Pharmacological evaluation of a polyherbal formulation for its antispasmodic activity

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Object: The present study was aimed to evaluate pharmacologically to a polyherbal formulation containing volatile extracts of various umbelliferae plants. **Materials and Methods:** The umbelliferae family was found most effective against spasm by extensive literature review and study of various rational formulations. Plants material was procured from the local market of Rajasthan. Volatile extracts were isolated from powdered plant materials through Clevenger's method. The volatile extracts of following plants *Trachyspermum ammi*, *Cuminum cyminum*, *Anethum graveolens* and *Foeniculum vulgare* were used to make polyherbal formulation. **Results:** Antispasmodic effect of newly prepared polyherbal formulation demonstrated on guinea pig ileum *in vitro*; 50% inhibitory concentration (IC_{50}) was 172.5 ± 1.4 μl/ml. A very effective value identified, when compared to antispasmodic drugs, e.g. Atropine (IC_{50} was 166.7 ± 1.2 μl/ml). Oral administration of polyherbal formulation dose-dependently reduced intestinal transit in mice when compared to atropine at 0.1 mg/kg i.p. and formulation at (300 mg/kg) protected mice against diarrhea induced by castor oil significantly when compared to control and standard loperamide at a dose of 5 mg/kg orally. **Conclusion:** Polyherbal formulation inhibits acetylcholine and calcium chloride induced contraction of guinea pig ileum dose-dependently. The current research validates antispasmodic effect of newly developed polyherbal formulation. It also concluded that polyherbal formulation inhibits the contraction produced by various spasmogens like acetylcholine and calcium chloride. This suggests that the activity of developed formulation is nonspecific to any spasmogen.

Key words: Antispasmodic, atropine, guinea pig ileum, a polyherbal formulation

INTRODUCTION

Spasm is a sudden violent involuntary muscular contraction.[1] Gastrointestinal spasm is very common global problem in infants.[2] It can be accompanied by various other gastrointestinal disorders such as abdominal pain, colic, diarrhea, and constipation.[3] Still no single drug has proved to be effective in treating these conditions. Developing countries generally remains dependent on traditional medicinal plants for health care. Therefore, research teams are focusing on medicinal plants, because of their potent constituents to fight against various gastrointestinal disorders. In India, traditional infusion of some plants observed highly effective against infantile colics.[4,5]

Ayurveda, an Indian system of medicine, cited several plants, which are useful in various gastrointestinal disorders without any side-effect. Based on this fact, umbelliferae family has been identified most effective against gastrointestinal troubles. Umbelliferae plants selection based on the functional approach. Some umbelliferae plants *Criandrum sativum*, *Pimpinella anisum*, *Trachyspermum ammi*, *Apium graveolens*, *Cuminum cyminum*, *Anethum graveolens* and *Foeniculum vulgare* were studied. Among the various plants of umbelliferae, we observed marginal effect of four plants *Trachyspermum ammi*, *Cuminum cyminum*, *Anethum graveolens* and *Foeniculum vulgare* can provoke relaxation of guinea pig [Table 1].[6] A new polyherbal formulation was developed. It contains active constituents of various plants which were proved individually in the earlier research work effective for their antispasmodic activity. Following umbelliferae plants *Trachyspermum ammi*, *Cuminum cyminum*, *Anethum graveolens* and *Foeniculum vulgare* selected by the functional approach in earlier studies (data not shown here).[7]

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These volatile extracts of umbelliferae plants, however, are generally found to be pleasant in smell but difficult to swallow when taken orally. While oil can be made relatively bland in taste through sweetening, organoleptically it is still greasy tasting, relatively viscous, and very difficult for most people to stomach. A simple, easy to prepare a composition, which would remove such negative taste and mouthfeel impressions would, thus, be very useful, for example, in preparing

