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Effects of Digesti'Vas, Apolyherbal Formulation on Gastrointestinal-Related Measurements in Rodent Models

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Abstract: The present study evaluated the activity of Digesti'Vas on gastrointestinal motility, appetite and hepatoprotective effects in a rodent model. Digesti'Vas is a polyherbal formulation developed by Suguna Foods Pvt Ltd. Acute toxicity and gastrointestinal motility activity were evaluated in mice. Appetite activity and potential hepatoprotective effects were evaluated in rats. Since, Digesti'Vas did not exhibit acute toxicity when given orally up to a concentration of 2000 mg kg^{-1} bw, 200 m kg^{-1} body wt. (1/10th of 2000 mg kg^{-1}) was selected as the median dose. Digesti'Vas showed a significant increase in mean defecation period (346.2±65.83 min), comparable to the positive control, loperamide (383.0±37.83 min) in comparison to the normal control group (251.2±60.32 min). Digesti'Vas treatment also led to a significant increase in the percentage change in body weight (8.367±3.021 g) when compared to the normal control group (3.618±2.088 g) and a significant increase in percentage change in food intake (13.28±3.673 g) when compared to the normal control group (8.657±2.160 g). When measurements were done on various liver parameters, it was found that Digesti'Vas treated animals showed a significant decrease in serum ALP levels (401.8±10.04 U L^{-1}) compared to CCl_4 treated animals (493.2±108.4 U L^{-1}); comparable to the positive control, Silymarin (370.5±37.82 U L^{-1}). Digesti'vas significantly decreased serum SGPT levels (57.03±66.01 U L^{-1}), compared to CCl_4 treated animals (420.8±214.1 U L^{-1}); in agreement with levels seen in Silymarin treated animals (61.39±37.87 U L^{-1}). Furthermore, Digesti'Vas significantly decreased SGOT (6.953±3.712 U L^{-1}) levels compared to the CCl_4

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treated group (29.65±5.765 U L^{-1}), in keeping with levels seen after treatment with Silymarin (10.71±6.135 U L^{-1}). This study suggests that Digesti'Vas, a herbal preparation that is formulated by Suguna Foods Pvt Ltd., possesses antimotility, hepatoprotective and increased appetite effects in a rodent model. Future studies investing the role of Digesti'Vas in alleviating gastrointestinal disorders are certainly warranted.

INTRODUCTION

Gastrointestinal (GI) diseases are common public health issues worldwide and gut motility disorders including indigestion and constipation are considered to be major causes of ill-health. Indigestion (dyspepsia) is a condition of impaired digestion. The symptoms of indigestion includes upper abdominal fullness, heartburn, nausea, belching (release of gas from the esophagus and stomach through mouth) and upper abdominal pain. Indigestion is usually caused by gastro esophageal reflux disease or gastritis. It is also due to peptic ulcer disease and cancer[1].

Various drugs are used to treat indigestion and other GI disorders. Some of them include, mepyramine, cimetidine, thioperamide, ranitidine, nizatidine, famotidine, lansoprazole, pantoprazole, rabeprazole, omeprazole, magnesium trisilicate, aluminium hydroxide, alginates, fluoroquinolone antibiotics, theophylline, tetracycline, digoxin and amitriptyline[2]. However, these therapeutics are not without adverse effects.

In addition, many medication used for other illnesses are also known to cause indigestion and other gastrointestinal distress. Drugs such as aspirin, non-steroidal anti-inflammatory drugs, antibiotics (metronidazole, macrolides), antidiabetic drugs (metformin), antihypertensive medications (losartan), cholesterol lowering agents (clofibrate), antidepressant drugs (fluoxetine), Parkinson drugs (levodopa), corticosteroids, estrogens, digoxin, etc. are known to cause some form of GI distress.

It has been recommended that lifestyle changes and dietary modifications may reduce some of these problems. For example, eating smaller more frequent meals are preferable to large meals as reducing the amount of food in the stomach reduces gastric distension, thus, reducing reflux. Weight reduction is advised if the patient is overweight. Smoking and alcohol intake should also be reduced[3].

