Abstract

World Health Organisation defines diarrhoea as the “passage of loose or watery stools at least three times in a 24 hours period”. Diarrhoea involves both an increase in the motility of the gastrointestinal tract, along with increased secretion, and a decrease in the absorption of fluid and thus a loss of electrolytes and water. The rapid movement of faeces through the intestine results in abnormally frequent and watery stools. Absorption of water, nutritive elements, and electrolytes is decreased and the patient usually complains of abdominal cramps and generalized weakness.

Diarrhoea is a commonly occurring disorder, which needs a prompt treatment to avoid complications arising from loss of body water. Several antidiarrhoeals are available in both modern and traditional medicines. Synthetic antidiarrhoeals used in modern medicines however show adverse effects like paralytic ileus, nausea, vomiting, abdominal cramps. Their chronic use as in ulcerative colitis leads to the risk of physical dependence. Larger doses of synthetic modern drugs have been reported to cause CNS side effects. Despite the availability of vast spectrum of approaches for diarrhoeal management, a vast majority of the people in developing countries rely on herbal drugs for the management of diarrhoea. WHO has encouraged studies for the treatment and prevention of diarrhoeal diseases using traditional medical practices.

Considering the significance of herbal formulations in diarrhoea over allopathic drugs which are having side effects, antidiarrhoeal activity and phytochemical screening of herbal formulations like Mebarid, Enterocin, Kutajarishta and crude drug like Black pepper was performed with the following objectives:

i) To study the antidiarrhoeal activity of herbal formulations like Mebarid, Enterocin, Kutajarishta and Black pepper and its comparison with standard drug.

ii) To study the possible mechanisms of action of antidiarrhoeal activity of selected herbal formulations and Black pepper.

iii) To evaluate the possibility of application of pharmacological methods in the biostandardisation of the herbal antidiarrhoeal formulations.

iv) To study the effect of Black pepper on antidiarrhoeal activity of Mebarid, Enterocin and Kutajarishta.
v) To perform the phytochemical analysis of Mebarid, Enterocin, Kutajarishta and Black pepper.

vi) To isolate the Piperine from Black pepper and characterise it.

vii) To perform the antidiarrhoeal activity of Piperine.

To achieve the objectives of the study, Mebarid, Enterocin, Kutajarishta, Black pepper and Piperine were studied for acute oral toxicity as per revised OECD guidelines number 423. Antidiarrhoeal effect of Mebarid, Enterocin, Kutajarishta, Black pepper and Piperine was evaluated in castor oil and magnesium sulphate induced diarrhoea in mice. Effect of Mebarid, Enterocin, Kutajarishta, Black pepper and Piperine was studied on intestinal propulsive movement and intestinal fluid accumulation in mice. Effect of Black pepper on antidiarrhoeal activity of Mebarid, Enterocin, and Kutajarishta on castor oil and magnesium sulphate induced diarrhoea, castor oil induced gastrointestinal motility and small intestinal secretions were studied.

Additionally, effect of Glibenclamide, Isosorbide dinitrate and Yohimbine on antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and Black pepper on castor oil induced diarrhoea in mice was studied. Effect of Mebarid, Enterocin, Kutajarishta and Black pepper on stimulant effect of Acetyl choline, Nicotine, Histamine and Calcium in isolated guinea pig ileum was also studied. To check the presence or absence of primary and secondary metabolites, all the formulations and Black pepper were subjected to battery of chemical tests.

In present study the results showed that Mebarid, Enterocin, Kutajarishta, Black pepper and Piperine successfully inhibited the castor oil and magnesium sulphate induced diarrhoea, indicating its antidiarrhoeal action by antisecretory mechanism and due to increase in the absorption of water and electrolyte from the gastrointestinal tract. It was also supported by inhibitory effect of Mebarid, Enterocin, Kutajarishta, Black pepper and Piperine on intestinal motility and intraluminal fluid accumulation induced by castor oil.

