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EVALUATION OF ANTI-DIARRHOEAL POTENTIAL OF LEAF EXTRACTS OF *OCIMUM SANCTUM* LINN. IN EXPETIMENTAL RATS

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Ocimum sanctum, anti-diarrhoeal potential, castor oil, Loperamide

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ABSTRACT: Diarrhoea is a condition that involves frequent passing of loose or watery stools. According to the WHO, approximately 3.5million deaths each year are attributable to diarrhoea. Medicinal plants have been used as traditional remedy for diarrhoea for years long and there is renewed interest from the discovery of novel compounds from plants to flight against diarrhoea. WHO also encourages studies on diarrhoea which include research on traditional herbs. The present study was designed to investigate antidiarrhoeal potential of leaf extracts of *Ocimum sanctum* (Lamiaceae. The present study is aimed to evaluate the leaf extracts of Ocimum sanctum (Lamiaceae) for acclaimed anti-diarrhoeal activity using albino rats. Anti-diarrhoeal activity of *Ocimum sanctum* was evaluated by castor oil-induced diarrhoea in rats. Loperamide was used as a standard drug. The study revealed that, the alcoholic extract and aqueous extract of *Ocimum sanctum* possessed significant antidiarrhoeal activity in castor oil-induced diarrhoea, compared to the control group. *Ocimum sanctum* showed significant anti-diarrhoeal activity as compared to loperamide and can be recommended for further studies.

INTRODUCTION: The medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice. In traditional systems of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum sanctum* Linn (known as Tulsi in Hindi), a small herb seen throughout India, have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc.

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The *Ocimum sanctum* Linn. has also been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions [1].

It is much branched small herb and 30 to 75 cm in height. All of tulsi parts are used in medicine, especially fresh and dried leaves. Leaves are oblong, acute with entire or serrate margin, pubescent on both sides and minutely gland-dotted. The leaves are green in colour with aromatic flavour and slightly pungent taste. Flowers are purplish in colour in the form of racemes. Nutlets are subglobose, slightly compressed, pale brown or red in colour. Seeds are reddish black and subglobose [2].

Diarrhoea is associated with an increased frequency of bowel movements with the production of soft or watery stools. It may be defined as the passage of more than 300ml of liquid faeces in 24 hours. This results in fluid and electrolytes loss that may lead ultimately to death, particularly in children [1]. Pain, urgency, perianal discomfort and incontinenence often accompany it. Low-volume, painful, bloody diarrhea is known as dysentery [3].

Since stool weight is largely determined by stool, most cases of diarrhoea result from disorders of intestinal water and electrolyte transport. From a mechanisteic perspective, diarrhoea can be caused by an increased osmotic load within the intestine (resulting in retention of water with in the lumen) excessive secretion of electrolytes and water into the intestinal lumen; exudation of protein and fluid from the mucosa, and altered intestinal motility, resulting in rapid transit. In most instances simultaneous effects of multiple processes are, leading to a net increase in stool volume and weight accompanied by changes in percent water content [4].

Diarrhoea may be due to a specific disease of the intestine or secondary to a disease outside the intestines. For instance, bacillary dysentery directly affects the gut, while diabetes mellitus causes a neuropathic diarrhoeal episode. Diarrhoea can be divided in to acute or chronic forms. Infectious diarrhea is often acute; diabetic diarrhoea has the pathophysiologic causes that help in the identification of specific treatments [5].

From the literature it was found that *Ocimum sanctum* has also been traditionally indicated for treatment of diarrhoea. Hence leaf extracts of this plant was selected for the study of anti-diarrhoeal activity in castor oil induced diarrhoea in experimental rats.

MATERIALS AND METHODS:

Plant material: Leaves of *Ocimum sanctum* collected in the month of February and were identified by Mr. K. Saravanan, Asso. Prof. and Head, Dept. of Pharmacognosy, Sanjay College of Pharmacy, Mathura and dried in shade at room temperature then subjected to size reduction to a fine powder with the help of mixer grinder.

Chemicals*:*
Loperamide is gift samples from Torrent Pharmaceuticals Ltd, Ahmedabad, India. Castor oil was purchased from Sigma fine chemicals, Mumbai, India.

