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Evaluation of the Haematological Properties and Effects of Crude Rauwolfia Vomitaria

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ABSTRACT

The effect of the ethanolic root extract of Rauwolfia vomitaria on the histology of the ovary of female albino wistar rats was investigated. The animals were divided into 5 groups with 5 rats in each group. Group A was the control group while the other 4 groups (B, C, D and E) were experimental groups. Group A animals were given distilled water and normal rat feed, Group B animals were given 50mg/kg of Rauwolfia vomitaria, Groups C,D and E were given 100mg/kg, 150mg/kg and 200mg/kg of Rauwolfia vomitaria respectively. Administration was done orally and it lasted for 21 days. At the end of administration, the rats were sacrificed and Ovaries from all the groups were carefully dissected out, fixed immediately in Bouin's fluid and sent to Laboratory for histopathological analysis. 2-3mm in thickness were section out, and re-fixed in neutral buffered formalin solution, processed to paraffin sections, cut at 5micron using Rotary microtome and evaluated under digital microscope. Result of histopathology showed that the ovaries from group A revealed normal cellular architecture of theca granulosa cells, vascular, follicular cells, stroma containing reticular and fusiform cells, primary and secondary follicles with distinct area oocyte, cumulus oophorus, zona pellucida, corona radiata, follicular antrum, there is no evidence of cellular abnormality seen, Ovaries from group B revealed slight area of cellular degeneration with other cellular content within normal limit as compared to control group, the group C revealed slight oocyte disappearance with other cellular pattern within normal limit as compared to control, Group D revealed moderate cellular abnormalities, theca cell degeneration, granulosa degeneration and formation of follicular atresia as compared to control group while Group E revealed severe cellular abnormalities, theca cell degeneration, granulosa degeneration and formation of follicular atresia as compared to control.

Keywords— Rauwolfia Vomitaria, Histopathology, Wistar Rat and Ovaries

I. INTRODUCTION

The use of herbal remedies to ameliorate various ailments appears to be the current trend in the society. This is due in part to the recognition of the value of the traditional medical system particularly of Asian origin and the identification of medicinal plants of indigenous pharmacopoeias shown to have significant healing power either in their natural state or as a source of new pharmaceuticals. Generally, these formulations are considered moderate in efficacy and thus less toxic than most synthetic pharmaceutical agents (Elvin, 2001).

It has been widely reported that a larger number of these tropical plants and their extracts have therapeutic effects including for infertility or in the treatment of cancer (Elvin, 2001). One of such herbs frequently used is Rauwolfia vomitaria belonging to the Apocynaceae family (Elvin, 2001). Rauwolfia vomitaria is a shrub of up to 8m in height. It has flowers that are sweet scented and the fruits are reddish in colour (Abbiw, 1999). It was given the generic name Rauwolfia in memory of a 16th century German physician, sir Leonhart Rauvolf, who travelled widely to collect medicinal plants. The English name of the plant is swizzler plant.

Reports show that this herb lowers blood pressure (Amole, 2005) and possesses analgesic properties (Ogunjere and Amole, 2001). Antioxidant effects have also been reported (Akpanabiatu et al., 2009) and antipsychotic effects (Obembe, 2003). In Nigeria, herbalists use it as a purgative. Children with jaundice and cerebral cramps are treated with this plant (Zohan, 1982). Rauwolfia vomitaria is considered endangered (Oni et al., 2001). However, inspite of the widely reported beneficial effect of the plant, there is need to carry out a research to ascertain its safety as a herbal remedy and specifically to determine if it has any cytotoxic effect on the ovary.