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an oral dosage form of volatile extracts. Therefore, in the present study volatile extracts of following plants *Trachyspermum ammi*, *Cuminum cyminum*, *Anethum graveolens* and *Foeniculum vulgare* were used to make polyherbal formulation, evaluated it for antispasmodic activity *in vitro* and intestinal transit rate *in vivo*.[8]

MATERIALS AND METHODS

Collection and Authentication of Plants

Plants of umbelliferae (*Apiaceae*) family *Trachyspermum ammi*, *Cuminum cyminum*, *Anethum graveolens* and *Foeniculum vulgare* were collected from the local market of Pratapgarh, Rajasthan. The plants materials were identified by Prof. (Dr.) S. K. Pandey, Scientist, KNK College of Horticulture, Mandsaur, Madhya Pradesh, India. The voucher specimen MIP/Pharmacognosy/2014/10, 11, 12, 13 are submitted in Department of Pharmacognosy for future reference.

Chemicals and Reagents

All chemicals, reagents and solvents used for the study were of analytical grade and procured from Puneet Enterprises, Ratlam, Madhya Pradesh, India. Atropine was purchased from Sigma-Aldrich Chemical, St. Louis, MO, USA and acetylcholine from Merck India Ltd., Mumbai, India.

Extraction of The Essential Oil

The dried materials of selected umbelliferae plants were grinded to get a fine powder using a grinder (Voltas-300). The grinded powder was then assembled for hydro-distillation to remove volatile extract with the help Clevenger's apparatus. Extraction was carried out by hydro-distillation of 500 g of the powdered drug with 1000 ml of tap water for 8 h to obtain volatile extract of each plant separately. The yield of the extracts was in range 2–4% of total dried material. The same procedure was repeated for other umbelliferous plants. Light yellowish colored extract was obtained having characteristic odor and taste. Volatile extracts were dried over anhydrous sodium sulfate to remove the moisture and stored in a dark glass bottle and kept at 4°C for further analysis.[9-11]

Preparation of Polyherbal Formulation

The present work relates to edible compositions which contain large amounts of volatile extracts, are highly palatable when ingested orally. These compositions are particularly useful as carriers for volatile extracts. This work relates to pleasant, nongreasy tasting edible compositions, preferably in liquid form, comprising from volatile extracts, a highly potent lipid-soluble sweetener and lipid soluble flavorant. The volatile extracts were mixed by mechanical stirrer, and Saccharin (o-benzoic sulfimide) was pulverized manually with a mortar and pestle, to enhance its dissolution, and was added to the continued agitation. Dissolution of the saccharin appeared complete after about 30 min. The flavor was then added to the oil mixture with agitation which was continued until the mixture, was homogeneous [Table 2].[12]

Standardisation of Polyherbal Formulation

Gas Chromatogram of Polyherbal Formulation

One microliters of 1% v/v solution of developed polyherbal formulation in chloroform were injected into a 20 M stainless-steel column Zb-5 (30 m × 0.25 mm × 0.25 µm). Nitrogen was used as the carrier gas at 1.3 ml/min and a flame ionization detector was used. The temperature of the oven, injector and detector were 190°C, 210°C and 280°C respectively. Oven temperature programming starts from initial temperature 90°C hold for 1-min, ramp of 10°C to 190°C and hold for 3 min. The chromatogram was recorded with the help of chromatography data station.[13]

Pharmacological Evaluation of Polyherbal Formulation

Guinea pigs of average weight and either sex were used for *in vitro* antispasmodic study. Albino mice were used to study the effect of the polyherbal formulation on intestinal transit time in mice. The animals were maintained on synthetic pelleted feed (Lipton India Ltd., Mumbai, India) and water

Table 1: Functional ratio of umbelliferae plants (value of 50% inhibitory concentration are expressed as geometric mean with 95% confidence interval

