Research Article

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EVALUATION OF *HELIANTHUS ANNUUS* L. LEAVES EXTRACT FOR THE ANTIDIARRHEAL AND ANTIHISTAMINIC ACTIVITY

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ABSTRACT

The objective of this study was to evaluate the anti diarrheal and antihistaminic activity of ethanolic extract of the leaves of *Helianthus annuus* L. at the dose of 250 mg/kg and 500 mg/kg body weight using castor oil induced diarrhoea and gastrointestinal transit model for anti diarrheal activity and histamine induced bronchoconstriction on guinea pigs and microshock model on rabbits for antihistaminic activity. At various doses (250 and 500mg/kg body weight), the extract showed a remarkable antioxidant activity when compared to ascorbic acid. Results also confirmed that *Helianthus annuus* L. decreases the severity of diarrhoea and possess the anti-histaminic potential to treat the allergic disorders.

Keywords: Sunflower, Diarrhea, Histamine, Castor oil.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is famous for its unique nature of heliotropism and is the world's second most important source of edible oil. *Helianthus annuus* L. is a folk remedy for several diseases such as; bronchitis, cough, diarrhea, dysentery, fever, flu, inflammations, laryngitis, malaria, menorrhagia, pleuritis, rheumatism, scorpion stings, snakebite, splenitis, wounds etc.[1] *Helianthus annuus* L. contains carbohydrates, flavonoids, tannins, alkaloids, saponins, phytosterols, steroids and fixed oils[2] and the leaves of this plant have these contents more than other parts[3]. It also has efficient quantities of antioxidants like tocopherols etc. to produce free radical scavenging activity.[4] Several research studies were carried out to find out the different pharmacological activity on the different parts of the *Helianthus annuus* L. like; antidiabetic, antitumor, antioxidant, anti asthmatic, anti-inflammatory, hypolipidemic and antibacterial activity.[5-11] As we know diarrhea is very common gastric problem which occurs due to low gastric motility, low water absorption, microbial infection etc. and might be fatal, if not controlled.[12] Histamine is an organic nitrogenous compound release in body and involved in all inflammatory and allergic diseases.[13] On the basis of its traditional uses and active constituents like; flavonoids, alkaloids, phenols etc.[14], leaves of this plant can be utilized to evaluate anti diarrheal as well as antihistaminic activity due to its high phytoconstituent concentration.[15]

MATERIAL AND METHODS

Collection and authentication of plant material

The leaves of the *Helianthus annuus* L. were collected in the month of October from the Smriti Forest, Jaipur, Rajasthan, India. The plants were authenticated by the taxonomist from Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India. The herbarium voucher specimen number was (RUBL.20711) submitted to the institute wherein the study was carried out.

Chemicals and standard drugs

Ethanol (solvent for extraction), Castor oil (laxative agent), DPPH, Ascorbic acid (standard antioxidant), Histamine, Loparamide (standard reference anti diarrheal drug), Atropine sulphate (standard reference anti diarrheal drug) and Chlorpheniramine maleate (standard reference antihistaminic drug) were used.

Preparation of plant extract

Leaves of plant *Helianthus annuus* L. were shade dried at room temperature. Leaves were reduced to coarse powder and passed through a 40 no. sieve to obtain desired particle size. The coarse powder of the *Helianthus annuus* L. leaves (100 g) were extracted with the 95 % ethanol by cold maceration process for period of 72 hours into the conical flask.[16] The filtrate obtained was evaporated to dryness.

$$\% \text{ yield} = (\text{weight of extract/weight of dried powder}) \times 100$$

Phytochemical screening

Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, carbohydrate, phenolic compounds, flavonoids, saponins, steroids, tannins, etc. by using standard procedures.[17]

***In-vitro* antioxidant activity using DPPH as standard**

The stable radical DPPH has been used widely for the determination of primary anti-oxidant activity. DPPH, after accepting electron or hydrogen radical, is converted into stable DPPH-H form. When this conversion occurs, deep violet color of DPPH turns into light yellow color. Unconverted DPPH is detected by UV spectrophotometer at 517 nm against blank and percent inhibition was calculated. Free radical scavenging activity of the ethanol extracts was substantiated by DPPH assay. Sample was prepared in ethanol at different concentrations of 500, 200, 100, 50, 10, 5, and 1 µg/ml. Sample of 1 ml of each concentration was added to 3 ml of 0.004 % ethanol solution of DPPH. Incubation period of 30 min was allowed at room temperature in dark place to complete any reaction that is to be occurred. Then absorbance was measured by UV spectrophotometer at 517 nm against blank. Ascorbic acid was used as standard free radical scavenger and activity of the extract was compared with it.[18]

