ORIGINAL PROF-2156

TERMINALIA CHEBULA RETZ;

SPASMOGENIC AND SPASMOLYTIC PROPERTIES

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ABSTRACT… Objective: To study the spasmogenic and spasmolytic properties of Terminalia chebula. Diagnostic parameter of smooth muscle activity was used for the determination of characteristic spasmogenic and spasmolytic activity of T. chebula. **Design:** Experimental study. **Setting:** This experimental study was carried out at the Department of Pharmacology, Faculty of Pharmacy, Karachi University. **Period:** From 2005 to 2006. **Material & method:** This experimental study was conducted on isolated smooth muscle of rabbit's intestine. Segment of small intestine (jejunum and ileum) was mounted in Tyroid's solution filled organ bath with maintained temperature at 37°C to record dose response activity. **Results:** It is observed that at the dose of 1 mg/ml there is a slight decrease in the response (0.88 cm 0.035 cm) as compare to control (1.15cm 0.040 cm). At 10 mg/ml there is a relaxing response of smooth muscle activity (0.85cm 0.08cm) from control (1.08cm 0.125cm). While the relaxation is prominent at the doses of 20 mg/ml (0.9cm 0.5cm) and 25 mg/ml (0.55cm 0.035cm) from control (1.4cm 0.155cm) and (1.3cm 0.07cm) respectively with (p value 0.05). The effect of ethyl acetate fraction shows initially relaxation and this effect disappeared after the administration of acetylcholine $1x10^{-2}$M, but full response of acetylcholine is not produced due to occupancy of muscarinic receptor by the extract. **Conclusions:** The effect of T. chebula showed a spontaneous decrease in the movement of smooth muscles of rabbit's intestine as compared to control experiments.

Key words: Spasmogenic, Spasmolytic, Small intestine, T. chebula.

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INTRODUCTION

In current years, interest on herbs research has improved around the world and a large number of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Fructus Chebulae consists of the dried fruits of Terminalia chebula Retz. or T. chebula Retz. var. tomentella Kurt. (Combretaceae)[1-3]. Terminalia chebula Retz. (Combretaceae) is commonly known as chebulic myrobalan. It grows throughout central Asia and some other parts of the world[4]. Seeds of T. chebula are used in traditional medicine to treat kidney and urinary disorders[5] and can also be used as homeostatic, laxative, antitussive, diuretic, and cardiotonic remedy[6]. The semi-ripe seed is considered as a purgative[7] and its gastro-protective effect against indomethacin-induced gastric ulceration has been reported[8]. Contents of T. chebula includes fructose, amino acids, succinic acid, betasitosterol, resin purgative along with principle of anthraquinone[9,10]. Flavonol, glycosides, triterpenoids, coumarin conjugated with gallic acids called chebulin as well as other phenolic compounds were also isolated[10].

Terminalia bellerica, Terminalia chebula and Emblica officinalis are used by Kavirajes for stimulation of appetite, as digestive aid, and for acidity. It is interesting that these plants have been shown to demonstrate healing activities against indomethacin-induced gastric ulceration in rats[8]. Additionally, extracts of Terminalia chebula have been reported to have antibacterial activity against Helicobacter pylori, a known agent for gastric ulcer[11].It demonstrate the development of duodenal ulcers and produces a cytoprotective effect on the gastric mucosa in vitvo[12].

In the Ayurvedic system of traditional medicine, Terminalia chebula is given for improving gastrointestinal motility. This has been scientifically validated[13].

From many studies conducted on T. chebula, explained its 'anti-vata' or 'anti-spasmodic' properties by decreasing abnormal blood pressure along with

intestinal spasms too. This suggests its traditional value for spastic colon and other intestinal disorders[14].

In the last few decades, there has been a considerable growth in the field of herbal medicine. It is getting popularized in developing and developed countries due to its natural origin and lesser side effects[15-20]. T. chebula also popularly known as Harde in India is one such medicinal herb, used commonly in many ayurvedic preparations.

The medicinal properties of T. chebula and several other herbal plants have been documented in the ancient Indian literature[21-25]. T. chebula belongs to the family Combretaceae and is found throughout India especially in deciduous forests and areas of light rainfall. Its yellowish-brown fruits are included in the Indian pharmacopoeia under the category astringent. It possesses laxative, diuretic, cardiotonic and hypoglycemic properties. A combination drug Triphala, a composite mixture of T. chebula, T. bellerica, and Emblica officinalis, is a very popular traditional medicine used for the treatment of many chronic diseases such as ageing, heart ailments and hepatic diseases, etc[22,26,27]. Six different extracts and four other compounds of T. chebula fruit demonstrated antioxidant activity at different potency[28].

