

Immunogenic Properties of *Ricinus Communis* Var Minor Seed on Female Albino Rabbits

Shindang, C. O.¹, Shindang, J.² and Enweani, I. B.³

¹College of Health Technology, Zawan, Plateau State. ²Department of Medical Laboratory Sciences, University of Jos, Jos, Plateau State. ³Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus, Anambra State

(Received 03.05.11, Accepted 26.07.12)

Abstract

The immunogenic properties of *Ricinus communis* var minor seed was determined after feeding 7 healthy virgin albino white rabbits with varying doses of 0.5g – 0.9g dried ground *Ricinus communis* var minor seed included in their feed (5g/100g body weight). Booster doses of the same weight were further administered after 14 days. Changes in the physiological and psychological baseline parameters were noted. There was a drop in the average baseline Packed Cell Volume (PCV) from 34.8% to 26.3%, and an increase in the White Blood cell Count (WBC) count and lymphocytosis in the differential count. Antibody response was shown by increased precipitation reaction against *R. communis* extract which served as antigen. Furthermore, the rabbits were restless after the administration of booster dose resulting in the death of 3 of the rabbits. In addition, there was loss of weight from 1.39kg to 1.25kg within 14 days of sensitization dose. This study showed that *R. communis* var minor seed has immunogenic and contraceptive properties. Its use should be controlled.

Key words: *Ricinus communis* var minor seed, immunogenic, female albino rabbits, contraceptive

Correspondence: celtinas2008@yahoo.com

Introduction

The resort of some Nigerian women to using some herbs as anti- contraceptive measures is common especially in rural communities. This was borne out of fear and suspicion of the effect of synthetic contraceptive drugs (Awosika, 1993). It has been reported that some have adverse side effects which include heart risk (Realin, 1985), susceptibility to STD's (Bromham, 1993) and lowering of activity of the immune system (Daly *et al*, 1994). Before the advent of orthodox medicine, man has been exploiting the medicinal properties of plants to cure certain ailments, oblivious of their active ingredients or adverse effect on the body chemistry.

Ricinus communis var minor seed is the smallest of the castor seed plants of the family Euphorbeaceae which has been reported to possess contraceptive properties (Okwuasaba *et al*, 1991, Kabele –Toge, 2003, Sardyakumary *et al*, 2003). It is a lectin, resembling immunoglobulin in behavior, having a high molecular weight, capable of agglutinating red blood cells of mammalian specie (Humphrey and White, 1973). In addition, it increased uterine premature opening of the vaginal endometrium in winster rats (Okwuasaba *et al*, 1997, Sandyakumary *et al*, 2003). The *R. communis* var minor seed was also reported by (Dafur *et al*, 2003) to be responsible for increased cytoplasmic vacuolation within the corpus lutea resulting in the production of numerous macrophages in winster rats without much adverse effect on the haematological indices. In addition, Badwin, (1995) demonstrated the toxic property of this seed; however, the immunogenic properties in man were not determined.

The objective of this study was to determine the immunogenicity of *R. communis* var minor seed in virgin albino rabbits as well as its effect on the haematological profile. Rabbit is chosen for this

research because of its size, blood volume and the accessibility of the vascular system via the ear vein. It is the breed of choice for polyclonal antibody production (Stills, 1994).

Materials and Methods

Ricinus communis var minor seed was certified by the Department of Pharmacology, University of Jos, Nigeria. The seed was treated according to the method described by Sani and Sule, (2007). The resultant wet ground pulp (500g) was dried in a hot-air (Gallenkamp) at 50^o C for 7 days.

Antigen Extract: To 10g weight of the ground wet pulp, 20ml of sterile distilled water was added and placed on a shaker for 3 hours at room temperature. It was filtered through Whatman no 1 filter paper. The filtrate was then evaporated at 65^o C in an evaporator. The extract was kept at 4^o C in a refrigerator and used as antigen for the immuno electrophoresis.

Preparation and Treatment of the Rabbits: A stock of nine healthy female virgin white albino rabbits of same average age and weight (1.34kg) from the Nigerian Institute of Trypanosomiasis Research (NITR) Vom were used. They were maintained at a temperature of 24^oC -28^oC in an experimental room for 2 weeks. Their baseline weight, activity level, behavior, vital signs, body temperatures and feeding habit were noted Suckow *et al*, (2002). In addition, blood samples to ascertain their baseline Packed Cell Volume (PCV), White Blood Cell count (WBC) and differential white blood cell count and antibody level, were collected according to the method described by Lieve (1990) into EDTA bottles and were stored in the refrigerator maintained at 4^oC . The PCV, WBC and differential count were carried out according to methods described by Cheesbrough (2004) and Lieve (1990). Furthermore, immunoelectrophoresis to determine the baseline antibody was carried out as described by Kohn (1970).

