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HAEMATOLOGICAL AND SPERM COUNT CHANGES FOLLOWING EXPOSURE TO *HYPTIS SUAVEOLENS*, *CLEOME VISCOSA* AND *URENA LOBATA* IN RATS.

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SUMMARY

The plants *Hyptis suaveolens*, *Cleome viscosa* and *Urena lobata* are herbs commonly found growing in pasture and also used as medicinal plant in Nigeria. They were suspected to be toxic following a pilot toxicity study, and their toxic effects were thus evaluated on haematological parameters and sperm count of albino rats. The crude aqueous extract of the leaves of these plants were administered orally for 28 days and the haematological analysis of the rats treated with *Cleome viscosa* showed a significant decrease ($p < 0.05$) in packed cell volume, white blood cells, lymphocytes and platelets, while *Hyptis suaveolens* showed a slight but insignificant changes ($p > 0.05$) in the erythrocyte indices, white blood cell count and differentials. The neutrophils number also increased significantly ($p < 0.05$) in rats treated with *Urena lobata*. The sperm count of the rats treated with *Cleome viscosa* showed a significant decrease ($p < 0.05$) in sperm concentration, while sperm morphological analysis showed significant decrease ($p < 0.05$) in headless tail sperm cell abnormality. The rats treated with *Hyptis suaveolens* also showed a significant decrease ($p < 0.05$) in headless tail which may be of significant effect to their fertility, while the rats treated with *Urena lobata* extract showed a significant increase ($p < 0.05$) in headless tail sperm cell abnormality

Key words: *Cleome viscosa*, *Hyptis suaveolens*, *Urena lobata*, haematology, sperm count, toxicity.

INTRODUCTION

Urena lobata which is generally grown for its fibre is a plant of mallow family known as *Malvaceae* with many common names; hibiscus burr, aramina pink, Chinese burr, urena burr and burr mallow. It is an erect shrub, which invades low elevated disturbed areas (Wagner, *et al.*, 1999). The barbs on its seeds cling to fruits or seeds of other plants or clothing for dispersal. The methanol extract of the root is reported to exhibit a broad spectrum of concentration-dependent anti-bacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*,

Staphylococcus epidermis, *Micrococcus luteus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae* and *Vibrio cholera* (Mazunder *et al.*, 2000).

Hyptis suaveolens is a coarse, erect, branched and hairy annual herb of *Lamiaceae* family commonly called wild spikenard. It is often a contaminant in pasture grasses. The plant is bitter, minty and aromatic. It has been used as analgesic, decongestant and antipyretic. It also stimulates blood circulation. Decoction of

the herb as tea is effective for fever associated with cold and flatulence. Rats administered with extract of this plant are reported to exhibit some behavioural changes such as aggressive scratching of the body and head, drowsiness and sleep, depressed pulse, loss of vigour and apparent loss of weight (Akinloye, 2003). The plant is also good for the treatment of gonorrhoea (Jain and Singh, 1994), and conjunctivitis (Sikawar, 1994). The fresh leaves of this plant are used by local people as mosquito repellent especially at night.

The extracts of the plant have been reported to be larvicidal to *Anopheles stephensi* (Sharma, *et al.*, 1992) and reduced oviposition of *Callosobruchus maculatus* (Adedire and Lajide, 1999). The leaf extract has also been shown to reduce adult population of *Raphidopalpa foveicollis* (Ray, 2000), while its essential oil is active against a variety of fungal pathogens (Singh *et al.*, 1992).

Cleome viscosa is a plant of caper family otherwise known as *Capparaceae*, with commonly known as Asian spider flower. It is an annual plant common in hot humid and dry areas (Ahmed *et al.*, 1972). Its seeds contain glycocapparin and other thioglucosides from which methyl isothiocyanate and other mustard oils are released when the seeds are crushed (Kjaer, 1960; Ahmed *et al.*, 1972). The seeds and leaves have rubefacient and vesicant properties (Chopra and Badhwar, 1940; Behl *et al.*, 1966). The seeds are occasionally used for culinary purposes. The leaves are used in external applications for treatment of wounds and ulcers while the seeds are used for treatment of round worm infections (Saxena *et al.*, 2000). Occasionally the seeds are used by local people as remedy for fever and diarrhoea, and powdered roots are put on the lips to restore consciousness in

people who fainted. Smoke from its leaves is used to repel mosquitoes at night. The extract of the plant exhibited larvicidal activity against second and fourth instar larva of *Anopheles stephensi* (Saxena *et al.*, 2000).

The ease of obtaining these plants, their usual presence in pasture, and their extensive use for medicinal purposes has necessitated the need to investigate their toxicological effects.