Rauwolfia vomitaria is an evergreen plant of the Apocynaceae family and the genus Rauvolfia. It is a shrub or tree up to 8m. The branches are whorled and lumpy. It is called swizzler or serpent wood in English. In Nigerian local languages, it is called Asofoyeje (Yoruba), mmoneba (Efik) and Ira (Hausa). The parts of the plant that are commonly used for herbal remedies are roots, root bark, leaves and stem-bark (Kutalek and Prinz, 2007). It is a plant that is extensively grown in Congo and other parts of Africa on commercial basis for its medicinal value (Burkill, 2002). The plant is of different species. The Indian specie is called Rauwolfia serpentina. The African

specie of the plant, Rauwolfia vomitaria has double the quantity of reserpine when compared with the Indian specie (Kutalek and Prinz, 2007). The plant together with its related specie was named in memory of a great German botanist and physician, Sir Leonhart Rauvolf. It is mostly found in forest growth where fallow periods are prolonged. They also occur naturally in gallery forests. Rauwolfia vomitaria is associated with palms, Trema quincensis and Combretum spp; and is one of the last species to disappear in this particular serial stage (Bohringer et al., 2001).

Wild seedlings are successfully transplanted and cultivated. Natural stump regrowth is possible in this species (Bothringer et al., 2001). It can also be propagated using leafy stem cuttings or single node. According to Sharma et al., (2004) more than 20 alkaloids were reported to be derived from Rauwolfia vomitaria in the early 1900's. They include Reserpine, Semperflorine, Mitioridine, Ariane, Vomilenine, Ajmalidine, Rauwolfine, Seredamine,Picrinine, Pelinine, Purpeline, Rauvoxinine, Yohimbine, Tetraphylliane, Obscuridine and Obscurine.

The main alkaloid present in Rauwolfia is called reserpine. It was first discovered by Swiss Scientists, Schiller and Muller of CIB pharmaceuticals in Switzerland in 1952 (Sharma, 2004). Reserpine is a major constituent of anti-hypertensive drug (Okpako, 1991).

The root of Rauwolfia vomitaria has been used for centuries in Africa and India for treatment of snake bite, insomnia and insanity. However, it was until the 1950's that the structure of the anti-hypertensive agent reserpine was discovered. Reserpine is used in the treatment of hypertension because it can deplete catecholamine from sympathetic and peripheral nerve endings (Smith, 1997). These substances are normally involved in controlling peripheral resistance and heart rate by means of inhibiting the ATP/Mg++ pump responsible for sequestering of neurotransmitters into storage vesicles located in the presynaptic neuron.

Prajapatti et al., (2007) reported that Rauwolfia vomitaria is hypnotic, has sedative properties and is good for reducing blood pressure. The root is a good antidote to snake venom and the juice of the leaves of the plant is used for the treatment of corneal opacity of the eyes.

Kutalek and Prinz (2007), Rauwolfia vomitaria is used traditionally against snake bites, fever and nervous disorders. The pygmies of the Congo Basin administer Rauwolfia species together with traditional ash salt against diarrhoea and with red palm oil against elephantiasis of the legs. It is used for abortion because it contracts the uterus after administration (Burkill, 2002). In Nigeria, it is used for emetic or purgative purposes. Children with cerebral cramps and gastrointestinal disorders are treated with this plant. In Mali, the roots of Rauwolfia vomitaria are used to treat haemorrhoids and hepatomegaly. It is also good for treatment of hypertension, insomnia, scabies and malaria (Odugbemi, 2008).

Oyedeji (2007) reported that the plant is good for the treatment of insomnia. Rauwolfia vomitaria is good in treating impotency and nervous disorders. It should however be moderately used as it atimes weakens the patient (Adodo, 2006).

The plant has known to cause mental depression which may persist for several months after it has been withdrawn. The root bark extract according to Oni et al., (2001) are poisonous in high dosage. Notable side effects of Rauwolfia alkaloids are depression and parkinsonian syndrome (Okpako, 1999). The root bark according to Eluwa et al., (2010) are cardiotoxic to the developing heart in higher doses.