Treatment 1: Seven of the rabbits were fed with 5g/100g body weight of dried ground *Ricinus communis* var minor seed incorporated into the feed in order to sensitize the rabbits. The eighth and ninth rabbits were used as controls, and the dried ground seed was not added to the feed. The rabbits were observed for 14 days after which blood samples were collected and analysed for PCV, WBC differential and electrophoresis as previously described by Kohn, 1970;Suckow *et al*, 2002. Treatment was carried out on day one using 0.5g, 0.6g, 0.7g, 0.8g and 0.9g per test rabbit according to their weight. Daily observations were made for loss of weights, appetite, restlessness and any other changes.

Treatment 2: Booster dose of the same concentration was added to the rabbit feed of the seven rabbits. The body temperature, weight and physical reaction were recorded daily for 7 days. Blood samples were taken again to analyze for PCV, WBC differential and immunoelectrophoresis.

Results

All the rabbits used were healthy and had good appetite. They recorded an average normal PCV of 34.8% with normal WBC differential count (Neutrophil 31%, Lymphocyte 69%). There was no precipitin line shown for the counter current electrophoresis test indicating the absence of antibody (Table 1).

After the administration of sensitization dose to the test rabbits, it was observed that all the test rabbits were restless. Those that had high dose of *R. communis* var minor seed produced discharges via the mouth whereas the less dosed test rabbits showed less activity without any discharge. Average weight loss of 0.14kg with 4 deaths was recorded as shown in Table 2.

There was reduction in average baseline PCV to 26.3% with increase in lymphocytes. There was marked eosinophilia on administration of booster dose of the same concentration as the sensitization dose (Table 3).

Precipitin lines developed for test rabbits 5, 6 and 7 as recorded in Table 4.

Table 1: Pre-Treatment Weight, Haematological Status and Physiological Parameters of Test Rabbits

Parameters	Rabbit							Rabbit Control	
	1	2	3	4	5	6	7	8	9
Weight (kg)	1.42	1.40	1.40	1.34	1.38	1.35	1.37	1.37	1.43
Mean PCV (%)	34.7	35.0	34.7	34.7	35.0	34.7	35.0	34.7	35.0
Neutrophil (%)	35	32	30	28	36	24	30	29	28
Lymphocytes (%)	65	68	70	72	64	76	70	71	72
Eosinophil (%)	-	-	-	-	-	-	-	-	-
Basophils (%)	-	-	-	-	-	-	-	-	-
Monocytes (%)	-	-	-	-	-	-	-	-	-

KEY (-) = Absent

Table 2: Effect of Sensitization and Booster Doses of *R. Communis* Var Minor on Test Rabbits

Parameters	Rabbit							Rabbit control	
	1	2	3	4	5	6	7	8	9
Sensitisation dose with <i>R. communis</i> seed(g)	0.9	0.8	0.8	0.7	0.6	0.6	0.5	NA	NA
Pre-treatment weight(kg)	1.42	1.40	1.40	1.34	1.38	1.38	1.35	1.37	1.43
Physical observation after ingestion on Day2	Weak,restless, discharge from nose,mouth	Same as 1	Same as 1	Same as 1	Less active no discharge	Less active no discharge	Less active no discharge	Active	Active
Day 3	D	D	D	Weak, no appetite	Appetite restored	Appetite restored	Appetite restored	Active	Active
Day 7	NA	NA	NA	D	Active	Active	Active	Active	Active
Day 14 wt after sensitization dose(kg)	NA	NA	NA	NA	1.27	1.24	1.23	1.46	1.54
Booster dose on day 21(g)	NA	NA	NA	NA	0.6	0.6	0.5	NA	NA
Day35 Observation after booster dose	NA	NA	NA	NA	No abnormal changes	No abnormal changes	No abnormal changes	Active	Active
Wt(kg) after booster dose	NA	NA	NA	NA	1.49	1.52	1.50	1.62	1.71

KEY: NA=Not Applicable; D = Death

Table 3: Effect of Sensitization and Booster Doses on Packed Cell Volume and Leucocyte Differential Count of Test Rabbits

