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PHYTOCHEMICAL SCREENING AND GUT MOTILITY ACTIVITY OF ETHANOLIC EXTRACT OF WHOLE PLANT OF *SCUTIA MYRTINA* KURZ.

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ABSTRACT

Phytochemical and Gut motility activity of Ethanolic Extract of whole plant of *Scutia Myrtina* Kurz collected from Kolli Hills, Tamil Nadu was studied. Phytochemical studies revealed the presence of Alkaloids, Glycosides (Anthraquinone), Flavonoids and Tannins. For Gut Motility activity Extracts in concentration of 200mg/Kg and 400mg/Kg were studied in three animal models in comparison to Caster Oil and Glaxenna; (a) Gut motility activity in isolated Rat Intestine, (b) Propulsive Gut motility in mice & (c) Laxative activity in Mice. The Gut motility activity was assessed by different parameters depending on respective animal models. Caster Oil and Glaxenna were used as Standard drugs depending on the animal models. Gut motility in the test drug treated animals were found to be significant in all the models. Anthraquinone Glycosides present in the drug is probably responsible for the Gut motility activity.

INTRODUCTION: Laxatives are drug which promote defecation. Since normal size, frequency and consistency of fecal output are difficult to define objectively and may have a wide inter-individual variation depending on personal habit and sociological pattern, there is a tendency to abuse laxative drugs. Previously with an almost totally vegetarian diet (which has high fiber content) the pattern was different and now -a- days with more consumption of non – vegetarian food the pattern is changing towards the western culture, which coupled with a western life style leads to over 70% of patients presenting to Gastroenterology clinics with complaints of either "too much" or "too little".

Though laxative agents have their valid uses; majority of causes of constipation can be managed by increased dietary intake of fibers, regular exercise, adequate water intake and bowel training with reassurance. In some cases where constipation is a manifestation of organic disease or some other systemic ailment, then treatment of the underlying disease should be the primary focus of therapy. Constipation occurring as an adverse drug reaction (analgesics, aluminium containing antacids, anticholinergics, antihistaminics, MAO inhibitors etc.) can be managed by reducing the dose or cessation of drug.

Laxative acts:

- By their hydrophilic or osmotic nature, laxative can cause retention of fluids. In the colonic content, as well as increase the mass,

Or

- They may inhibit electrolyte and water absorption from the colon by direct Or indirect mechanisms e.g., activation of adenylate cyclase within the Colonic mucosa or by enhancing secretion of hormones such as cholecystokinin by Mg^{2+} salts,

Or

- Laxatives may enhance the motility of the colon, there by reducing the time. Available for absorption of electrolytes and water. Hence, the fecal mass Presenting at the rectum is more fluid.

Scutia myrtina is an erect, glabrous or minutely pubescent branched ever green herb which grows up to 75 – 80 cm height. Stem is striate, leaves are distant, and Surratt margin and ovate. Flowers are white in color. The seeds are small and yellowish brown in color. The aerial part of the plant was used for stomach problems. The root and Leaves of the plant traditionally used as antihelminthic. The alcohol extract of the aerial part of the plant posses antiviral activity. The root bark of *Scutia myrtina* is used for fever and also the infusion of the plant is used to treat malaria. In eastern Tanzania the root of this plant is used for the treatment of bilharzias, intestinal worms and fever. The leaves and root bark decoction is used for gonorrhea, bilharzias, and intestinal worms in Tanzania. It contains Anthraquinone Glycoside which led us to evaluate the Gut Motility activity in Experimental animal Models[1, 2, 3, 4, 5].

MATERIALS AND METHOD:

Phytochemical Evaluation [6]: The plant of *Scutia myrtina* was collected from Kolli hills, Tamilnadu, India in the month of July, 2009. The shade dried plant was grinded in mixer grinder to obtain particle size of coarser size. The grinded product was macerated with

absolute ethanol for 7 days following the process called simple maceration. After 7 days of maceration, evaporation of solvent was done to obtain semisolid product which was used for further *in vivo* studies. Reagents and Chemicals used were either of analytical grade (Qualigens) or were freshly prepared. Ethanolic extract of *Scutia myrtina* was subjected to qualitative analytical test for the detection of various chemical constituents viz. Alkaloids, steroids, carbohydrates, fixed oils, glycosides, tannins, proteins, saponin and flavonoids. And Alkaloids, Glycosides (Anthraquinone), Flavonoids and Tannins were found to be present.