The ovary is a paired almond shaped organ that produces reproductive hormones. It is located near the attachment of the broad ligament to the lateral pelvic wall (Moore and Dalley, 2003). Several ligaments support the ovary. The ovarian ligament connects the uterus and the ovary. The posterior portion of the broad ligament forms the mesovarium which supports the ovary and houses the arterial supply and venous drainage. The suspensory ligament of the ovary (infundibular pelvic ligament) attaches the ovary to the side of the pelvic cavity (Moore and Dalley, 2003). The surface is covered by a simple squamous or cuboidal epithelium, the germinal epithelium. Under the germinal epithelium is a layer of dense connective tissue, the tunica albuginea which is responsible for the whitish colour of the ovary (Juncqueira and Carneiro, 2005). Underneath the tunica albuginea is the cortex where ovarian follicles, structures that contain the oocytes are situated. The follicles are embedded in connective tissue (stroma) of the cortical region. This stroma is composed of spindle shaped fibroblasts that respond to hormonal stimuli in a different way from fibroblasts of other regions. Ovaries have been considered as the major organ that played key role in fertility. The most internal part of the ovary is the medulla containing a rich vascular bed within a loose connective tissue (Juncqueira and Carneiro, 2005). A lot of research has been done on Rauwolfia vomitaria. Despite its frequent and regular usage, there is no available literature which has shown the effect of the root extract on the ovary. Therefore, the research is undertaken to investigate the cytotoxic effect of the ethanolic root extract of Rauwolfia vomitaria on the ovary of female albino wistar rats.

II. MATERIALS AND METHODS

Drugs and Chemicals:

Sodium chloride, formaldehyde, sodium trioxocarbonade V, sodium bicarbornate, xylene, 70% alcohol, 90% alcohol, absolute alcohol, distilled water, hutches, concentrate feed, syringe and hypodermic needles, EDTA treated bottles, latex hand glove, weighing scale, graduated vials, measuring tape, they were all

procured from BDH Chemicals, England. All other chemicals were of analytical grade.

Procurement, Preparation and Concentration of Extract:

The roots of Rauwolfia vomitaria were obtained from a piece of farm land in Uruan Local Government Area of Akwa Ibom State. They were identified and authenticated by a botanist in the Department of Botany, University of Uyo, Uyo. The roots were washed with water to remove the impurities. The bark of the plant root was peeled off and dried under the sun for four days. The dried pieces were then pulverized using an electric kitchen blender and the powder obtained was stocked in a plastic container with plastic covers. The pulverized root bark was then extracted into 80% ethanol for three days at room temperature (26˚C -28˚C). This was then filtered using filter paper. The filtrate was then concentrated in a steam bath and the resulting residue was reconstituted in distilled water. The ethanolic root extract was then ready for use. The extract was then stored in a refrigerator from which a fresh solution was always prepared using Tween 80 and was heated when required.

Median Lethal Dose of root of Rauwolfia vomitaria:

The LD_{50} (median lethal Dose) of the root of Rauwolfia vomitaria was determined when using Lorke's method. Lorke's method (LD_{50}) was calculated as geometrical mean of the maximum dose producing 0% mortality (a) and the minimum dose producing 100 % mortality (b). ($LD_{50} = \sqrt{}$ ab (Lorke, 1983).

The acute toxicity of root of Rauwolfia vomitaria on wistar albino rats was determined by giving different doses of the plant extract based on body weight of the animals it was administered orally to the animals in five groups. The animal were monitored for the next three hours and examined after twenty-four hours for mortality.

After the establishment of LD_{50}, animals in group A- B were administered with 0mg/kg, 50mg/kg, 100mg/kg, 150mg/kg, and 200mg/kg body weight of ethanolic extract of the root of Rauwolfia vomitaria.

Experimental Animals:

Twenty sexually mature male wistar rats were used for the research work. Rats were gotten from University of Nsukka, Nigeria .The rats were left to acclimatize in the College of health sciences animal house in University of Uyo, Nigeria for seven (7) days. The rats were housed in a clean wooden cage and fed with rodent pelleted feed and clean drinking water ad libitum. Rats were identified by different color marking on their tails. All rats were handled according to guiding principles in the care and use of animal's standard care of laboratory animals. 25 female albino wistar rats were used in the study. All animals were kept and fed ad libitum at the Animal house of the College of Health Sciences, University of Uyo. All experiments were done following the experimental guidelines of Institutional Animals ethics committee (IAEC).