Name	IC_{50} (µl/ml)	Functional ratio (Fr)
Anethum graveolens	80.4±1.1	1
Cuminum cyminum	96.7±1.2	1.20
Foeniculum vulgare	106.5±1.2	1.32
Trachyspermum ammi	111.5±1.2	1.38
Apium graveolens	464.1±1.0	5.77
Pimpinella anisum	610.3±1.0	7.59
Coriandrum sativum	747.1±1.2	9.29
M4 (*Anethum graveolens*, *Cuminum cyminum*, *Foeniculum vulgare and Trachspermum ammi*)	172.5±1.4	2.14
M7 (*Anethum graveolens*, Cuminum *cyminum*, *Foeniculum vulgare*, *Trachyspermum ammi*, *Apium graveolens*, *Pimpinella anisum* and *Coriandrum sativum*)	196.5±1.6	2.44

Table 2: Composition of the polyherbal formulation, as given in the following table, was prepared

Name	Family	Final concentration v/v
Trachyspermum ammi Linn.	Apiaceae (Umbelliferae)	12 ml
Cuminum cyminum Linn.	Apiaceae (Umbelliferae)	
Anethum graveolens Linn.	Apiaceae (Umbelliferae)	
Foeniculum vulgare Mull.	Apiaceae (Umbelliferae)	
Saccharin (O-benzoic sulfimide)		0.006 ml
Peppermint concentrate[1]		0.3 ml

[1]A mixture of 4 ml of volatile oil (Moksha Lifestyle Products, 24/157 Shakti Nagar, G.T. Karnal Road, Delhi, India) and 1 ml artificial peppermint flavor (Loba chemie Pvt. Ltd.).

ad libitum, in a 12-h/12-h light/dark cycle at a temperature of 21–23°C. The study protocol was approved by Institutional Animal Ethical Committee. CPCSEA guidelines were adhered to during maintenance and experiment.[14,15]

Tyrode solution composition (mM): NaCl, 137; KCl, 2.7; $CaCl_2$, 1.8; NaH_2PO_4, 0.4; $MgSO_4$ 0.25; $NaHCO_3$, 11.9; glucose, 11.1 de Jalon solution composition (mM): NaCl, 154; KCl, 5.6; $CaCl_2$, 0.55; $NaHCO_3$, 6.0; glucose 2.78.[13]

Methods

Guinea pigs of either sex were fasted 24 h before the study. Then the animals were sacrificed to isolate the ileum. The guinea pigs were sacrificed by exsanguinations as per CPCSEA recommended guidelines. The *in vitro* method was performed using guinea pig ileum. Guinea pig ileum was used to study the effect of volatile extracts and polyherbal formulation on acetylcholine-induced contractions.[16]

The *in vitro* method was performed using guinea pig ileum. Guinea pig ileum was used to study the effect of developed polyherbal formulation on calcium chloride and acetylcholine-induced contractions.

Male guinea pigs were killed by stunning and bleeding. Ileum were removed and suspended under a constant tension of 1 g in 15 ml organ baths containing Tyrode at 37°C.[17] Before the experimental procedures, the organs were allowed for 30–40 min equilibration period with changes of medium at every 5 min. Volatile extracts were dissolved in 0.5% DMSO. 200 µl diluted volatile extracts were used as the maximum volume in the baths, at which volume there was no solvent effect. During the experimental phase, volatile extracts were added to the bath and after 1-min incubation period the agonist was added. Contractions were recorded using an isotonic transducer connected to a polyrite (Model 201, Recorders and Medicare, India).