GI motility is the movement of the digestive system and the transit of the contents within it. Neurohormonal mechanisms, pathogens, malnutrition, chronic diseases and drugs can alter gastrointestinal physiology resulting in changes in either secretion or absorption of fluid by the intestinal epithelium. Altered motility contributes in a general way to this process as the extent of absorption by and large, parallels transit time. Prokinetic agents, organophosphate pesticides, nerve gases, surgery, irritation bowel syndrome, collagen vascular disease and diabetes are some of the pathophysiological conditions that may alter intestinal motility and transit time. Antimotility compounds such as diphenoxylate, loperamide, opium alkaloids, anticholinergics, etc. have been used against diarrheal disorders but have exhibited side effects after prolonged use[4].

Acetylcholine, the vagal neurotransmitter, enhances while atropine, a known anticholinergic agent, decreases intestinal motility and secretion. Although, various derivatives and congeners of atropine (such as propantheline, isopropamide and glycopyrrolate) have been advocated in patients with peptic ulcer or with non-specific diarrhoea, the prolonged use of such agents is limited by other manifestations of parasympathetic inhibition such as dry mouth and urinary retention[5]. Thus, there is a need to identify new compounds and evaluate their antimotility activity in order to develop selective inhibitors that not only decrease gastric secretion and intestinal motility but also show minimal anticholinergic and other adverse side effects[6].

The liver is the key organ regulating homeostasis in the body. It is involved in almost all the biochemical pathways related to growth, nutrient supply, energy provision and fight against disease. The liver is expected to not only perform physiological functions but also protect against the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field in recent years, liver problems continue to be on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate. Presently, a few hepatoprotective drugs are available for the treatment of liver disorders.

The use of plants as sources of medicines are human substance has been in vogue since antiquity. According to a survey of World Health Organization (WHO), the practitioners of traditional system of medicine treat about 80% of patients in India, 85% in Myanmar and 90% in Bangladesh. Thus, there is great interest in traditional systems of medicine for remedies of gastric and hepatic disorders[7]. The present investigation was designed to study the effects of Digesti'Vas, a polyherbal formulation on digestion, appetite, weight, GI motility and liver functions in rodent models.

MATERIALS AND METHODS

Chemicals and drugs: Standard drugs such as loperamide and silymarin were procured from the

medical store. Digesti'Vas, a polyherbal formulation developed by Suguna Foods Pvt. Ltd. was provided to us.

Biochemical kits: The biochemical kits used for various measurements were purchased from Anjan distributors, authorized supplier of ERBA Diagnostics Mannheim.

Experimental animals: Swiss Albino mice, weighing 20-30 g and Sprague Dawley rats, weighing 150-200 g were used in these studies. All the animals were procured from Adita Biosys Pvt Ltd., Tumkur, CPCSEA Registration No: 1868/PO/Bt/S/16/CPCSEA (with health certificate of the animals) and were maintained under controlled condition of temperature (23±2°C), humidity (50±5%) and 12 h light and dark cycles. The animals were randomized into experimental and control groups and housed in sanitized polypropylene cage containing sterile paddy husk as bedding. They had free access to standard food pellets and water. Assignment of animals to experimental and control groups was made in such a way that each group had mean and total body weights similar to the other groups. The acute oral toxicity study was carried out according to the guidelines established by OECD. An ethical clearance was obtained from the Institutional Animal Ethics Committee (PESCP/IAEC/33/2016) and the study was conducted according to the guidelines of CPCSEA, New Delhi.

Acute oral toxicity study: Swiss albino mice weighing between 20-30 g were used for the acute toxicity study. The purpose of this study was to determine the LD50 of Digesti'Vas. Based on the results of this study, the median dose was selected and used for the remainder of the experiments[8].

Prior to dosing, animals were fasted overnight, weighed and the dose calculated according to the body weight. Single animals were dosed in sequence usually at 48 h intervals. Using the default progression factor, doses were selected from the sequence 1.75, 5.5, 17.5, 55, 175, 550 and 2000 mg. As no estimate of Digesti'Vas's lethality is available, the dosing range chosen was between 175 and 2000 mg kg^{-1}. The LD50 was calculated based on observations of physical and behavioural changes that were made for 14 days following the administration of the highest dose (2000 mg kg^{-1} body weight). The results indicated that mortality was not observed at 175, 550 and 2000 mg kg^{-1} body weight doses. Given that Digesti'Vas did not exhibit acute toxicity when given orally at a concentration of 2000 mg kg^{-1} body weight, 200 mg kg^{-1} body weight (1/10th of 2000 mg kg^{-1}) was selected as the median dose.