Experimental Sites:

The study was done at the College of Health Sciences Animal House, University of Uyo, Uyo, Akwa-Ibom State Nigeria.

Experimental Protocols:

Rats were grouped into four (5) groups according to their weights with five rats housed per cage.

Carefully grouped rats were labeled as follows.

Group A: Control (Administered with distilled water only).

Group B: Ethanolic extract of Rauwolfia vomitaria root (50mg/kg).

Group C: Ethanolic extract of Rauwolfia vomitaria root (100mg/kg).

Group D: Ethanolic extract of Rauwolfia vomitaria root (150mg/kg).

Group E: Ethanolic extract of Rauwolfia vomitaria root (200mg/kg).

A total of 25 female albino wistar rats weighing between 100g -200g were assigned into five groups containing five rats in each group. Group A served as the control group and group B, C, D and E served as the experimental groups. Group A which was the control group received distilled water. Groups B, C, D and E were treated with the root extract for a period of 21 days with each of the groups receiving 50mg/kg, 100mg/kg, 150mg/kg and 200mg/kg concentration of the extract respectively. After the last day of the extract administration, the animal feed was taken out. The rats were anaesthetized with chloroform and sacrificed. The animals were dissected and the ovaries were removed and then weighed.

Sample Collection for Histopathological Analysis:

At the end of the stipulated 14 days of administration of the extract, the rats were subjected to a 12 hours fast but had access to water, sacrificed using chloroform vapour. Ovaries were carefully harvested out from the rats, harvested organs were carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were immediately fixed in Bouin's fluid transported to the Histopathology laboratory, After 72 hours, 2-3 mm in thickness were dissected out and post fixed in Neutral Buffered Saline and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene.

Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of

molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the kidney, liver and pancreas.

The sections were designated "vertical sections". Serial sections of 5 µm in thickness were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides and later stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed Histopathologically under digital light microscope.

III. RESULTS AND DISCUSSION

Results

Histopathological Findings

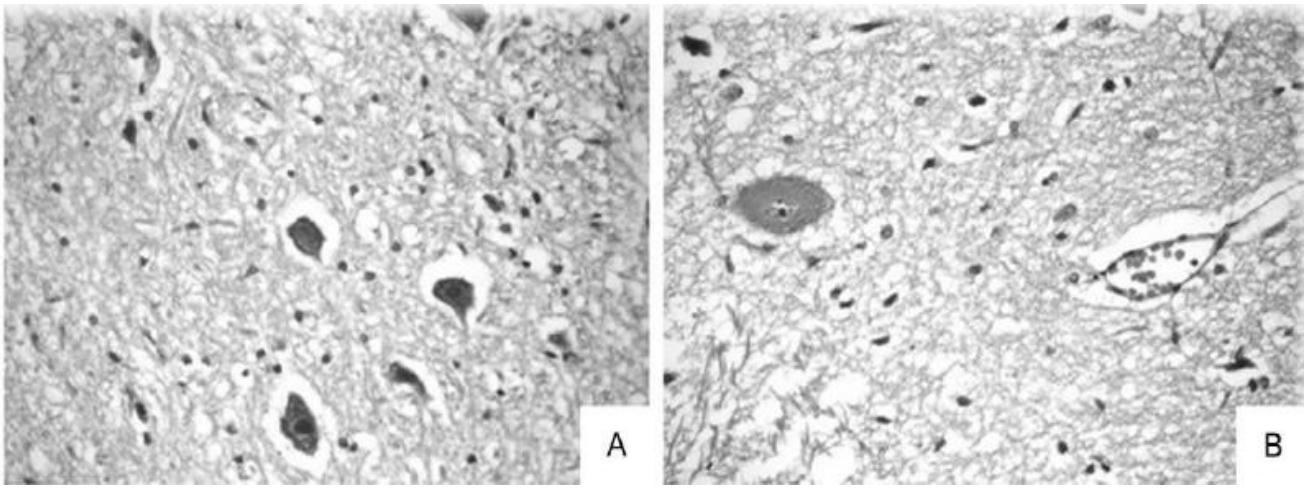

Plate 1: Section of the Ovary of the control group treated with Distilled water at Magnification A (X100) and B (X400) stained with H and E.