Acetone extract has powerful antioxidant activity in comparison with alphatocopherol and HPLC analysis with diode array detection revealed the occurrence of hydroxybenzoic acid derivatives, hydroxycinnamic acid derivatives, flavonol aglycones and their glycosides, as main phenolic compounds[29].

Gallic acid (GA) and Chebulic Acid (CA) were separated from the extract of the herbal medicine Kashi (myrobalan, the fruit of T.chebula) as main ingredient that antagonize the cytotoxic T-lymphocyte-mediated cytotoxicity. Granule exocytosis in response to anti-CD3 stimulation was also blocked by GA and CA at the equivalent concentrations[30].

T. chebula fruit and seeds exhibited dose dependent reduction in blood glucose of streptozotocin induced diabetic rats both in short term and long term study and also had renoprotective activity[31,32]. It has anti bacterial inhibitory action against Klebsiella[33]. Ethanol extract of T. chebula fruit showed strong antibacterial activity against multidrug-resistant uropathogenic Escherichia coli and phenolics were found to be responsible for this antibacterial activity[34,35]. T. chebula has also retroviral reverse transcriptase inhibitory activity[36]. Tannins from T. chebula are effective against potato virus x[37]. The acetone extract of T. chebula seeds showed anti plasmodial activity against Plasmodium falciparum[38].

METHODOLOGY

Collection and recognition of plant material

The plant material was purchased from the local market and is recognized by Prof. Asif Bin Rehman, Department of Pharmacology, Faculty of Pharmacy, University of Karachi. A model coupon sample has been placed in the department.

Extraction and fraction

The dried plant material (5 Kg) was soakd in ethanol solution (70%) for 02 weeks. This method was repeated for next 01 week. Filtrate was dried up under reduced pressure at room temperature. A thick dark reddish brown pasty residue (crude extract) was collected. Later on crude extract of T. chebula was fractionated in 04 different fractions (Ethylacetate, Chloroform, n-butanol and aqueous) by the standard extraction method.

DATA ANALYSIS

Data was analyzed by using Microsoft Office Excel Worksheet 2003 and SPSS version 17.0. For comparison of numeric output student t – test was used and for comparison of qualitative output response pearson's Chi- square test and Z – test of proportion was used Statistical significance will be

taken at $p < 0.05$.

RESULTS

The pharmacological activity of the drug T. chebula was performed in vitro on isolated rabbit intestine. The effect of the crude extract of T. chebula and its different fractions (ethyacetate, chloroform, n-butanol and aqueous) were observed through the concentration and relaxation of rabbit intestine. The effect of the crude extract of the drug was observed at the doses of 01, 05, 10, 15, 20 and 25 mg/ml. Table-I represents the dose related response of crude extract of T. chebula on isolated smooth muscle of rabbit. Fig-1 shows the response (%) of crude extract and the Fig-2 shows the dose response along SEM. Table-II represents the effect of different fractions of the drug on isolated rabbit intestine.

Dose (mg/ml)	Control (cm)	Response(cm)	Response in %	p-value
01	1.15±0.040	0.88±0.035	23.47	0.037*
05	1.08±0.09	0.85±0.06	21.29	0.0433*
10	1.08±0.125	0.85±0.08	21.29	0.0433*
15	1.5±0.060	0.75±0.035	20.62	0.0418**
50	1.4±0.155	0.9±0.5	35.71	0.0217**
25	1.3±0.07	0.55±0.035	57.69	0.0452***

Table-I. Dose related response of crude extract of terminalia chebula on isolated rabbit intestine.
The result are expressed in ± SEM at P ≤ 0.05
**Significant, **Moderate significant, ***Highly significant*

Fractions	Dose (mg/ml)	Control(cm)	Response (cm)	Response in %	p-value
Ethylacetate	15	1.05±0.035	0.89±0.035	42.23	0.028**
Chloroform	15	1.05±0.035	0.59±0.09	43.80	0.013**
n-butanol	15	0.99±0.035	0.59±0.09	40.40	0.047*
Aqueous	15	1.04±0.035	0.9±00	13.46	0.063