Black pepper increased the antidiarrhoeal effect of Mebarid, Enterocin and Kutajarishta in castor oil and magnesium sulphate induced diarrhea in mice. Black pepper also enhanced the inhibitory effect of Mebarid, Enterocin and Kutajarishta on castor oil induced motility and intestinal secretions in mice.
Potassium channels, nitric oxide pathway and $\alpha_2$ adrenergic receptors plays important role in the diarrhoea. Glibenclamide (potassium channel blocker), Isosorbide dinitrate (nitric oxide donor) has reduced the antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and Black pepper while antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and Black pepper was not influenced by Yohimbine ($\alpha_2$ adrenergic receptor blocker). The results indicated that Mebarid, Enterocin, Kutajarishta and Black pepper produced the antidiarrhoeal effect through potassium channels and nitric oxide pathway while no effect was seen on $\alpha_2$ adrenergic receptors.

Mebarid produced relaxation of guinea pig ileum. Mebarid decreased stimulant effect of acetyl choline, nicotine, histamine and calcium on isolated guinea pig ileum. Enterocin produced relaxation of guinea pig ileum. Stimulant effect induced by acetyl choline on isolated guinea pig ileum was not significantly changed by Enterocin. But Enterocin decreased stimulant effect of nicotine, histamine and calcium on isolated guinea pig ileum. Kutajarishta produced relaxation of guinea pig ileum. Stimulant effect of acetyl choline and nicotine on isolated guinea pig ileum was not significantly changed by Kutajarishta. But Kutajarishta decreased stimulant effect induced by histamine and calcium on isolated guinea pig ileum. Black pepper produced relaxation of guinea pig ileum. Stimulant effect of acetyl choline, nicotine, histamine, and calcium on isolated guinea pig ileum was not significantly changed by Black pepper.

Phytochemical study of Mebarid and Enterocin showed the presence of carbohydrates, steroids, triterpenoids, alkaloids, flavonoids and tannins, as major constituents while Kutajarishta and Black pepper showed the presence of carbohydrates and alkaloids as major constituents. Piperine isolated from ethanolic extract of Black pepper was confirmed by its colour, melting point, UV absorbance, Rf value of crude extract and chemical test.

The investigation revealed that Mebarid, Enterocin, Kutajarishta and Black pepper produced antidiarrhoeal effect through its antisecretry and antimotility effect. The experimental models used in the present work for the antidiarrhoeal study can be used for the biostandardisation of Mebarid, Enterocin, Kutajarishta and Black pepper. Due to the simplicity and reproducibility of these methods, it can be used for the biostandardisation of different antidiarrhoeal herbal formulations and crude drugs where other methods of standardization cannot be used.
Antidiarrhoeal effect of Mebarid may be through potassium channels, nitric oxide pathway, muscarinic receptors, ganglionic receptors, histamine receptors and calcium channels but not through the $\alpha_2$ adrenergic receptors. Antidiarrhoeal effect of Enterocin may be via potassium channels, nitric oxide pathway, ganglionic receptors, histamine receptors and calcium channels but not through the muscarinic receptors and $\alpha_2$ adrenergic receptors. Kutajarishta produced antidiarrhoeal effect which may be via potassium channels, nitric oxide pathway, histamine receptors and calcium channels but not through the muscarinic receptors, ganglionic receptors and $\alpha_2$ adrenergic receptors. Black pepper produced antidiarrhoeal effect may be through potassium channels and nitric oxide pathway but not through the muscarinic receptors, ganglionic receptors, histamine receptors, $\alpha_2$ adrenergic receptor and calcium channel. Thus, the mechanism of action of antidiarrhoeal effect of Mebarid, Enterocin, Kutajarishta and Black pepper showed that it is mediated by multiple pathways.

The results also indicated the additive effect of Black pepper on the antidiarrhoeal effect of Mebarid, Enterocin, and Kutajarishta. Thus indicating the usefulness of Black pepper as the active constituent of antidiarrhoeal herbal formulations. The additive effect produced by Black pepper may be due to its inherent antidiarrhoeal activity and bio-enhancing effect.

Antidiarrhoeal effect of Mebarid, Enterocin, Kutajarishta and Black pepper may be due to presence of different phytochemicals such as alkaloids, tannins, flavonoids and terpenoids. Antidiarrhoeal activity of Black pepper is attributed to the Piperine which is the active constituent of Black pepper. As Piperine produced the significant antidiarrhoeal activity it may be used as a structural template to develop new antidiarrhoeal agents.