Calcium Antagonism by Polyherbal Formulation

A guinea pig ileum preparation was set up as described by Magnus[18] and for evaluating Ca^{++} antagonism activity of the formulation; the method described by Van den Broucke and Lemli[19] was followed. About 2–3 cm long lumen was suspended in an organ bath of 25 ml capacity containing depolarizing solution to make the muscle membrane permeable for Ca^{2+} ions. Composition of salt solution is g/lt: NaCl 1.58; $NaHCO_3$ 1.26; KCl 7.46; $MgCl_2$ $6H_2O$ 0.25; Glucose 1.98. The preparation was aerated and allowed to stabilize for 30 min before starting the experiment. In order to remove intra and extracellular Ca^{2+}, the longitudinal muscle strip was washed during 1-h with the depolarizing solution. Contraction of the tissue preparation to different doses of calcium chloride was recorded using isotonic fine movement transducer with a tension of 0.5 g, connected to student physiograph. Dose-response curves were obtained with calcium chloride. Then developed formulation was added to the organ bath in increasing concentration. The effect of this formulation to antagonize Ca^{2+} (14.4×10^{-2} M) concentrations in the guinea pig ileum was evaluated [Figure 1].

Effect Polyherbal Formulation on Agonist Induced Contraction

A guinea pig ileum About 2–3 cm long lumen was suspended in an organ bath of 25 ml capacity. The preparation was aerated and allowed to stabilize for 30 min before starting the experiment. Contraction of the tissue preparation to different doses of acetylcholine was recorded using isotonic fine movement transducer with a tension of 0.5 g, connected to student physiograph. Dose-response curves were obtained with acetylcholine. Then developed formulation was added to the organ bath in increasing concentration. The effect of this formulation to antagonize acetylcholine-induced concentrations in the guinea pig ileum was evaluated.

Effect of Polyherbal Formulation on Intestinal Transit Time in Mice

The passage of an orally administered charcoal meal through the gastrointestinal tract in mice is used as parameter for evaluating intestinal motility in an *in vivo* test The evaluation was carried out according to the procedure given in Vogel and Vogel;[16] Janssen and Jageneau.[20] Albino

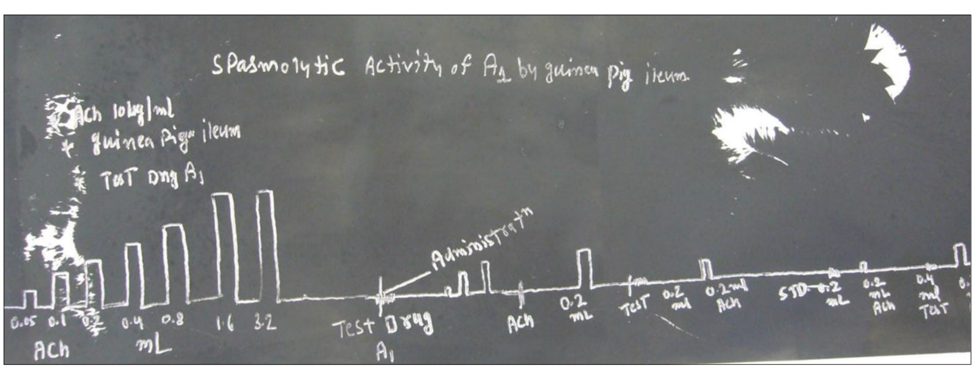

Figure 1: Effect of developed polyherbal formulation on $CaCl_2$ induced contractions

mice (20–25 g) were randomly divided into five groups of 6 mice each. The animals were starved for 24 h prior to the experiment but were allowed access to water. One group of animals was given 20 ml/kg of normal saline orally while remaining three groups received orally polyherbal drug at a doses of 50, 100, 200 mg/kg. The last group received atropine (0.1 mg/kg.). After 60 min of drug administration through oral route (15 min after drug administration through i.p.) charcoal meal (0.2 ml of a 4% suspension of charcoal in 2% carboxymethylcellulose solution) was administered to each animal orally. The animals were killed 30 min later, and the abdomen was opened. Percentage distance (from pylorus to caecum) traveled by the charcoal plug in both the extract and normal saline-treated groups were determined. As the intestines of the mice used were all of similar length, it was considered justifiable to use the distance travelled by the charcoal meal as an index of intestinal transit. In this way, the intestinal transit was measured for different groups of mice.