Table-II. Effect of different fractions of terminalia chebula on isolated rabbit intestine
The result are expressed in ± SEM at P ≤ 0.05
**Significant, **Moderate significant, ***Highly significant*

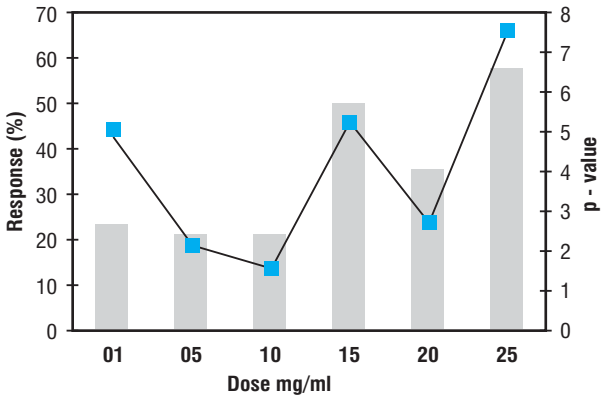

Fig-1. The in virto experiments shows the percent of response of crude extract of terminalia chebula on isolated rabbit intestine

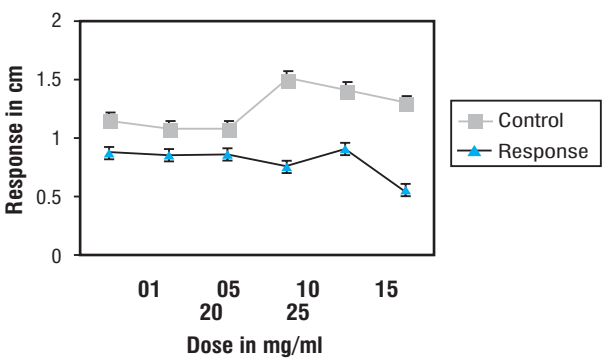

Fig-2. The in vitro experiments showing the dose response along with SEM of crude extract of terminalia chebula on isolated intestine of rabbit

The activities of the different fractions of crude extract of T. chebula were tested at the dose of 15 mg/ml. Further studies carried out on the fractions of crude extract of T. chebula. The ethylacetate, chloroform, n-butanol and aqueous fractions shows interesting results. Each of the fractions was tested at the dose of 15 mg/ml. The (%) response of the fractions is given in Fig-3 and the dose of response along SEM is given in Fig-4.

DISCUSSION

Terminalia chebula is a commonly advocated agent in Ayurveda for improving gastrointestinal motility. One of the study conducted by Tamhane et al., Terminalia

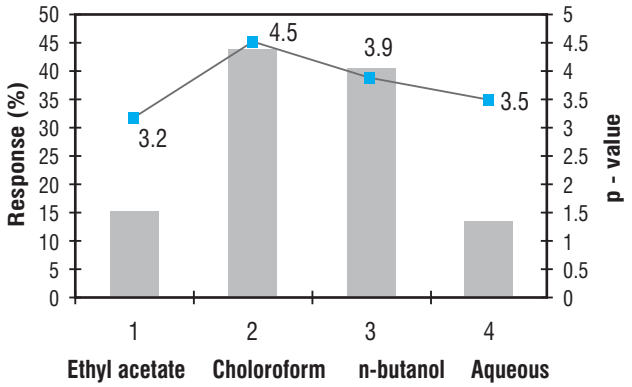

Fig-3. The in vitro experiments showing the percent of response of different fraction of terminalia chebula on isolated intestine of rabbit

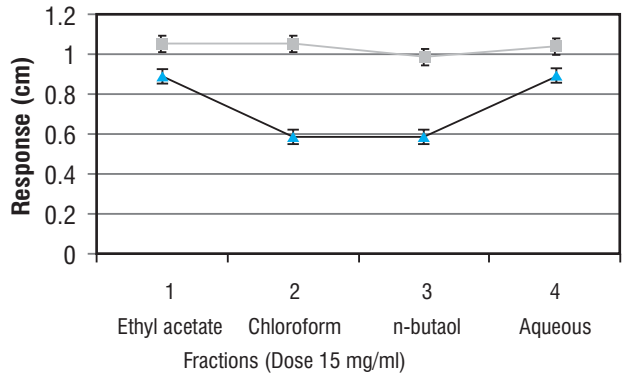

Fig-4. The in vitro experiments showing the dose response along with SEM of different fractions of Treminalia chebul on isolated intestine of rabbit

chebula was given in a dose of 100 mg/kg/day for 15 days orally. Metoclopramide and atropine have established prokinetic and antikinetic activities respectively and are therefore included for comparison. Metoclopramide significantly increased the gastric emptying and atropine inhibited the motility. Terminalia chebula was found to increase the percent gastric emptying Thus from this study it appears that Terminalia chebula can serve as a useful alternative to prokinetic drugs available today.[13]