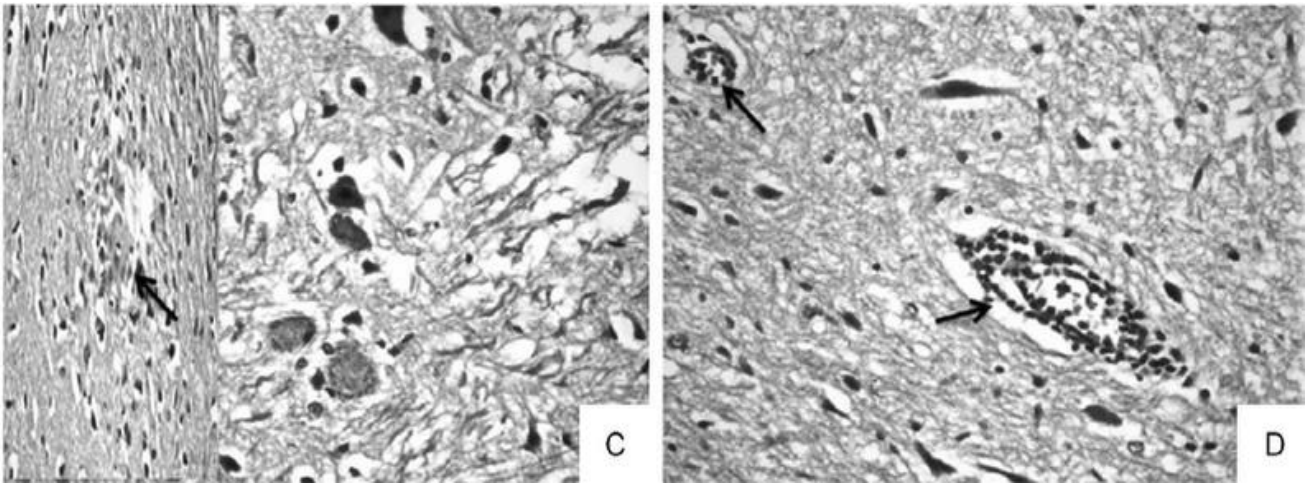

Plate 2: Section of the Ovary treated with Rauwolfia vomitaria 50mg/kg at Magnification C (X100) and D (X400) stained with H and E