MATERIALS AND METHODS

Animals and test procedure

Forty albino rats weighing between 180-220g of both sexes were used for the study. They were kept in rat cages and fed with rat pellets manufactured by Ladokun and Sons Livestock Feeds Nigeria Limited. The rats were allowed feed and clean water *ad libitum*. The animals were divided into four groups of ten rats each for the treatments and control. Based on a pilot toxicity study, the rats in each group received 250mg/kg of the crude extract of the plant meant for each group, while the control group received 3ml/kg of distilled water by weight.

Preparation of the crude aqueous extracts of the plants

Ten percent aqueous extracts of the fresh leaves of the plants were always prepared by harvesting 10g leaves which were macerated using mortar and pestle in 100cm³ of distilled water. The solutions were filtered and the filtrates administered to the rats orally at the same period of the day using stomach canula for 28 days.

Determination of haematological parameters

Blood was collected by cardiac puncture from diethyl ether anaesthetized rats into heparinised bottles for haematological studies. Haemoglobin concentration and

Red blood cell count were determined using cyanomethaemoglobin and haemocytometer methods respectively as described by Jain, (1986). White blood cell differentials were also determined from Giemsa stained slides, while Packed cell volume was carried out using the conventional method of filling the capillary tubes with blood as described by Schalm *et al*, (1975).

Sperm count and morphological study

The sperm concentration was measured by the use of haemocytometer, while sperm morphology was evaluated microscopically using eosin-nigrosin stained semen samples (Zemjanis, 1970; Bradford, 1996).

Statistical analysis

The result of the haematological analysis and sperm count were expressed as the mean \pm standard error of mean. The difference between the experimental and control groups were determined statistically using students' t-test and differences were considered significant at $p < 0.05$ level (Bailey, 1992).

RESULTS

The result of this study with respect to the haematological changes showed that *Cleome viscosa* caused a significant decrease ($p < 0.05$) in packed cell volume (Table I), total white blood cell, lymphocytes and platelets levels (Table II). The plant also

caused insignificant reduction ($p > 0.05$) in red blood cell count and haemoglobin concentration (Table I). *Hyptis suaveolens* caused significant decrease ($p < 0.05$) in red blood cell and mean corpuscular volume (Table I), while no significant changes was observed in the white blood cell and differentials (Table II). The plant *Urena lobata* did not cause any significant change in haematological parameters, while a significant decrease ($p < 0.05$) was observed in the levels of lymphocytes and platelets (Table II). Also, significant increase ($p < 0.05$) was observed in the neutrophil level (Table II). The aqueous extract of *Cleome viscosa* caused a significant decrease ($p < 0.05$) in the sperm concentration, headless tail and bent tail, sperm cell abnormality (Table III). *Hyptis suaveolens* caused a significant decrease ($p < 0.05$) in headless tail sperm cells (Table II), while the extract of *Urena lobata* caused a significant increase ($p < 0.05$) in the headless tail sperm cells (Table III).

Table I: The effect of the aqueous extract of *Cleome viscosa*, *Hyptis suaveolens* and *Urena lobata* on erythrocyte counts and other haematological indices in rats.

Parameter	<i>Cleome viscosa</i>	<i>Hyptis suaveolens</i>	<i>Urena lobata</i>	Control
RBC x 10 ⁶ (/ul)	7.2 ± 0.9	9.5 ± 0.5 ^b	8.2 ± 0.3	8.1 ± 0.4
PCV (%)	33.9 ± 3.2 ^a	40.0 ± 0.9	39.3 ± 1.8	40.4 ± 1.6
HbC (g/dl)	10.3 ± 1.0 ^a	12.4 ± 0.3	11.7 ± 0.5	12.6 ± 0.5
MCV (fl)	48.5 ± 3.3	43.5 ± 3.4 ^b	48.2 ± 3.1	50.8 ± 3.9
MCH (Pg)	14.7 ± 0.9	13.1 ± 1.0	14.3 ± 0.8	15.7 ± 1.2
MCHC (g/dl)	30.5 ± 1.4	31.1 ± 0.6	29.9 ± 0.9	31.1 ± 0.4

Superscripted items implies a significant difference (p<0.05) between mean values of test and control animals.

Table II: The mean ± S.E.M of total white blood cell count and white blood cell differentials of rats administered with aqueous extract of *Cleome viscosa*, *Hyptis suaveolens* and *Urena lobata*.