TABLE 1: SHOWING RESULTS OF PHYTOCHEMICAL SCREENING

CHEMICAL CONSTITUENTS	ETHANOLIC EXTRACT
Alkaloid	+ve
Carbohydrates	-ve
Glycosides	+ve
Saponins	-ve
Protein	-ve
Phytosterols	-ve
Tannins	+ve
Flavonoids	+ve
Anthraquinone	+ve

Gut Motility Activity:

Animals: Healthy adult albino rats of Wistar strain weighing 180 - 250g and Swiss albino mice 15 – 20 gm of either sex were used for this study. The animals were obtained from animal house, IRT Perundurai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at temperature of 24 ± 2 ^{o}C and relative humidity of 30 – 70 %. A 12: 12 light: dark cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/S Hindustan Lever Ltd., Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics committee (688/2/C-CPCSEA) of NCP and were in accordance with the guidelines of the IAEC. Approval was obtained from the above for animal studies in this project by (Proposal No.: NCP / IAEC / PG - 26/2009).

Collection of Plant & Preparation of the Extract: The plant of *Scutia myrtina* was collected from Kolli hills, Tamil Nadu, India. The shade dried plant was grinded in mixer grinder to obtain particle size of coarser size. The grinded product was macerated with absolute ethanol for 7 days following the process called simple maceration. After 7 days of maceration, evaporation of solvent was done to obtain semisolid product which was used for further *in vivo* studies.

Drugs and Chemicals: Glaxenna collected from market, is a laxative containing Senna as active constituent manufactured and marketed by Glaxo Lab. Castor Oil purchased was marketed by Nice pharmaceuticals Ltd, Other Chemical used were of Analytical Grade and reagents were freshly prepared.

Gut Motility Activity in Isolated Rat Intestine [7]: Albino Wistar rats (200 - 300g) of either sex were obtained from the Animal House. Each rat was starved for about 12 hours prior to the experiment, but was allowed to have free access to water. This was to ensure that the

intestines were free of faecal materials (Akah *et al*. 1997). The rat was killed by cervical dislocation and the intestines were quickly dissected out and freed from other connective tissues. They were placed inside a beaker containing aerated Tyrode solution: NaCl 136; KCl 2.7; MgCl2 1.8; CaCl21.8; NaHPO4 0.3; NaHCO3 12.0 and Glucose 5.6mM which was maintained at 37 ^{o}C (Akah *et al.,* 1997). Effects of Extracts and agonists on the Tissues: 2 – 3 cm of the required segment of the intestines was cut and mounted vertically inside a 20ml organ bath containing Tyrode solution which was maintained at 37^{0}C by a thermostat-bearing heater and aerated with air from an aerator.

The tension on the tissue was adjusted to 1 g in order to maintain its muscle tone. The tissue was allowed to equilibrate for one hour during which the tyrode solution was replaced at a ten minute interval. Graded doses (0.25 – 20.0 mg/ml) of the ethanol extract or infusion of the plant material were applied to the tissue and its responses were recorded by a kymograph. Contact time of study was between 30 and 45 seconds, because the responses of the tissues in some cases were not immediate.

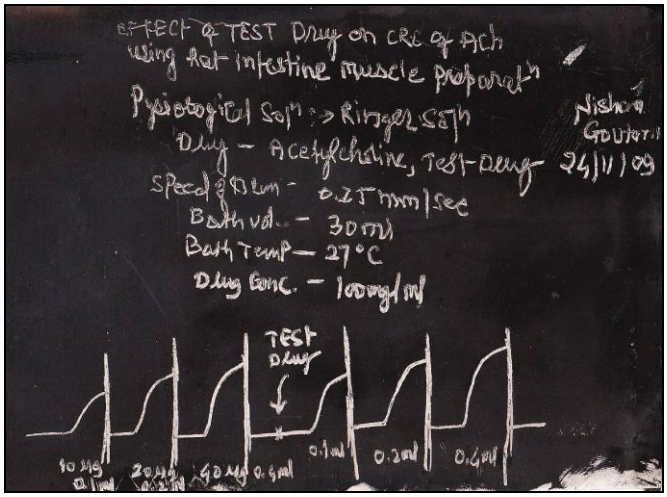
The tissue was washed at least twice with fresh tyrode solution after each dose to ensure that the tissue was free of the drug. The tissue was allowed to rest for between 10-15 minutes before the next dose of the extract or infusion was applied (Akah *et al*., 1997). The dose of the extract which produced the maximum response was taken as the Working Dose against which graded doses of agonists were tested. The tissue was always pretreated with the required dose of the agonists for 3 minutes before the application of the Working Dose of the extracts or infusions. Each agonist was used until the dose which produced the maximum reduction in tissue response to the drugs was obtained. This dose was tested against the Working Dose in quadruplicate.