Statistical Analysis

Data were expressed as mean ± standard error of mean significance was assessed by Student's *t*-test or ANOVA, followed by Dunnet's test. The minimum level of significance was fixed at $P < 0.05$.[21]

RESULTS

Standardisation of Polyherbal Formulation by gas chromatography

Gas chromatographic analysis was performed under specific conditions and results thus obtained [Table 3] were compared. The compounds are listed along with corresponding peak number, retention time, name of component and area. Some peaks remain unidentified due to the unavailability of pure standards. Some constituents cuminaldehyde, thymol, carvone, *t*-anethole and limonene were identified in polyherbal formulation. A fingerprint chromatogram of drug is presented in Figure 2.

Effect of Polyherbal Formulation on Agonist Induced Contractions

Dose-dependent inhibition by polyherbal formulation on the contractile response of guinea pig ileum induced by acetylcholine (10^{-7} M) [Figure 3]. The 50% inhibitory concentration (IC_{50}) against acetylcholine-induced contraction on guinea pig ileum was 50.0 mcg/ml. The developed composition inhibited the tone of spontaneously contracting intestine in a concentration-dependent manner thereby confirming the antispasmodic activity of developed composition on guinea pig ileum. Polyherbal composition contains potent spasmolytic components, which are evident in the spontaneous contracting preparation of guinea pig ileum.

Calcium Antagonism

The effect of polyherbal formulation at doses of 10–50 mcg/ml caused a moderate spasmolytic effect in isolated guinea pig ileum suspended in a depolarizing solution and the inhibition was dose-dependent and reversible shown in Figures 1 and 4.

Effect on Intestinal Transit Time in Mice

The results of charcoal meal test showed that developed formulation caused a significant decrease in gut motility when compared to normal saline. At 50–200 mg/kg p.o.,

Table 3: Standardization of polyherbal formulation by gas chromatography

Peak	Time (min.)	Component name	Area (µV.s)
1	1.588	Methanol	5767582
3	3.653	Unidentified	758404
4	4.041	Limonene	1779214
6	4.368	Carvone*	1986726
7	4.735	Thymol	136192.4
8	4.910	Unidentified	192385.3
11	5.973	Unidentified	72839.42
12	6.388	Cuminaldehyde	3581044
13	6.509	Unidentified	53068.69
14	6.821	Anethole	3347586