	Rabbit							Rabbit Control	
	1	2	3	4	5	6	7	8	9
Pretreatment mean PCV(%)	34.7	35.0	34.7	34.7	35.0	34.7	35.0	35.0	34.7
Sensitization dose effect	D	D	D	D					
Mean PCV(%)	NA	NA	NA	NA	22	30	27	34	34
Neutrophil(%)	NA	NA	NA	NA	14	19	16	31	28
Lymphocytes(%)	NA	NA	NA	NA	86	81	84	69	72
Eosinophil (%)	NA	NA	NA	NA	-	-	-	-	-
Basophil (%)	NA	NA	NA	NA	-	-	-	-	-
Monocyte(%)	NA	NA	NA	NA	-	-	-	-	-
Booster dose effect	NA	NA	NA	NA	-	-	-	-	-
Mean PCV(%)	NA	NA	NA	NA	35.5	36.2	35.0	35.5	35.0
Neutrophil(%)	NA	NA	NA	NA	10	16	4	32	30
Lymphocyte(%)	NA	NA	NA	NA	74	66	72	68	70
Eostnophil(%)	NA	NA	NA	NA	16	18	24	-	-
Basophil (%)	NA	NA	NA	NA	-	-	-	-	-
Monocyte(%)	NA	NA	NA	NA	-	-	-	-	-

KEY (-)= Nil, NA= Not Applicable, D= Death

Table 4: Antibody to *r. Communis* var minor extract using counter current electrophoresis

	RABBITS	BLEEDING PERIODS			
		1 ST	2 ND	3 RD	4 TH
TEST ANIMALS	1	-	D	D	D
	2	-	D	D	D
	3	-	D	D	D
	4	-	D	D	D
	5	-	+	++	+
	6	-	+	++	+
	7	-	+	++	+
ANIMAL	8	-	-	-	-
CONTROL	9	-	-	-	-
Control	feed				
against serum 5					

KEY

D = Death; (-) = Absence of precipitin line; (+) = Presence of precipitin line; 1st bleeding = pretreatment; 2nd bleeding = day 14(Sensitization); 3rd bleeding =day 28 (Booster dose); 4th bleeding = day 35

Discussion

The rabbits used for this study had baseline parameters which indicated that they were healthy and did not have any antibody against the immunogenic properties of *R. communis* var minor seed prior to the study. Their cellular activity did not show that the animals reacted against any immunogen. These parameters served as good basis for further comparison with experimental data.

The results showed that the *R. communis* var minor seed had immunogenic properties as it elicited an immune response in the host. Weir (1973) described an immunogen as a substance which when appropriately administered into a host cell, provokes a demonstrable specific immunogenic effect either with the production of antibody or changed cellular activity.

Restlessness discharges of mucous from the nose and eyes of the rabbits were indications of hyper sensitivity reaction which resulted in the death of four rabbits. Isichei *et al* (2000) and Johnson *et al* (2005), reported that ingestion of the *R. communis* seed when administered to human, results in symptoms such as vomiting, diarrhea, followed by severe dehydration, and in some cases, liver, spleen and kidney may fail leading to death.

Furthermore, the seed being in particulate form probably exposed its immunogenic properties which elicited antibody production. The effect of sensitization which brought about loss of appetite could have been responsible for the fall in the PCV as the animals did not feed well. However, Humphrey and White (1973) had described *R. communis* seed as a lectin. It is known that lectins cause haemoagglutination of mammalian red blood cells and can also be mitogenic. It could therefore be inferred that the fall in the PCV at sensitization as observed was a result of haemoagglutination factor of the *R. communis* var minor seed.

The weight gain and increased PCV value could be that the defence mechanism of the rabbits after having been primed became alert and on administration of the booster dose, quickly recognized the antigen (Weir, 1973)

R. communis var minor seed has been reported to possess contraceptive property with adverse effect on uterus resulting in termination of fetus in early pregnancy (Sardyakumary *et al*, 2003). Its contraceptive effect, coupled with its immunological property, probably led to the alteration of haematological indices in the experimental rabbits.

The administration of *R. communis* var minor seed is shown to elicit immune response with the attendant risks. Thus, the use of this seed should be controlled through adequate enlightenment campaign. In addition, records of mortality and morbidity as a result of use of this seed should be kept.

References

Awosika, F. (1993). Local medicine plants and Health of the consumer. *J. Clin Pharm and Herbal Medicine* 7: 3-4.