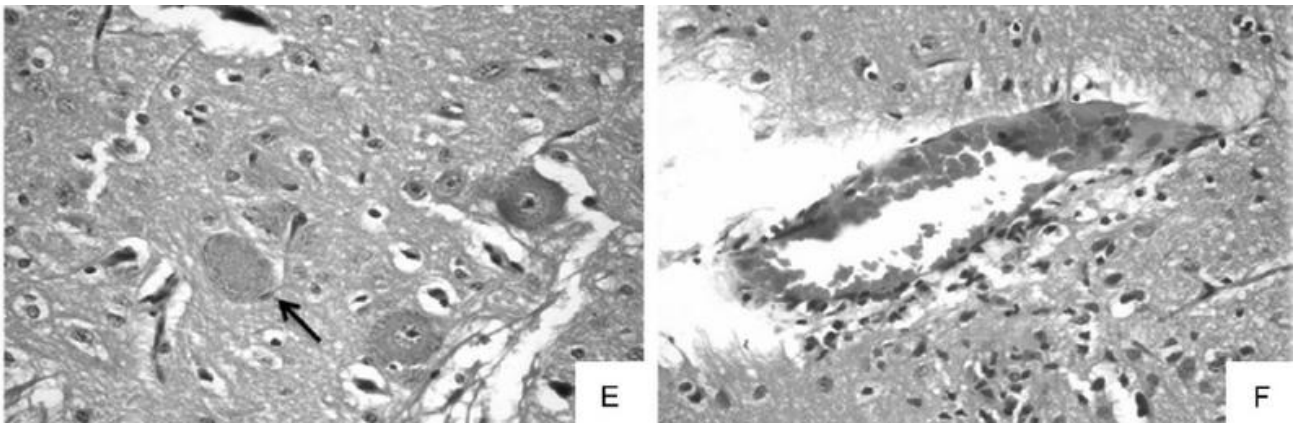

Plate 3: Section of the Ovary treated with Rauwolfia vomitaria 100mg/kg at Magnification E (X100) and F(X400) stained with H and E

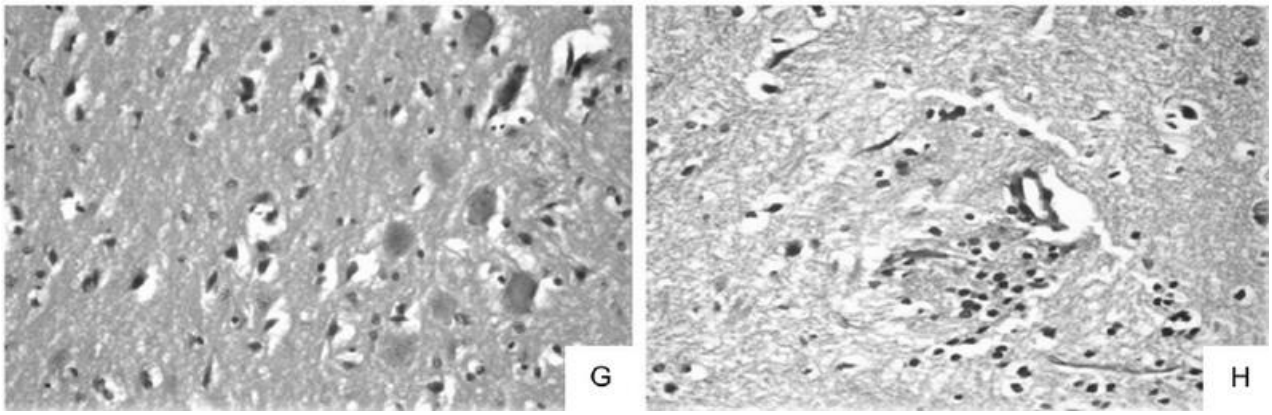

Plate 4: Section of the ovary treated with Rauwolfia vomitaria 150 mg/kg at Magnification G(X100) and H (X400) stained with H and E.