*As per literature review

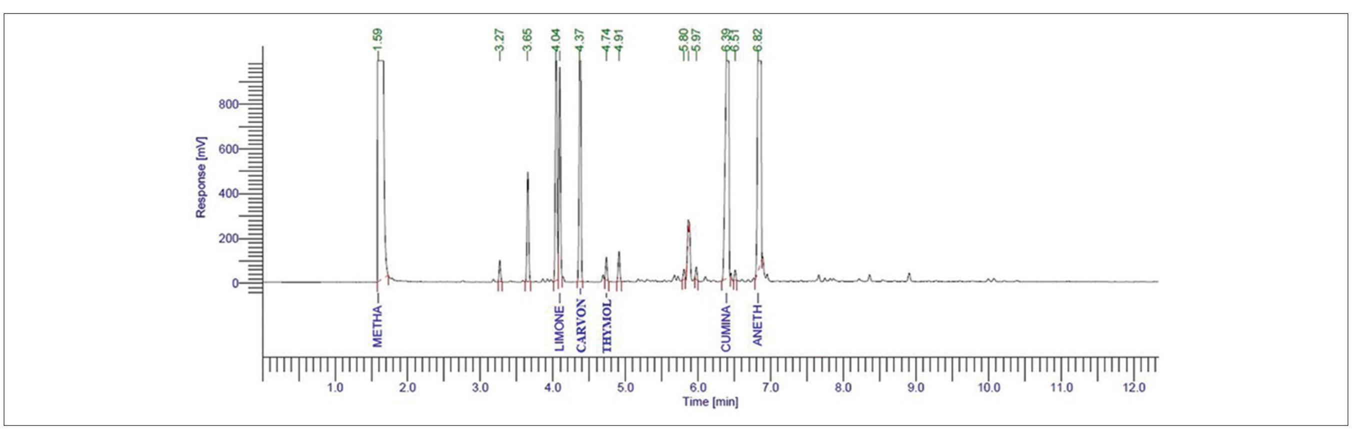

Figure 2: Gas chromatogram of polyherbal formulation

polyherbal formulation dose-dependently reduced intestinal transit time in mice. The effect on this parameter was significant at a dose of 50, 100, 200 mg/kg p.o. of developed drug. The mean percentile inhibition was 72.24 ± 4.77%, 64.75 ± 5.082%, 60.17 ± 5.7045% respectively whereas atropine at 0.1 mg/kg i.p. produced 56.68 ± 5.912% inhibitions [Figure 5].

Selection of Umbelliferae Plants

The effect of *Anethum graveolens* was more important (IC_{50} = 80.4 ± 1.1 µl/ml) than *Trachyspermum ammi*, *Cuminum cyminum* and *Foeniculum vulgare*, whereas *Coriandrum sativum*, *Pimpinella anisum* and *Apium graveolens* exhibited weak effects (respectively $IC_{50:}$ 747.1 ± 1.2, 610.3 ± 1.0 and 464.1 ± 1.0 µl/ml). The current research on the basis of functional ratio reveals that lowest IC_{50} with *Anethum graveolens* (IC_{50} of 80.4 ± 1.1 µl/ml) and the value of 1 was given to this volatile extract whereas the highest functional ratio of 9.29 was attributed to *Coriandrum sativum.*

DISCUSSION

Pleasant-tasting, nongreasy, edible compositions, preferably in liquid form, comprising edible volatile extracts, a high potency, lipid soluble sweetener, such as saccharin, and a lipid soluble flavorant, are exposed. The present composition also relates to the process for making the above-described palatable compositions wherein the sweetener is solubilized in the oily material, such as by agitation, and the flavorant is subsequently added in a manner so as not to lose its volatile components.

The use of various spasmogens, with different mechanisms to cause intestinal contraction, can provide information on the pharmacological basis of antispasmodic activity of developed formulation. Present composition did not show any agonistic property like acetylcholine. **Acetylcholine** is known to act on the smooth muscle cells of the guinea pig ileum, interfering with specific receptors for these spasmogens.[21] Acetylcholine-induced spasms are due to muscarinic M3 receptor activation, which is a characteristic of vagal stimulation in the body. Hence, mostly all endogenous colic pain like biliary, gastrointestinal, ureter arise due to such acetylcholine-induced spasms.

Since the availability of calcium is a basic determinant of muscle contraction, possible calcium antagonism by polyherbal formulation was also studied. Inhibition of smooth muscle contractility may be the result of two different actions. A neurotropic one as inhibition of neurotransmitter release from nerve terminals or blockade of specific membrane receptor sites.

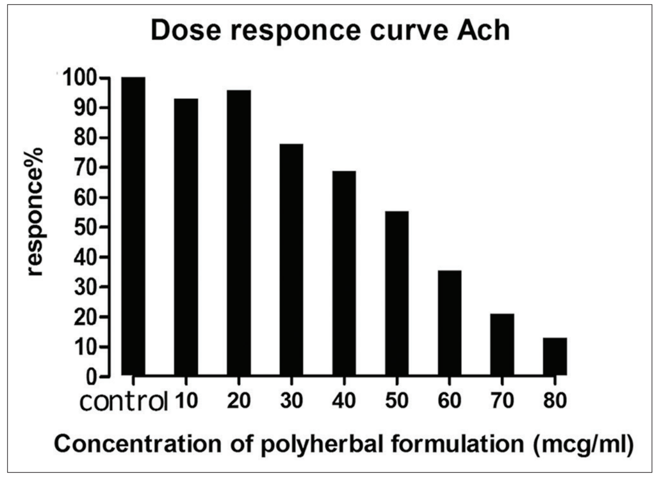

Figure 3: Effect of developed composition on Ach (17.6 × 10^{-8} M) induced contractions on guinea pig ileum. Values expressed as mean ± standard error of mean. *P < 0.05 versus control