Badwin, S. M., Adam, S. F. and Hapka, H. (1995). Comparative toxicity of *Ricinus communis* and *Jatropha curcas* in Brown Hisex chicks DTW-Dtsch-Tierarztl-wochenchr 102 (2): 75-77.

Bromham, D. R. (1993). Intrauterine contraceptive device—A reappraisal. *British Med Bull* 49 (1) 100 – 123.

Cheesbrough, M. (2004). Haematological tests: District Laboratory Practice in Tropical Countries Part 2.2nd Ed. 2004. Cambridge University Press. 296-391.

Dafur, S.D., Ekwere, E.O., Okwuasaba, F.K., Isichei, C.O., Ekweunchi, M.M., Onoruwwe, O., Olayinka, A.O. (2003). The effects of *Ricinus communis* on Reproductive organs of Wistar rats and haematological indices in Women Volunteers. *J. Highland Medical Research* (3):31-34

Daly, C. C., Helling-Giese, G. E. and Mati, J.K. (1994). Contraceptive methods and the transmission of HIV: Implications for Family planning. *Genitourin Med.* 70 (2): 110-117.

Humphrey, J. H. and White, R. G. (1973). Antigens: Immunogen and Hapten: Immunology for students of Medicine. 2nd Ed 1973. Oxford Blackwell Scientific Publ. London pp 235 – 236.

Isichei, C.O., Das, S.C., Ogunkeye, O.O., Okwuasaba, F.K., Uguru, V.E., Onoruwwe, 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. *Phytotherapy Research* 2000;14:40-42.

Johnson, R.C., Lemire, S.W., Woolfitt, A.R., Ospina, M., Perry, K.P., Olson, C.T. and Barr, J.R. (2005). Quantification of Ricinine in rats and human urine: A biomarker for Ricin Exposure. *J. Anal Toxicol.* 29:149-155

Kabelle-Toge, B.B. (2003). The seeds of castor oil plant (*Ricinus communis* Linn) as a Herbal oral contraceptive – A review. *Nig. J. Pharmacy* 34: 27-32

- Khon, J. (1970). Method for the detection and identification of alpha Fetoprotein in serum. *J. Clin Pathol* 23 733-735.
- Lieve, O. (1990). Haematology of Rabbits: Laboratory investigations as a diagnostic aid: Disease of Domestic Rabbits. 2nd Ed.1990.Oxford. Blackwell Scientific Publ. pp.42-43
- Okwuasaba, F.K., Osunkwo, U. A., Ekwenchi, M. M., Ekpenyong, K. I., Onkwukeme, K. E., Olayinka, A. O., Uguru, M. O. and Das, S. C. (1991). Anti-conceptive and oestrogenic effects of a seed extract of *Ricinus communis* var minor. *J. Ethnopharmacology* 34: 141- 145.
- Okwuasaba, F.K., Das, S.C., Isichei, C.O., Ekwenchi, M.M., Olayinka, A.O., Uguru, V.E., Dafur, S.J., Ekwere, E.O. and Parry, O. (1997). The anti –conceptive and effect on uterus of ether extract of *Ricinus communis*. *Journal of Phytotherapy Research*. 10, : 97-100.
- Realini, N. (1985). Oral contraceptives and cardiovascular disease, a critique of the epidemiological studies. *AM J. Obstetrics and Gynaecology* 152: 729-798.
- Sani, U. M. and Sule, M. I. Anti-fertility activity of methanol Extracts of three different seed varieties of *Ricinus communis* Linn (Euphorbiaceae) Nig. J Pharm. Sci. 6,(2), 78-83
- Sardyakumary, K., Bobby, R.G. and Indira, M. (2003). Antifertility effects of *Ricinus communis* (Linn) on rats. *Journal Home*, 17 (5):508-511.
- Still, F. (1994). Polyclonal Antibody Production IN: The Biology of laboratory Rabbit. Manning, P.J, D.H Ringler and Newcomer 2nd Ed. 1994.San Diego, Academic Press; pp 435-448.
- Suckow, M A., Brammer, D W., Rush, H. G. and Chrisp, C.E. (2002). Biology and Diseases of Rabbits: Laboratory Animal Medicine, 2nd Ed, 2002 New York Academic Press.
- Weir, D. M. (1973). Antigens: Immunology for undergraduates 3rd Ed. Publ. Churchill Livingstone London. pp18-26.