Propulsive Gut Motility in Mice [8]: The passage of a charcoal meal through the gastrointestinal tract in mice is used as parameter for intestinal motility and to study the effect of laxatives. Groups of 6 mice weighing 15 – 20 gm are fed standard diet for 3 days. Eighteen hours prior to the experiment food, but not water, was withdrawn. The animals are treated orally 60 min before administration of the charcoal meal (0.2 ml of a 4% suspension of charcoal in 2% carboxy methylcellulose solution).

The mice are sacrificed after 60 min. 6 animals were there in each group, (IV Groups). The entire intestine is immediately removed and immersed in 5% formalin to halt peristalsis; then washed in running water. The distance the meal has traveled through the intestine as indicated by the charcoal is measured and expressed as percent of the total distance from the pylorus to the caecum. The charcoal passage test can be used for evaluation of laxative activity as well as for inhibition of intestinal motility.

Laxative Activity in Rat [9]: Group of at least six rat weighing 150-200 gm of either sex, were fed on standard diet for 3 days, with water *ad libitum.* In this model, Group I served as normal control received normal saline, (1 ml/kg, p. o.), Group II served as solvent control (0.5% CMC, 1 ml/kg, p. o.), Group III served as Standard received Castor oil (0.2 ml/Rat, p. o.) whereas Group IV and Group V animals received ethanolic extract of *Scutia myrtina* (200 mg/kg and 400 mg/kg, p. o.) respectively.

Then each rat was kept for observation under a transparent cage, the floor of each was lined with blotting paper and observed for 5 hrs. The parameters observed were, numbers of wet faecal pellets, total number of faecal pellets output. Then calculations were made for the correspondent percentages and later by comparison with respective control group.



EFFECT OF ETHANOLIC EXTRACT OF *SCUTIA MYRTINA* IN ISOLATED RAT INTESTINE

TABLE 2: PROPULSIVE GUT MOTILITY IN *SCUTIA MYRTINA* TREATED ANIMAL MODEL

TREATMENT	After 1 Hr Intestinal Transmission (%)
Normal Transit (Normal Saline, 1 ml/kg)	38.34±0.99
Solvent control (0.5% CMC, 1 ml/kg)	46.5±1.5**
Standard (Castor oil, 0.2 ml/mice)	69.84±1.5**
Scutia myrtina (200 mg/kg)	54.34±1.5**
Scutia myrtina (400 mg/kg)	67.84±1.2**

Values are mean ± SEM; No. of animals in each group = 6; * P value <0.05; ** P value <0.01 compared with the corresponding control

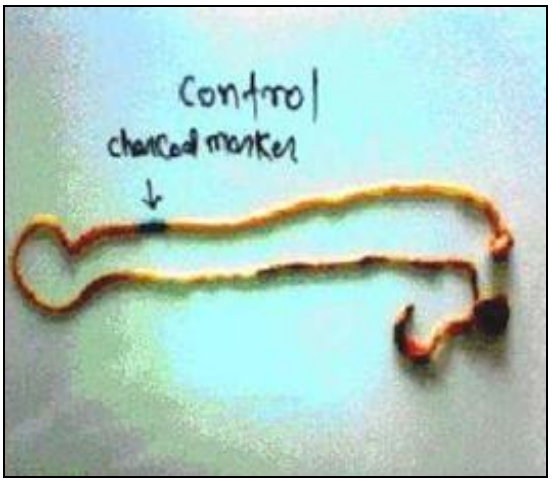


FIG. 1: PROPULSIVE GUT MOTILITY-CONTROL (0.5% CMC-1 ml/kg)