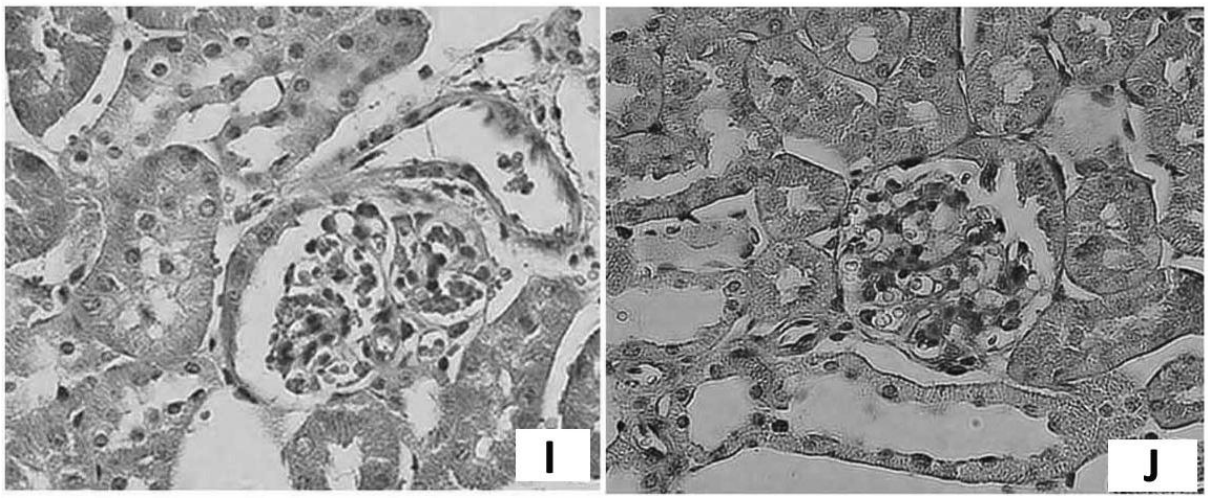

Plate 5: Section of the ovary treated with Rauwolfia vomitaria 200mg/ml at Magnification I(X100) and J (X400) stained with H and E.

Group 1: Control Ovary showing normal cellular architecture of theca granulosa cells, vascular, follicular cells, stroma containing reticular and fusiform cells, primary and secondary follicles with distinct area oocyte, cumulus oophorus, zona pellucida, corona radiata, follicular antrum, there is no evidence of cellular abnormality seen.

Group 2: Ovaries treated with 50mg/kg of Rauwolfia vomitaria reveal slight area of oocyte degeneration with other cellular pattern within normal limit as compared to control group.
Group 3: Ovaries treated with 100mg/kg of Rafiola vomitira revealed complete oocyte disappearance with other cellular pattern within normal limit as compared to control group.
Group 4: Ovaries treated with 150mg/kg of Rafiola vomitira revealed severe cellular abnormalities, theca cell degeneration, granulosa degeneration and formation of follicular atresia as compared to control group.
Group 5: Ovaries treated with 200mg/kg of Rafiola vomitira revealed severe cellular abnormalities, theca cell degeneration, granulosa degeneration and formation of follicular atresia as compared to control group.

IV. DISCUSSION

An extensive literature search did not reveal any study on the effect of the extract of Rauwolfia vomitaria on the ovary and this study appears to be a pioneering effort with regard to the effect of the herb on the ovary. The results obtained from this work have indicated that Rauwolfia vomitaria is toxic to the body when the dosage is high. Animals treated with Rauwolfia vomitaria were observed to be weak and drowsy. The drowsiness could be attributed to the sedative and hypnotic effect of Rauwolfia vomitaria as reported by Prajapatti et al., (2007).

The animals in groups A which received distilled water showed no cellular abnormality in the ovary. The follicular cells, vascular tissue, Zona Pellucida and corona radiata were all normal. Animals in group B which were treated with 50mg/kg of Rauwolfia vomitaria revealed normal cellular architecture of the ovary as compared to the control group. Normal cellular architecture was also observed in the animals in group C which were treated with 100mg/kg of Rauwolfia vomitaria. Severe cellular abnormalities, theca cell degeneration and granulosa cell degeneration were observed in experimental animals of group D and E that received 150mg/kg and 200mg/kg of Rauwolfia vomitaria respectively.

Some of the herbal remedies have been linked with haematological toxic effects, neurotoxic, nephrotoxic, carcinogenic effects and allergic reactions thus the belief that anything natural is safe is incorrect (Dasgupta, 2003). Rauwolfia vomitaria has been reported to have some beneficial effects as it is useful in the treatment of diarrhoea, fever and other ailments such as nervous disorders. However, this study has shown that Rauwolfia vomitaria has detrimental effects on the ovary especially when administered in higher doses orally. This corroborates the findings of some earlier researchers like Eluwa et al., (2010) who reported on the toxicity of this plant when consumed in higher doses.

V. CONCLUSION

It has been observed that administration of Rauwolfia vomitaria causes alteration or degeneration of the histological architecture of the ovary which appears to be exacerbated with increasing dosage.

Conflict Interests

The authors declared that they have no competing interests.

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