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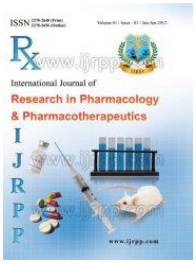

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Antidiarrhoeal and antibacterial effects of areca nut and lime combination in experimental animals

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ABSTRACT

The work was designed to evaluate the anti-diarrheal effects of aqueous extract of areca nut in combination with lime against castor oil induced diarrhea model and also to evaluate the anti-bacterial effect by disc diffusion method.

Keywords: Areca catechu, lime, Anti diarrheal effect, Anti bacterial effect.

INTRODUCTION

Diarrhea is one of the leading death-causing diseases, especially in developing countries, so this is the most concerning issue for these countries. In view of this, WHO has initiated a Diarrheal Disease Control Program to study traditional medical practices and other related aspects [1].

Children's are more prone to diarrhea, which is the second leading cause of death in children under 5 years of age [2]. The causative agents of diarrhea in humans are *Shigellaflexneri, Staphylococcus aureus, E.coli, Salmonella typhii, and Candid albicans*[3, 4].Diarrhea is a GIT dysfunction, which is considered as a common symptom of infection and one of the most important cause of intestinal motility disorder [5].

Ancient time on wards medicinal plants played an invaluable role in the development of invaluable therapeutic agents. There are many medicinal plants that possess anti diarrheal activity with lesser side effects than the conventional drugs. Tannins, alkaloids, flavonoids and terpenoids are the major constituents that are primarily responsible for anti diarrheal activity of this medicinal herbs [6].

Diarrhea causes high motility, which causes an increase in the rate of movement of intestinal contents. Castor oil is known to induce GIT enter pooling which is seen in same as that of diarrhea. The effect of castor oil is mediated by recinolic acid, which can cause a hyper secretory response in the gut, leading to diarrhoea [7]. For this study aqueous extract of areca nut and lime combination were selected and tested for anti diarrheal activity and anti bacterial activity.

MATERIALS AND METODS

Preperation of Areca Nut Extract

Areca nut was collected during June 2015 from Kerala (Kannur). The plant material was identified and authentified by Dr.Biju P. The aqueous extract of

areca nut was prepared by boiling 100g nut in 500ml of distilled water for 1 hour. The extract was concentrated by evaporation[8]. The extract was then suspended in normal saline and diluted to desired concentration.

Preperation of Lime Extract

The *Citrus limon* were collected during June 2015 from Karnataka. The fruit was taxonomically identified and authentified by Dr.Biju P. The fresh juice was prepared by peeling its outer covering and the remaining inner portions were squeezed. The juice obtained was filtered and the filtrate is used.

ANIMALS

Experimental rats of either sex weighing 150-250g were housed at 25.5° C, relative humidity 50.5% in a well–ventilated animal house under 12:12h light dark cycle. All the rats were provided with commercially available standard pellet diet, water and libitum. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of (CPCSEA). The institutional ethical committee approved the experimental protocol (SDCP/IAEC-06/2014-2015).

ACUTE TOXICITYSTUDIES

The dose selection of Areca nut extract (ANE) and lime juice combination were based on acute toxicity studies, carried out according to OPPTS (Office of Prevention, Pesticide and Toxic Substance) following the limit test procedure. The animals were fasted over night prior to the studies. Mice were divided into two groups of three each. Test dose of 2 g/kg body weight and 5g/kg body weight were given orally to either group of mice. Mice were observed for 72 hours for mortality. $1/10^{th}$ and $1/50^{th}$ of the maximum safe dose corresponding to 500 and 100 mg/kg body weight were selected as high and low doses respectively.

ANTIDIARRHOEAL ACTIVITY

Faecal excretionrate[9]

The Albino rats of either sex will be divided in to 4 groups of 6 animals (n=6)

- Group I –rats will be treated as normal control without any drug treatment.
- Group II- animals will be treated with Loperamide (2mg/kg orally).
- Group III (AH500+LH500) – High dose of areca nut extract + lime. (Dose as per acute toxicity study)
- Group IV (AL100+LL100) - Low dose of areca nut extract + lime. (Dose as per acute toxicity study)

Food will be withdrawn from cage 3 h before commencement of experiment. The pellets discharged by the rats at 1^{st}, 3^{rd},5^{th},and the 7^{th} hour after treatment are collected and weighed immediately followed by taking its dry weight (after24h,at50^0C)further wet to dry ratio of pellets discharged 7h after treatment as calculated to assess the effect of extract on absorption of fluids.