Animals*:*
Albino rats (Wistar strain) of either sex weighing between 150-200 g and Albino mice 16-25g were used for the study. The animals were acclimatized for 7 days under standard husbandry condition. i.e.

Room temperature	-	26 ± 2^0 C
Relative humidity	-	45-55%
Light/ dark cycle	-	12:12 h

The animals were fed with a synthetic standard diet from Amrut Laboratories & Pranav Agro Industries Ltd. Sangli. Water was allowed *ad libitum* under strict hygienic conditions. All animal studies were performed in accordance to guidelines No. 425 of CPCSEA and Institutional Animal Ethical Committee (IAEC) of Sanjay College of Pharmacy, Mathura, Uttar Pradesh (India). CPCSEA registration number was 1334/01/10/CPCSEA and all the procedures were followed as per rules and regulations.

Preparation of extracts:
Preparation of alcoholic extract:
The leaves powder was packed in a soxhlet apparatus and extracted with 95% alcohol for 18 h. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50^{o}C to get alcoholic extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated [6].

Preparation of aqueous extract:
About 100 g of powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 7 days with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at 50^{o}C [6]. These two extracts were stored in airtight containers in a refrigerator below 10^{o}C. The two

extracts were examined for their colour and consistency. Their percentage yield was calculated with reference to air-dried powder sample used for the extraction.

Toxicity studies:

The acute toxicity of *O. sanctum* was determined by using albino mice of either sex (16-20 g), maintained under standard husbandry conditions. The animals were fasted for 3h prior to the experiment and were administered with single dose of individual extracts of *O. sanctum* and observed for the mortality up to 48 h study period (Short term toxicity). Based on the short-term toxicity profile, the next dose of the individual extracts was determined as per OECD guidelines No. 425. From the LD_{50} doses 1/20,1/10 and 1/5 doses were selected and considered as low, medium and high dose respectively [7].

Castor oil induced diarrhoea: [8, 9]

In the present study albino rats of either sex weighing 150-200 g were used. They were divided into 9 groups of each containing six animals. They were fasted for 18-24 hrs prior to the test with free access to water.

Group 1: Control (10 ml/kg of 5% w/v of gum acacia P.o)

Group 2: Toxicant (Castor oil 1ml/100g P.o).

Group 3: Standard (Loperamide 3 mg/kg P.o)

Group 4: AELOS (low dose, {100 mg/kg} p.o)

Group 5: AELOS (medium dose, {200 mg/kg} p.o)

Group 6: AELOS (high dose, {400 mg/kg} p.o)

Group 7: AQELOS (low dose, {100 mg/kg} p.o)

Group 8: AQELOS (medium dose, {200 mg/kg} p.o)

Group 9: AQELOS (high dose, {400 mg/kg} p.o

1 hour after the above treatment all the groups were received with castor oil (1ml/100g p.o). Each rat was then housed separately in cage over clean filter paper. Then diarrhoea was observed for a period of 4 h. During this period, number and wet weight of diarrhoeal dropping were noted. Using mean weight of stools, percentage of diarrhoea and percentage protection was calculated. Anti diarrhoeal activity was determined interms of percentage protection.

The percentage protection was calculated by the following formula:

$$\% \text{ Protection} = \frac{\text{Total weight of stool in control animals} - \text{Total weight of stool in drug treated animals}}{\text{Total weight of stool in control animals.}} \times 100$$

Stastical analysis:

All the recorded results are expressed as mean ± SEM from 6 animals. Statistical difference in mean was analyzed by using one-way ANOVA (analysis of variance) followed by Post hoc test (Dunnett's 't' test). P< 0.05*, 0.01** and 0.001*** were considered as statistically significant.

RESULT:

Alcoholic and aqueous extracts of leaves of *O.sanctum* were subjected for phytochemical screening and were found to contain sterols, flavanoids and triterpenes in alcoholic extract and triterpenes, flavonoids in aqueous extracts of *O.sanctum.*

When compared to normal control animals in castor oil induced diarrhoeal model animals treated with castor oil have shown with a significant increase in diarrhoea and faecal weight was significantly increased by 65.33% Standard drug (Loperamide) has offered significant protection against castor oil induced diarrhoea, and diarrhoea was inhibited by 57.64%.