Parameter	<i>Cleome viscosa</i>	<i>Hyptis suaveolens</i>	<i>Urena lobata</i>	Control
Total WBC x 10 ³ /ul	15.5 ± 1.8 ^a	14.4 ± 3.0	14.2 ± 1.8	19.2 ± 2.7
Neutrophils (%)	3.5 ± 0.5	4.7 ± 1.5	28.0 ± 12.6 ^b	4.6 ± 0.4
Lymphocytes (fl)	6.9 ± 1.3 ^a	9.6 ± 1.5	7.9 ± 2.6 ^b	14.4 ± 3.6
Monocytes (pg)	0.5 ± 0.1	0.2 ± 0.1	0.7 ± 0.3	0.6 ± 0.5
Eosinophil (%)	-	-	0.4 ± 0.3	0.4 ± 0.01
Platelets (%)	508.0 ± 77.2 ^a	575.0 ± 14.3	501.3 ± 35.7 ^b	583.2 ± 16.6

Superscripted items indicates a statistically significant values (p<0.05) between the test and control groups.

Table III: The mean \pm S.E.M of sperm concentration and sperm cell abnormality of rats administered with *Cleome viscosa*, *Hyptis suaveolens*, *Urena lobata* and the control group.

	Sperm concentration $\times 10^5$ (cell/ml)	Tailless head (cell/ml)	Headless tail (cell/ml)	Rudimentary tail (cell/ml)	Bent tail (cell/ml)	Curved tail (cell/ml)	Bent mid piece (cell/ml)	Curved mid piece (cell/ml)
Control	56.0 \pm 1.9	5.3 \pm 0.6	6.0 \pm 0.7	-	5.7 \pm 0.6	12.3 \pm 0.9	11.3 \pm 0.8	11.7 \pm 0.6
<i>Cleome viscosa</i>	52.0 \pm 1.2 ^a	4.7 \pm 0.8	2.0 \pm 0.5 ^b	-	1.7 \pm 0.9 ^c	12.0 \pm 1.0	9.7 \pm 1.1	9.3 \pm 0.8
<i>Hyptis suaveolens</i>	55.3 \pm 1.7	4.8 \pm 0.6	0.8 \pm 0.6 ^b	1.0 \pm 0.5	6.8 \pm 3.0 ^c	12.8 \pm 0.6	9.5 \pm 1.1	10.5 \pm 3.1
<i>Urena lobata</i>	55.3 \pm 5.3	5.0 \pm 0.9	8.3 \pm 1.4 ^b	-	4.3 \pm 1.4	13.0 \pm 2.4 ^d	9.3 \pm 2.4	11.7 \pm 2.8

Superscripted items indicates a statistically significant values (p<0.05) between the test and control groups.

DISCUSSION

The aqueous extract of *Cleome viscosa* caused a significant decrease in the packed cell volume, but an insignificant decrease in the levels of haemoglobin concentration and red blood cell count. These effects may suggest that the plant can produce anaemia. The reduced levels of the mean corpuscular volume and mean corpuscular haemoglobin concentration show the plant to produce normocytic normochromic anaemia (Radin *et al.*, 1986; Clark, 1988) in the treated animals. The statistically significant decrease in the total white blood cell and lymphocytes also showed that the continuous administration of this plant extract to animals may compromise the cell mediated immune process of the animals (Young, 1989; Paul, 1993) since the T-lymphocytes are very important in the ability of the animal to counteract infection.

The haemoglobin concentration and packed cell volume of the animals administered with *Hyptis suaveolens* reduced insignificantly, while the red blood cell significantly increased. The significant increase in the neutrophil level of the animals administered with extract of *Urena lobata* may account for the use of the plant for medicinal purposes (Keenwe and Bekalo, 1996) especially its antibacterial activity. The mean corpuscular volume and mean corpuscular haemoglobin concentration values of the plant also showed normocytic normochromic anaemia. *Urena lobata* showed adverse effects on the spermatogenesis as manifested in the significant increase in the number of headless tail sperm cells. The headless tail abnormality according to Blom (1948) is a primary sperm cell abnormality which is caused as a result of disruption in the course

of spermatogenesis which may be due to plant toxicity or chemical poisoning.

The significant decrease in the number of tailless head and bent tail sperm cells showed *Cleome viscosa* to have a protective activity on the sperm cells. The extract of *Hyptis suaveolens* also showed a significant decrease in the headless tail sperm cells, and it also showed a protective activity towards the sperm cells.