Assessment of GI motile activity in mice: Eighteen mice weighing between 20-30 g were divided into three groups comprising of six mice per group. All mice were weighed and food deprived with free access to water. Three hours after food deprivation, Group 1 received normal saline 10 mL kg^{-1} orally (control group). Group 2 received loperamide 5 mg kg^{-1} orally (positive control group). Group 3 received Digesti'Vas 200 mg kg^{-1} orally (test group)[9, 10].

After 90 min, 0.3 mL of an aqueous suspension of 5% charcoal was administered to each animal, orally. About 60 min later, they were given free access to food. The animals were observed at 5 min intervals until faeces with charcoal was eliminated (maximum time of observation was 450 min). Charcoal was observed in the faeces using normal light when it was easily visible or using a microscope to help the identification of the black spots. The results were based on the time of charcoal elimination in the faeces.

Assessment of appetite activity in rats: The rats were divided into two groups containing six rats in each group. Prior to the start of the experiment all the rats were housed individually in stainless steel mesh cages with individual food cups for weighed diets and they were housed in light controlled room (12 h light/dark cycles) with free access to drinking water. All the animals were maintained on a control diet for 7 days as an acclimatization period and then regrouped based on their feeding pattern. Group 1 animals were fed a normal pellet diet (control group). Group 2 received 200 mg kg^{-1} of Digesti'Vas orally along with a normal pellet diet (test group). The treatment was carried out for a period of 15 days[11].

The body weights of the rats was measured before and after the experiment. The average food intake per day was measured over a period of 15 days.

Assessment of protective effect by Carbon tetrachloride (CCl_4) induced hepatotoxicity in rats[12,13]: The polyherbal drug, Digesti'Vas was tested for its effects on liver function. CCl_4 has been used previously by others as well as our lab to induce hepatotoxicity. CCl_4 causes liver damage by forming covalent binding as well as oxidative damage. Silymarin has been used as a standard hepatoprotective drug. Silymarin contains at least seven flavoligands and the flavonoid taxifolin. The hepatoprotective and antioxidant activity of silymarin is caused by its ability to inhibit the free radicals that are produced from the metabolism of CCL_4.

For this experiment, rats were divided into four groups containing six rats in each group. Rats in group 1 (control group) received normal saline (1 mL kg^{-1}) orally. Rats in group 2 (hepatotoxic group) were administered CCl_4 in olive oil (30% v/v) 1 mL kg^{-1} i.p. Rats in group 3 (test group) received Digesti'Vas 200 mg kg^{-1} orally. Rats in group 4 (positive control) were treated with

the standard drug, silymarin 100 mg kg^{-1} orally. Groups 3 and 4 received the drug treatment along with CCl_4 in olive oil (30% v/v) 1 mL kg^{-1}. CCl_4 in olive oil was administered every 72 h. The treatment was carried out for a period of 10 days.

Serum parameters: The blood was collected, incubated in an upright position for a period of 30-45 min to allow clotting and centrifuged using a cold centrifuge for a period of 15 min at 1000-2000 rpm. Using a clean pipette, the supernatant serum was aspirated and poured into another tube and examined for serum parameters such as SGOT, SGPT and ALP. Serum ALP measurements are of particular interest in the investigation of hepatobiliary disease. Increased levels of SGOT (AST) are associated with liver diseases or damage. Increase in (ALT) SGPT levels is found to be greater in hepatocellular diseases compared to AST. Thus, this study measured the effects of Digesti'Vas on these enzymes to determine whether it would show hepatotoxic or hepatoprotective effects.

Estimation of aspartate aminotransferase (AST) (SGOT): SGOT catalyzes the reversible transfer of α-amino group between aspartate and glutamate. It is an important enzyme in amino acid metabolism. SGOT is found in the liver, heart, skeletal muscle, kidneys, brain and RBC's. AST levels increase in liver diseases, myocardial infarction, muscular dystrophy and cholecystitis whereas the levels decrease in patients undergoing renal dialysis and those with vitamin B_6 deficiency.

Estimation of Alanine aminotransferase (ALT) (SGPT): Normally, ALT is found inside the liver cells. However, if the liver is inflamed or injured, ALT is released into the bloodstream. Measuring blood levels of ALT provides information about the health of the liver cells. To a lesser extent, ALP is also present in the kidney, heart, skeletal muscle, pancreas, spleen and lungs. ALP levels increase in liver diseases such as cirrhosis, carcinoma viral or toxicity hepatitis and obstructive jaundice and decrease in renal dialysis patients and those with vitamin B_6 deficiency.