FIG. 2: PROPULSIVE GUT MOTILITY-STANDARD (CASTOR OIL TREATED 0.2 ml/mice)

FIG. 4: PROPULSIVE GUT MOTILITY –TEST (*SCUTIA MYRTINA* TREATED- 200 mg/kg)

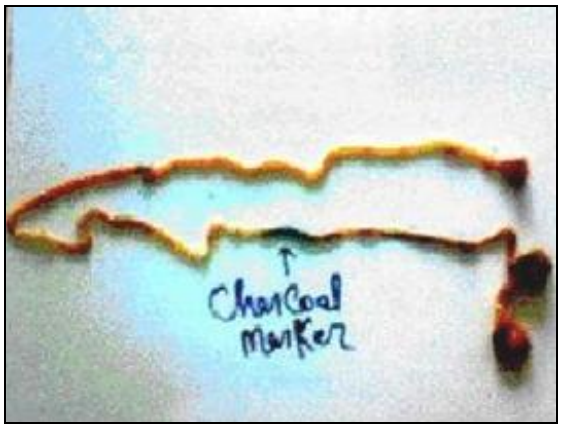


FIG. 4: PROPULSIVE GUT MOTILITY- TEST (*SCUTIA MYRTINA* TREATED- 400 mg/kg)

TABLE 3: LAXATIVE ACTIVITY IN SCUTIA *MYRTINA* TREATED ANIMAL MODEL

Treatment	Total number of Faeces	Total number of wet Faeces
Normal Transit (Normal Saline, 1 ml/kg)	14.17±0.75	4.5±0.22
Solvent control (0.5% CMC, 1 ml/kg)	17.67±0.88*	6.17±0.33**
Standard (Glaxenna, 1ml/rat)	24.17±1.0**	9.83±0.48**
Scutia myrtina (200 mg/kg)	21.34±1.0**	7.67±.0.34**
Scutia myrtina (400 mg/kg)	23.17±1.0**	8.67±0.34**

Values are mean ± SEM; No. of animals in each group = 6; * P value <0.05; ** P value <0.01 compared with the corresponding control

RESULTS:

Gut Motility Activity in Isolated Rat Intestine: Effect of *Scutia myrtina* on isolated rat intestine was studied (plate no.6) CRC of acetylcholine was recorded on isolated rat intestine. *Scutia myrtina* increases the CRC of acetylcholine on isolated rat intestine. It indicates that *Scutia myrtina* potentiates the CRC induced by acetylcholine on isolated rat intestine. *Scutia myrtina* potentiates the effect of acetylcholine on isolated rat intestine which indicated the contraction of rat intestine.

Propulsive Gut Motility in *Scutia myrtina* treated Animal Model: Results suggest that *Scutia myrtina* extract at the dose level of 200 mg/kg and 400 mg/kg produced a significant gut motility (P value <0.01), which is also evidenced by significant increase in % motility at the dose of 200 mg/kg and 400 mg/kg (54.34 and 67.84) respectively. The activity at both the doses levels were comparable and equipotent as that of castor oil treated group (P value <0.01).

Laxative Activity in Rat: Result suggests that *Scutia myrtina* extract at the dose level of 200 mg/kg and 400 mg/kg produce a significant laxative activity (P value <0.01), which is also evidenced by significant increase in laxative effect at the dose of 200 mg/kg and 400 mg/kg (7.67 and 8.67) respectively. The activity at both the dose levels were comparable and equipotent as that of Glaxenna treated group (P value <0.01).

DISCUSSION: The above results indicates that *Scutia myrtina* potentiates the effect of acetylcholine on isolated rat intestine which indicated the contraction of rat intestine. As well as it has significant Gut motility and thus Laxative activity, may be due to the presence of Anthraquinone Glycosides.

CONCLUSION: Finally from the study we can state that *Scutia Myrtina* has Significant Gut Motility activity which may be due to the presence of Anthraquinone Glycosides and can be further explored to give it a shape of Laxative Formulation.

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