The leaf extracts of *O.sanctum* at low, medium and high doses (100,200 and 400 mg/kg) too have reduced the castor oil induced diarrhoea. The percentage inhibition recorded with AELOS was found to be 27.39%, 31.04%, and 55.44% and with AQELOS was found to be 15.52%, 33.26% and 55.84% with low, medium and high doses respectively (**Table 1** & **Fig. 1**).

TABLE 1: ANTI DIARRHOEAL ACTIVITY OF LEAF EXTRACTS OF *O. SANCTUM* ON CASTOR OIL INDUCED DIARRHOEA IN RATS.

Groups	Treatment &dose	weight of stools after 4 h(g) mean ± SD	% change
1	Normal control (5% gum acacia 10 ml/kg)	5±0.7071	——
2	Toxicant control (Castor oil 1ml /100 g p.o)	8.266±0.911	65.33%
3	Standard (Loperamide 3 mg/kg p.o)	3.501±1.205**	57.64%
4	AELOS (100 mg/kg p.o)	7.02±0.54*	27.39%
5	AELOS (200 mg/kg p.o)	5.7±1.13 **	31.04%
6	AELOS (400 mg/kg p.o)	3.68±0.31**	55.44%
7	AQELOS (100 mg/kg p.o)	6.983±0.722*	15.52%
8	AQELOS (200mg/kg p.o)	5.51±0.61**	33.26%
9	AQELOS (400 mg/kg p.o)	3.65±0.37**	55.84%

AELOS- Alcoholic extract of leaves of *O. sanctum*
AQELOS -Aqueous extract of leaves of *O.sanctum*
n= 6 Significant at $P<0.05$*, $P<0.01$** and ns-not significant vs. control group.

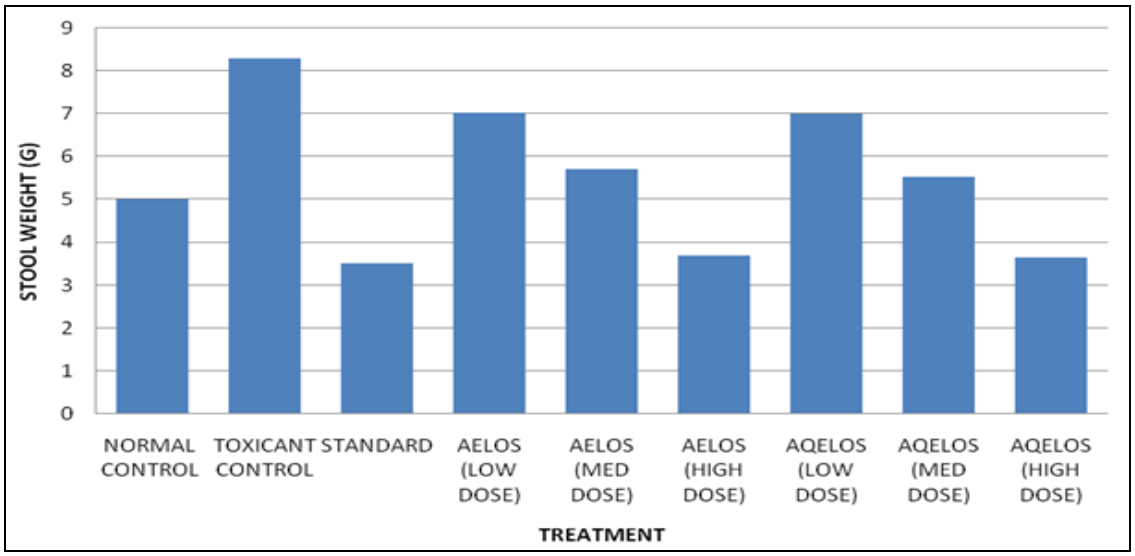

FIG.1: EFFECT OF AELOS AND AQELOS ON CASTER OIL INDUCED DIARROEA IN RATS

AELOS- Alcoholic extract of leaves of *O.sanctum*
AQELOS -Aqueous extract of leaves of *O.sanctum*

It is well known that ricinoleic acid an active component of castor oil induces changes in mucous permeability, electrolyte transport and intestinal peristalsis leading to hyper secretion of the intestinal mucosa, leading to prostaglandins release which causes an increase in net secretion of water and electrolytes in to the small intestine. Ricinoleic acid causes irritation and inflammation gastric mucosa. The mechanism has been associated with dual effects on gastro intestinal motility as well as on water and electrolyte transport.