In our study, it was observed that at the dose of 1 mg/ml, there is slight decrease in the response. This shows a negligible smooth muscle relaxing activity. At the dose of 05 mg/ml, an increase in the muscle activity is found i.e contraction of smooth muscle. Relaxing response of isolated tissue was observed at the dose of 10 mg/ml. The dose of 15 mg/ml shows more or less same relaxing response as dose 0f 10 mg/ml while the relaxation of the tissue of rabbit intestine was prominent at the doses of 20 and 25 mg/ml.

The overall response of crude extract of T. chebula on isolated tissue of rabbit intestine was described in the tone of muscle.

The ethylacetate and chloroform fractions of the crude extract of T. chebula show relaxation of isolated tissue of rabbit intestine. This effect was not observed in aqueous fraction. The relaxation of isolated tissue of rabbit intestine also found in n-butanol fraction but the response was les potent then chloroform and ethylacetate fractions. This effect was same as it was observed in crude extract at the dose of 25 mg/ml. By observing these data it was found that the relaxing effect of rabbit intestine that appeared in ethylacetate and chloroform fractions was also present in the crude extract, but the other two fractions does not have the effects like crude extract. This is due to the separation of chemical constituents of the drug. It also indicates that a single drug can have different responses if its constituents are separated. The result of these data also indicates that crude extract of the T. chebula decreases the motility of intestine; therefore, it has valuable importance in the treatment of intestinal disorders. The fractions of crude extract of T. chebula show only relaxation of isolated tissue. The relaxing effect of ethylacetate and chloroform fractions can help in the case where the intestinal motility is noticed. The standard drugs along fractions of crude extract of T. chebula were tested for further screening of the fractions of T. chebula.

The Fig-5 shows the effect of ethylacetate fraction of T. chebula on pretreated isolated tissue of rabbit intestine with atropine $1x10^{-2}$M. this shows the cumulative

relaxing response of isolated tissue of rabbit intestine. The Fig-6 shows the effect of ethylacetate fraction on pretreated and post treated tissue with acetylcholine $1x10^{-2}$M. When ethylacetate fraction was administrated to the tissue, it decreases the response of tissue and produces relaxation of intestinal smooth muscle. This effect was almost disappeared, but the full contractile response of acetylcholine was not produced due to occupancy of muscarinic receptors. In the reverse manner first tissue was treated with acetylcholine $1x10^{-2}$M then with testing drug, it decreases the response of tissue same as when atropine was given after acetylcholine. This result shows that the effect of the drug may be produce through muscrinic receptors.

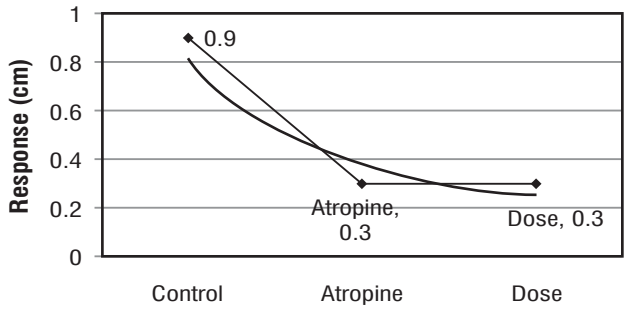

Figure 5: Effect of ethyl acetate fraction of terminalia chebula, pretreated tissue with atropine in 1x 10^{-2}M concentration