Castor - oil induceddiarrheoa[10]

Albino rats of either sex will be divided into 5 groups (n= 6) and fasted for 18 h

- Group I (Normal control) - animals kept as normal control without any drug treatment.
- Group II (Toxic control) - animals kept as toxic control without any drug treatment.
- Group III (standard) - Animals treated with Loperamide (2mg/kg orally).
- Group IV (AH500+LH500) – High dose of areca nut extract + lime. (Dose as per acute toxicity study)
- Group V (AL100+LL100) - Low dose of areca nut extract + lime. (Dose as per acute toxicity study)

One hour after the above treatment, the rats from group II to V will be administered 1ml of castor oil orally by gavages. The animals will be transferred into cages containing plastic sheets at the base and then will be kept for observation of different parameters up to 4 hour with a change of sheets after every hour. Weight of plastic sheet before and after defecation will be noted and compared to control.

Castor oil induced intestinal fluid accumulation [11]

Rat will be grouped in the same way as in the castor oil induced diarrhea. After 30 min of above

treatment the rats will be given orally1 ml of castor oil through gavages. Later 30min after castor oil administration, all rats will be sacrificed by cervical dislocation and small intestine is dissected from the pylorus to caecum and the volume of its content will be measured. The intestinal fluid was analyzed for Na^+ and K^+ Concentration using semiautoanalyzer.

Castor oil induced gastrointestinal transit test using charcoalmeal[12]

The adult rats will be fasted for 18 hours and grouping is done in the similar manner as in the castor oil induced diarrhea with only a difference that in Group III in place of Loperamide; Atropine sulphate with a dose of 5mg/kg orally will be administered. After 30min of the above treatment the animals will be administered 1ml of castor oil orally by gavages. Followed by this, 1ml of 5% deactivated charcoal suspended in10% in aqueous Tragacanth gum will be given to rats 30 min after the castor oil administration. After 30 min of charcoal meal administration rats will be sacrificed by cervical dislocation, the abdomen will cut, opened and the small intestine is carefully removed and the distance travelled by the charcoal plug from the pylorus is measured.

ANTI BACTERIAL STUDY

Disc diffusion method[13]

The study is performed on reference bacterial strains i.e. *Escherchia coli, Pseudomonas arugenosa, and Staphylococcus aureus.* Muller Hinston Agar (MHA) plates will be used as a nutrient medium. The suspension of the bacterial strains will be spread on the surface of MHA agar plates which will be then allowed to dry for 5min.The extract to be tested will be then applied on 6mm sterile disc on Whatermann's filter paper no I in different concentration. The disc will be placed on the surface of the nutrient medium and the extract allowed to diffuse for 5 min. These plates are then incubated for 24hr at 37^oC and inhibition zones around the disc will be examined triplicate. For determining MIC the extract will be first diluted with equal volume of nutrient broth which is further mixed in wells of microtitre plate/Briefly .1ml of standardized inoculumswillbeaddedineachtubeandtheplatesareincubatedaerobicallyat37^0C. For 18-24h the minimum concentration showing no visible bacterial growth will be considered as MIC.

RESULT AND DISCUSSION

The result of the present study show that the (AH+LH) & (AL+LL) have anti-diarrheal effect established by decrease in total number and total weight of faeces including weight of wet feaces, decreased diarrhea score, decreased weight and volume of intestinal content and decreased Na^+ and K^+ concentration of intestinal fluid, decreased % intestinal transit characterized by the decreased distanced travelled by charcoal plug (marker).

In the presence of lime, arecoline and guvacoline the major alkaloids present in Areca nut are hydrolyzed into arecaidine and guvacine, respectively, which are strong inhibitors of GABA uptake level which decreases the intestinal motility which gives an anti-diarrhoeal effect.

Th anti-bacterial study is carried out by disc diffusion method. The zone of inhibition and MIC of the areca nut in combination with lime extract both AL+LL (100+100) & AH+LH (500+500) were compared with standard drug ciprofloxacin is found to be potential anti-bacterial effect.

CONCLUSION

Thus investigational finding conclude that areca nut in combination with lime juice possess potential benefits in treating animals with diarrhea induced by castor oil and having anti-bacterial effect.

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