PGE2 also inhibits the absorbtion of water and electrolytes. The presence of flavonoids and tannins already reported for their antidiarrhoeal activity has been proven in this experiment. The antidiarrhoeal activity of flavonoids has been ascribed to their ability to intestinal motility and hydroelectric secretion which is known to be altered in intestinal condition. *In-vitro* and *in-vivo* experiments shown the flavonoids are able to inhibit intestinal secretary response induced by prostaglandin E2 [10].

DISCUSSION: The leaf extracts of *Ocimum sanctum* have been reported to possess anti tussive, hypoglycemic, antioxidant, antistress, antiulcer, anti-inflammatory, antiasthmatic, immune stimulatory and neuroprotective effects and for regulation of thyroid function. During acute

toxicity studies the extracts (alcoholic and aqueous) of *O.sanctum* were found non toxic and they did not induced any toxic effect / or mortality even up to the dose level of 2000 mg/kg. Phytochemical studies with these leaf extracts revealed the presence of sterols, flavonoids and triterpenes in both the extracts [11, 12].

Diarrhoea is one of the leading causes of death in developing countries. The inhibition of experimental diarrhoea and the reduction in faecal output by a substance are the basis of the pharmacological evaluation of a potential antidiarrhoeal agent. Many antidiarrhoeals act by reducing the gastrointestinal motility and or the secretions. It is well known that ricinoleic acid, an active component of castor oil, induces changes in mucosal permeability, electrolyte transport and intestinal peristalsis, leading to hyper secretory response and diarrhoea. Ricinoleic acid causes irritation and inflammation of the intestinal mucosa, leading to prostaglandin release, which causes an increase in the net secretion of water and electrolytes into the small intestine. Inhibitors of prostaglandin biosynthesis delay castor oil induced diarrhoea. It has been shown that PG-E type causes diarrhoea in experimental animals as well as in human beings. The mechanism has been associated with duel effects on gastrointestinal motility as well as on water and electrolyte transport. PG-E_2 also inhibits the absorption of glucose, a major stimulus to the intestinal absorption of water and electrolytes.

The antidiarrhoeal activity of the alcoholic and aqueous extracts was comparable to the standard drugs Loperamide and Atropine. The antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion, which are known to be altered in this intestinal condition. *In vitro* and *in-vivo* experiments have shown that flavonoids are able to inhibit the intestinal secretary response induced by PG-E_2. In addition, flavonoids possess antioxidant properties, which are presumed to be responsible for the inhibitory effects exerted upon several enzymes, including those involved in the arachidonic acid metabolism. The activity might be due to sterols, flavonoids and triterpenes present in these extracts which could have contributed to the antidiarrhoeal activity as these are already reported for their antidiarrhoeal activity [13, 14].

CONCLUSION: The preliminary phytochemical analysis of the AELOS and AQELOS revealed the presence of sterols, flavanoids and triterpenes. From the studies it can be concluded that AELOS and AQELOS showed a significant anti-diarrhoeal effect against castor oil induced diarrhoea. The medium and higher doses of AELOS and AQELOS (200 and 400 mg/kg) treated groups showed better anti-diarrhoeal activity when compared to standard drug loperamide (3 mg/kg p.o) treated group.

The leaf extract contains sterols, flavanoids and triterpenes which may be able to have contribution to the anti diarrhoeal activity. Further research needed to identify the specific constituents responsible for this activity and it may lead to a new hope against this world wide problem.

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CONFLICT OF INTEREST: There is no conflict of interest.

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