REFERENCES

- ADEDIRE, C.O AND LAJIDE, L. (1999): Toxicity and oviposition deterency of some plant extracts on cowpea storage bruchid, *Callosobruchus maculatus*. Fabricuc zeitschrift-fur-pflanzenkheiten-und-pflanzenschutz. **106: 6**, 647 – 653.
- AHMED, Z.F., RIZK, A.M., HAMMONDA, F.M. AND SEIF EL-NASR, M.M. (1972): Glucosinolates of Egyptian Capparis species. *Phytochemistry*. **11**: 251 – 256 (1972).
- AKINLOYE, O.A.(2003): An evaluation of the toxic effects of *Hyptis suaveolens* in rats. *Nigerian Journal of Natural Products and Medicine*, **7**: 49 – 52.
- BAILEY, N.T. (1992). *Statistical method in Biology*, 2nd Ed. pp. 28 – 41. Cambridge University Press Cambridge.
- BEHL, P.N., CAPTAINS, R.M., BEDI, B.M.S AND GUPTA (1966): Skin irritant and sensitizing plant found in India New Delhi. *Indian J. Agric Sci*.
- BRADFORD P. S. (1996): *Large animal internal medicine* 2nd edition. Mosby pp. 241.
- BLOM, E. (1948). Om spermaundersogelsemetoder hos tyren (On methods of evaluating bull semen). *Medlemsbl. Danske Dyrlaegeforening*, **31**: 446.
- CHOPRA, R.N. AND BADHWAR, R.L.(1940): Poisonous plants of India. *J. Agric. Sci.* **10 (1)**: 1-44
- CLARK, M.R. (1988): Senescence of red blood cell: Progress and problems. *Physiol. Rev.* **68**, 503.
- JAIN, N.C. (1986): *Schalm's Veterinary Haematology*. 4th Ed. Lea and Fabiger, Philadelphia pp. 564-572.
- JAIN, S.P. AND SINGH, S.C. (1994): Ethno-medicobotanical survey of Ambikapur district. *Ethnobiology in human welfare; Abstracts of the fourth international congress of Ethnobiology*, Lukknow, Uttar Pradesh, India.
- KEENWE, M. AND BEKALO, I. (1996): *Ethnoveterinary Medicine in Kenya, A field manual of traditional animal health care practices*. Published by ITK and IIRR, Nairobi.
- KJAER, A. (1960): Naturally derived isocyanates (mustard oils) and their parent glycosides. *Fortschr. Chem. Org. Natstoffe*. **18**: 122 – 176.
- MAZUNDER, U.K., GUPTA, M., MANIKANDAN, L. AND BHATTACHRYA, S. (2000): Antibacterial activity of *Urena*

- lobata* root. Fitoterapia. **72**, 8: 927-929.
- PAUL, W.E. (1993): Infectious disease and the immune system. Sci. Am. 90.
- RADIN, M.J., EUBANK, M.C., WEISER, M.G. (1986): Electronic measurement of erythrocyte volume and volume heterogeneity in horses during erythrocytes regeneration associated with experimental anaemias. Vet. Pathol. **23**, 656 – 660.
- RAY, D.C. (2000): Field studies on *Raphidopalpa foveicollis*. Extent of damage caused and evaluation of certain insecticides and plant extracts. Indian Journal of Environment and Ecoplanning. Vol. **3** (3): 579 – 586.
- SAXENA, B.R., KOLI, M.C. AND SAXENA, R. (2000): Preliminary ethnomedical and phytochemical study of *Cleome Viscosa* L. ethnobotany **12**: 47 – 50.
- SCHALM, O.W., JAIN, N.C. AND CARROLL, E.J. (1975): Veterinary Haematology. 3rd Ed. Lea and Febiger, Philadelphia.
- SHARMA, R.N., GUPTA, A.S., PATWARDHAN, S.A., HEBBALKAR, D.S., TARE, V. AND BHONDE, S.B. (1992): Bioactivity of Laminacea plants against insects. Indian Journal of Experimental Biology **30** (3): 244-246.
- SIKAWAR, R.L.S. (1994): Ethnoveterinary plant medicines in Morena district of MP. India. Ethnobiology in human welfare. Abstracts of the fourth international congress of Ethnobiology, Lukknow, Uttar Pradesh, India 17-21 November 1994, 289.
- SINGH, G., UPADYAY, R.K. AND RAO, G.P.(1992): Fungitoxic activity of the volatile oil of *Hyptis suaveolens*. Fitoterapia **63** (5): 462 – 465
- WAGNER, W.L., HERBST, D.R. AND SOHMER, S.H.(1999): Manual of the flowering plants of Hawaii (Revised Ed) University of Hawaii Press, Honolulu pp. 802 – 882.
- YOUNG, J.D. (1989): Killing of target cells by lymphocytes: A mechanistic review. Physiol. Rev. **69**, 250.
- ZEMJANIS, R. (1970): Diagnostic and therapeutic techniques in Animal Reproduction 2nd edition pp. 150.