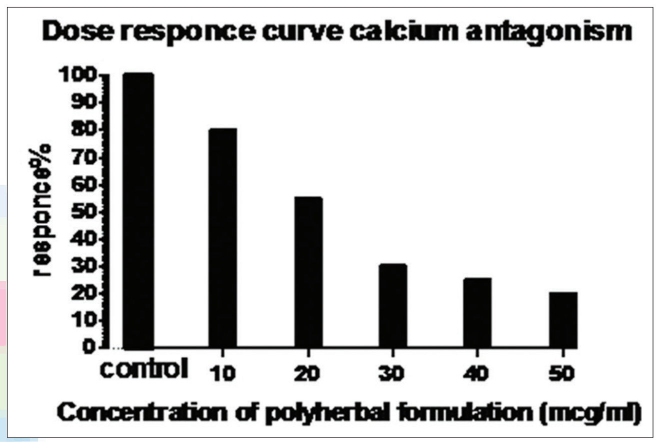

Figure 4: Effect of developed formulation on $CaCl_2$ (14.4 × 10^{-2} M) induced contractions on guinea pig ileum. Values expressed as mean ± standard error of mean. *P < 0.05 versus control

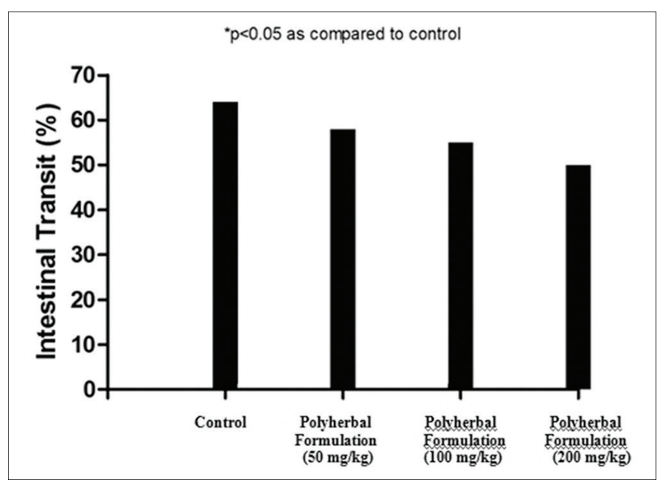

Figure 5: Effect of developed formulation on intestinal transit time

The results indicate that antispasmodic activity is caused by a direct and indirect action on the smooth muscle. It is suggested that muscle relaxant effect results from a decrease of the Ca^{2+} availability for muscle contraction by blocking the release of intracellular bound Ca^{2+} and prevention of the extracellular bound Ca^{2+} influx in the smooth muscle cell. Inhibition of the nerve action potential in the postganglionic nerve action potential in the postganglionic nerve fiber is proposed to be the indirect action of antispasmodic activity. Developed formulation decreases intestinal transit by inhibiting the gastrointestinal motility. The finding that the drug decreased peristaltic movement in the charcoal meal study corroborated with some of the results of *in vitro* studies.

The results of the present study indicate that developed composition possesses potent antispasmodic properties on the smooth muscles of the gastrointestinal tract. All contractions induced by various spasmogens with different pharmacological mechanisms to cause contractions were reduced or blocked, and the blockade was reversible. Hence, the antispasmodic activity is nonspecific in nature.[6]

CONCLUSION

All the above findings suggest that developed formulation is a nonspecific antispasmodic, which can be used in the treatment of various spasmodic disorders of the gastrointestinal tract and other viscera. The present study confirms the antispasmodic activity of the said constituents using modern pharmacodynamic experiments. Further studies are required to find the biochemical and molecular mechanism of action of developed polyherbal formulation.

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