Estimation of Alkaline Phosphatase (ALP): ALP is present in high concentrations in liver, bone, placenta, intestine and certain tumors. Physiologically elevated serum alkaline phosphatase occurs in pregnant women and in children. Increased levels of the enzyme also occur in liver diseases, bone diseases (rickets, Paget's disease), Hodgkin's disease and congestive heart failure. Decreased levels occur in hypophosphatasia and malnourished patients.

Alkaline Phosphatase (ALP) catalyzes the conversion of alkaline hydrolysis of a large variety of naturally occurring and synthetic substrates. ALP activity is present in most organs of the body and is especially associated with membranes and cell surfaces located in the mucosa of the small intestine and proximal convoluted tubules of the kidney, in bones (osteoblasts), liver and placenta. ALP exists in multiple forms, some of which are true isoenzymes, encoded at separate genetic loci.

Statistical methods: All data are expressed as the standard error of the mean (SE+mean). Comparisons among the control and treatment groups were made using Analysis of Variance (ANOVA) followed by a Bonferroni method of statistics using the graph pad prism statistical program. With all analyses, an associated probability (p-value) of <5% ($p<0.05$) was considered significant.

RESULTS AND DISCUSSION

The results of the present investigation indicate that Digesti'Vas possesses several properties that would make it a useful agent for GI disorders. For example, rats treated with Digesti'Vas exhibited a significant increase in mean defecation period (346.2±65.83 min) compared to normal control group (251.2±60.32 min) in a similar manner to the positive control drug, loperamide (mean defecation period, 383.0±37.83 min). Loperamide is an opioid-receptor agonist and acts on the μ-opioid receptors to decrease the activity of the myenteric plexus of the large intestine. This increases the amount of time food stays in the intestine, leading to its antimotility effect. Although, the mechanism by which Digesti'Vas exerts its effects are not known at this time, the results of our study suggest a significant antimotility effect of this herbal formulation.

When measurements were done on various liver parameters, it was found that Digesti'Vas treated animals showed a significant decrease in serum ALP levels (401.8±10.04 U L^{-1}) compared to CCl_4 treated animals (493.2±108.4 U L^{-1}). Rats treated with Silymarin showed levels to be (370.5±37.82 U L^{-1}). Similarly, Digesti'Vas treated animals showed a significant decrease in serum SGPT levels (57.03±66.01 U L^{-1}) when compared to the CCl_4 treated animals (420.8±214.1 U L^{-1}) and in agreement with levels seen in Silymarin treated animals (61.39±37.87 U L^{-1}). When serum SGOT levels were measured it was found that animals treated with Digesti'Vas had significantly decreased (6.953±3.712 U L^{-1}) levels compared to the CCl_4 treated group (29.65±5.765 U L^{-1}) in keeping with levels seen after treatment with Silymarin (10.7±6.135 U L^{-1}). These results suggest that Digesti'Vas is a safe herbal preparation on vital organs like the liver and shows protective effect on liver functions.

When experiments were conducted to study the effects of Digesti'Vas on food intake and weight gain, it was found that animals treated with the herbal formulation showed a significant increase in the percentage change in food intake (13±3.7 g) when compares to the control group (8.7±2.2 g). Similarly, Digesti'Vas treated animals

Table 1: Gastrointestinal motility (charcoal meal test) following treatment with Digesti'Vas

	Total charcoal defecation (MDP±SD)	
Groups	Minutes	Hours
1 (Normal control)	251.2±60.32	4.18±60.32
II Loperamide	383.0±37.83**	6.38±37.83**
III Digesti'Vas	346.2±65.83*	5.77±65.83*

Table 2: Effect of Digesti'Vas on ALP, SGPT and SGOT levels

N = 6	Treatments	ALP (U L^{-1})	SGPT (U L^{-1})	SGOT (U L^{-1})
1	Normal control	286±10.49	45.65±34.71	19.66±3.175
2	CCl_4	493.2±108.4^{a}***	420.8±214.1^{a}***	29.65±5.765^{a}**
3	CCl_4+Digesti'Vas	401.8±10.04^{b}*	57.03±66.01^{b}***	6.953±3.712^{b}***
4	CCl_4+Silymarin	370.5±37.82^{b}**	61.39±37.87^{b}***	10.71±6.135^{b}***

Table 3: Effects of Digesti'Vas on body weight and food intake

Groups	Change in body weight (g) (%)	Change in food intake (g) (%)
1 (Normal control)	3.618±2.088	8.657±2.160
2 (Digesti'Vas)	8.367±3.021*	13.28±3.673*

showed a significant increase in the percentage change in body weight (8.4±3 g) when compared to the normal control group (4±2.1 g). Thus, these results suggest that the effects of Digesti'Vas on body weight and food intake may be related to an increase in appetite (Table 1-3).