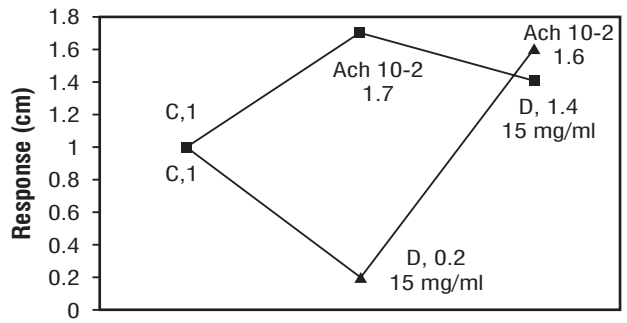

Figure 6: Effect of ethyl acetate fraction of terminalia chebula, pretreated and post treated tissue with acetylcholine $1x10^{-2}$M concentration

Similarly Fig-7 shows the effect of chloroform fraction of T. chebula on pre-treated and post-treated tissue with atropine $1x^{10-2}$M. Both result shows the cumulative response of the drug that is additional relaxation of smooth muscle of rabbit intestine. This is the synergetic effect of the drug which can help in various disorders of the gastrointestinal tract.

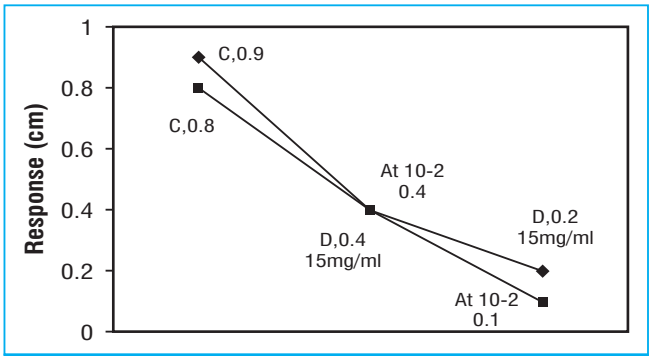

Figure 7: Effect of chloroform fraction of terminalia chebula, pretreated and post treated tissue with atropine 1x 10^{-2}M concentration

Another way to see the effect of drug is by aqueous fraction of T. chebula given in Fig-8. This represents the post-treated tissue with acetylcholine $1x^{10-2}$M. Here acetylcholine produces its full response which indicates that aqueous fraction of T. chebula does not have any prominent activity. The Fig-9 shows the effect of n-butanol fraction of T. chebula on post-treated with acetylcholine $1x^{10-2}$M. This fraction has relaxing response on isolated tissue. The result shows that acetylacholine $1x^{10-2}$M does not produces its full response due to blocking of muscarinic receptors.

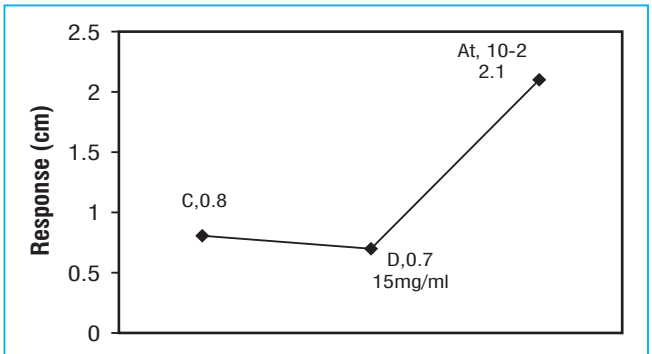

Figure 8: Effect of aqueous fraction of terminalia chebula, post-treated tissue with acetylcholine in $1x10^{-2}$M concentration

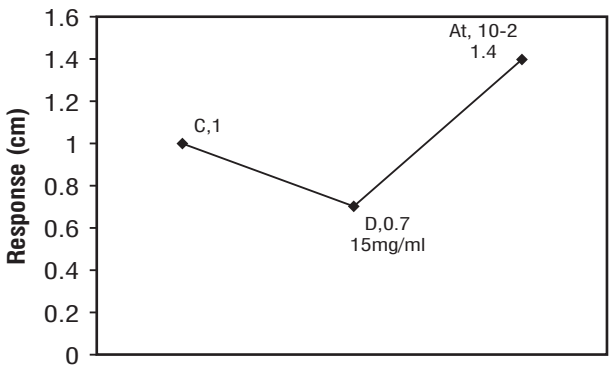

Figure 9: Effect of n-butanol fraction of terminalia chebula, post treated tissue with acetylcholine 1x10^{-2}M concentration

CONCLUSIONS

The over all effect of crude extract of T. chebula and its fractions produces a relaxation of isolated smooth muscle of rabbit intestine. Due to its safety margin, it has a beneficial role in gastrointestinal tract disorders.

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