was no associated rise in eosinophil count. Instead, there was a slight increase in the neutrophil count which is a response usually found in acute infections. This will need further proof, as it could be seen as an improvement in WBC values, particularly neutrophil count and so may offer

protection or help in combating infections. Traditional medicine in West Africa also employs castor oil as lotion in poultices to swellings and in South Africa the root is believed to cure toothache.

Lymphocytes. Lymphocyte levels in the test group was relatively constant but was found to increase steadily in the test group. Probably the extract increased the total body proteins and so cell count in view of the seed constituents of castor bean.

Eosinophils and basophils. These parameters were not found in the control group. No significant finding seen.

Monocytes. Now it is known that blood monocytes are equivalent to tissue macrophages. The finding in test subjects was relatively the same, an initial fall and later rise. It was noted that monocytes levels fell steadily in the first four weeks of the experiment after which values began to rise. In the control, there was a rise after the first week followed by a fall and later, a slight rise in the values. The fall was more obvious in the test animals but so also was the rise in values. It does appear that the extract alters monocytes values. It is known that tissue macrophages could be mobilized to the blood in the challenge of infection but more usefully, this phenomenon will reduce tissue reaction. It is possible that *Ricinus communis* may be useful in kelloidogenesis.

Finally, the use of castor oil is diverse; ranging from scientific fact to superstition. Due to the widespread use of castor bean, particularly among the Rukuba tribe of Plateau State, there is need for much more investigation into the properties and toxicity of the plant. This is more so in that the seeds are usually ingested whole.

Acknowledgement

We wish to thank the following persons for their contribution towards the study; Mr. Ameh of the animal house for all his support and in the acquisition of the animals; Dr Chris Isichei for his assistance in the laboratory analysis; laboratory staff of Faith Alive Foundation who carried out the blood analysis faithfully and promptly; Mr. John Ben and Mr. Sale Gotum of the Anatomy Department for their assistance in provision of equipment and anaesthesia used.

References

- Bashir M.E.H., Hubatsch I., Leinenbach H.P., Zepezauer M., Panzani R.C., Hussein I.H. (1998): Ric C1 and C3, the allergen 2S albumin storage proteins of *Ricinus communis*: Complete primary structures and phylogenetic relationships. *Int Arch. Allergen Immunol.* 115(1), 73-82.
- Chiej E. (1984): McDonald Encyclopaedia of Medicinal Plants. pp 262-263
- Clegg E.J. (1960): The age at which male rats became fertile. *J. Reprod. Fertil.* 1: 119-120.
- Das S.C., Isichei C.O., Okwuasaba F.K., Uguru V.E., Onoruvwe O., Olayinka A.O., Ekwere E.O., Dafur S.J. and Parry O. (2000) Chemical, Pathological and Toxicological studies of the Effects of Ricom-1013-J of *Ricinus communis* var. minor on women volunteers and Rodents. *Phytother. Res* 14, 15-19.
- Evans W.C (1996). Trease and Evans' Pharmacognosy. 14th ed. W.B. Saunders, London, 612 pp.
- Hutchinson J. and Dalziel J.M., (1958): The useful plants of tropical West Africa; Tale of the West Africa medicinal service being an appendix to the Flora of West Tropical Africa .320 pp.
- Irwin S.D and Lord J.M. (1990). Nucleotide sequence of a *Ricinus communis* 2S albumin precursor gene. *Nucleic Acids Res.* 18(19), 58-90.
- Isichei C.O.; Das S.C, Ogunkeye O.O., Okwuasaba F.K., Uguru V.E., Onoruvwe O., Olayinka A.O., Dafur S.J., Ekwere E.O. and Parry O. (2000). Preliminary clinical investigation of the contraceptive efficacy and chemical pathological effects of Ricom-1013-J. of *Ricinus communis* var. minor on women volunteers. *Phytother. Res.* 14, 40-43.
- McNeil R.T., Noronha C.C., Kusemiju T.O. and Okanlawon A.O. (2003): The anti-ovulatory effect of a seed extract of *Ricinus communis* Linn. *Nigerian Journal of Health and Biomedical Sciences* 2(1), 31-34
- Isichei C.O.; Das, S.C; Ogunkeye, O.O; Okwuasaba, F.K.; Uguru, V.E; Onoruvwe, O.; Olayinka A.O.; Dafur S.J. and Parry, O. (1997): Preliminary clinical investigation of the contraceptive efficacy and some biochemical effects RICOM-1013-J of *Ricinus communis* var. minor in human volunteers. Herbal medicine research workshop, University of Jos.
- Okwuasaba F.K.; Das S.C.; Isichei C.O; Ekwonchi M.M.; Onoruvwe O.; Olayinka A.O.; Uguru V.E.; Dafur S.J.; Ekwere E.O.(1997): The anticonceptive

- and the effect on uterus of ether extract 18312-J of *Ricinus communis*. *Phytother. Res.* 10. 97-100.
- Okwuasaba F.K.; Olayinka A.O.; Onoruvwe O.; Isichei C.O.; Uguru V.E.; Dafur, S.J. Ojobe O.; Parry O. (1997): Pharmacognostic studies of the seed of *Ricinus communis* Linn. RICOM 1013 J. Herbal Medicine Research Workshop, University of Jos.
- Okwuasaba, F.K.; Osunkwo, U.A.; Ekwenchi, M.M.; Ekpenyong K.I.; Onwukeme, K., Olayinka, A.O., Uguru, V.E. and Das, S.C. (1991): Anticonceptive and oestrogenic effect of a seed extract of *Ricinus communis* var. *minor*. *J. Ethnopharmacol.* 34: 141-145.
- Osunkwo, U.A.; Ibu, J. and Okwuasaba, F.K. (1988): Anticonceptive and oestrogenic effects of a medicinal plant (*Ricinus communis*) in rats. Abstract of 17th Annual Conference of the West African Society of Pharmacology, Port Harcourt, pp 41-44.