CONCLUSION

The results obtained from this study suggest that Digesti'Vas, a polyherbal formulation possesses antimotility and hepatoprotective properties. In addition, Digesti'Vas appears to increase food intake and subsequent weight gainby increasing appetite in rats. Taken together, the study suggests that Digesti'Vas may be a useful herbal formulation to alleviate GI problems related to digestion, liver function, GI motility and appetite in rats. Further studies on the mechanism by which Digesti'Vas exerts its beneficial effects are necessary before definite conclusions can be drawn. However, the data provide an insight into the possibility of using Digesti'Vas for the treatment of several GI related disorders.

REFERENCES

01. BMG., 2013. National institute for health and clinical excellence. Clinical guideline. BMG Rights Management Music Recording Company, Berlin, Germany. https://www.nice.org.uk/guidance/cg17/documents/dyspepsia-review-decision2
02. Rang, H.P., M.M. Dale, J.M. Ritter and R.J. Flower, 2007. Pharmacology. 7th Edn., Harcourt Publishers Ltd, Edinburgh, England, Pages: 362.
03. Blenkinsopp, A., P. Paxton and J. Blenkinsopp, 2013. Symptoms in the Pharmacy: A Guide to the Management of Common Illness. 6th Edn., John Wiley & Sons, New York, USA., ISBN-13:9781405180795,.
04. Harrison, T.R., 2005. Diarrhea and Constipation. In: Harrison's Principles of Internal Medicine, Harrison, T.R., E. Braunwald, J.L. Jameson and S.H. Dan Longo (Eds.). Mac-Graw Hill, New York, USA., ISBN:9780071391412, pp: 224-232.
05. Hardman, J.G. and L.E. Limbird, 2001. Goodmans and Gilmans the Pharmacological Basis of Therapeutics. 10th Edn., Macgraw Hill, New York, USA., ISBN:9780071354691, Pages: 2148.
06. Chitme, H.R., R. Chandra and S. Kaushik, 2004. Studies on anti-diarrhoeal activity of *Calotropis gigantean* R. Br. in experimental animals. J. Pharm. Pharm. Sci., 7: 70-75.
07. Palanivel, M.G., B. Rajkapoor, R. Senthil Kumar, J.W. Einstein and E.P. Kumar *et al*., 2008. Hepatoprotective and antioxidant effect of *Pisonia aculeata* L. against CCl_4-induced hepatic damage in rats. Scientia Pharmaceutica, 76: 203-215.
08. Khadke, S.S., D.R. Pachauri and S.D. Mahajan, 2011. An acute oral toxicity study of *Gnidia glauca* (Fresen.) Gilg. in albino rats as per OECD Guideline 425. IJPRIF, 2: 787-791.
09. Marona, H.R.N. and M.B.B. Lucchesi, 2004. Protocol to refine intestinal motility test in mice. Lab. Anim., 38: 257-260.
10. Paul, S. and D. Saha, 2012. Evaluation of antimotility effect of *Plumbago indica* (L.) on charcoal induced gastrointestinal motility in mice. Asian J. Res. Pharm. Sci., 2: 95-97.
11. Mithila, M.V. and F. Khanum, 2015. Effectual comparison of quinoa and amaranth supplemented diets in controlling appetite; a biochemical study in rats. J. Food Sci. Technol., 52: 6735-6741.
12. Mayuren, C., V.V. Reddy, S.V. Priya and V.A. Devi, 2010. Protective effect of *Livactine* against CCl_4 and paracetamol induced hepatotoxicity in adult Wistar rats. North Am. J. Med. Sci., 2: 491-495.
13. Ahmad, D., M. Gulfraz, M.S. Ahmad, H. Nazir and H. Gul *et al*., 2014. Protective action of *Taraxacum officinale* on CCl_4 induced hepatotoxicity in rats. Afr. J. Pharm. Pharmacol., 8: 775-780.