Activity of the sample was calculated from [(A1–A2)/A1] x100,

Where, A1 is the absorbance of the control and A2 is the absorbance of the standard/ sample extract

Castor oil induced diarrhea model

The mice of weight 20-25 g were divided in to the four groups with four animals in each group and were housed in labeled cages. Animals were given a period of time to adjust to the new environment provided with food and water. The Group I Animal were administered the 0.1 ml saline. Group II Animals received the reference standard drug loperamide (3 mg/kg body weight). Group III Animals were given ethanolic extract of *Helianthus annuus* L. at dose of 250 mg/kg body weight. Group IV Animals were given ethanolic extract of *Helianthus annuus* L. at dose of 500 mg/kg body weight. All animals were fasted for 24 h before the administration of drugs. After 60 minute of administration of doses each animal were feed 2 ml castor oil and placed in the cages and observed for the defecation.[19]

Small intestinal transit model

The mice of weight 20-25 g were divided in to the four groups with four animals in each group and were housed in labeled cages. Animals were given a period of time to adjust to the new environment provided with food and water. Group I Animal were administered the 0.1 ml saline. Group II Animals received the reference standard drug Atropine sulphate (5 mg/kg body weight). Group III Animals were given ethanolic extract of *Helianthus annuus* L. at dose of 250 mg/kg body weight. Group IV Animals were given ethanolic extract of *Helianthus annuus* L. at dose of 500 mg/kg body weight. They were fasted for 24 hours prior to the test, but were allowed free access to water. Thirty minutes after drug administration, 1 ml of charcoal meal (5 % activated charcoal in 10 % aqueous tragacanth) was administered to all the animals in the study and thirty minutes later, all the rats were sacrificed and the abdomen opened. The small intestine was dissected out and the distance covered by the charcoal meal in the small intestine from the pylorus to the caecum was measured and expressed as a percentage of the distance traveled.[20]

Histamine induced bronchospasm in guinea pigs

The guinea pigs were fasted overnight and were divided into four groups with four animals in each group. They were exposed to aerosol of 0.1 % Histamine using nebulizer with constant pressure 160 mm/Hg in histamine chamber and time for pre convulsion dyspnoea was recorded from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of asphyctic convulsions i.e. pre-convulsion time. As soon as pre convulsion dyspnoea commenced, animals were removed from the chamber and placed in fresh air to recover for 24 hours. After 24 hours the Group I serve as control and receive distilled water. Group II received chlorpheniramine maleate (2 mg/kg) and served as standard. Group III and IV received the ethanolic extract at the doses of 250 and 500 mg/kg respectively. These animals were again exposed to Histamine aerosol later at 4 h and 24 h to determine pre convulsion dyspnoea.[21] The percentage protection offered by the treatment was calculated by the following formula,

$$\% \text{ Protection} = \{1 - T1/T2\} \times 100$$

Where, T1 = time in second for pre convulsion dyspnoea before treatment; T2 = time in second for pre convulsion dyspnoea after treatment

Microshock in rabbit model

In this model rabbits in place of gunia pig were utilized. The rabbits were fasted overnight and groups which were previously defined in histamine induced bronchospasm, were exposed to aerosol of 0.2 % histamine using nebulizer with constant pressure 160 mm/Hg in histamine chamber and time for pre convulsion dyspnoea was recorded from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of asphyctic convulsions i.e., pre-convulsion time. With the procedure defined above, after 24 hours the Group I serve as control and receive distilled water. Group II received chlorpheniramine maleate (2 mg/kg) and served as standard. Group III and IV received the ethanolic extract at the doses of 250 and 500 mg/kg respectively. These animals were again exposed to Histamine aerosol later at 4 h and 24 h to determine pre convulsion dyspnoea.[22] The percentage protection offered by the treatment was calculated by the following formula;

$$\% \text{ Protection} = \{1 - T1/T2\} \times 100$$

Where, T1 = time in second for pre convulsion dyspnoea before treatment; T2 = time in second for pre convulsion dyspnoea after treatment

These animal studies were carried out after obtaining the approval of the Institute's Animal Ethics Committee (approval code no. 005/2009/CPCSEA/JNU).

Statistical analysis

All values are expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's tests using Graphpad Prizm 6 software. The results were considered statistically significant if probability factor, $P < 0.05$.[23]

RESULTS AND DISCUSSION

After the extraction, the % yield was found to be 3.82 %. The phytochemical screening of extract of *Helianthus annuus* L. showed the presence of carbohydrates, glycosides, saponins and alkaloids. The presence of phenolic and other flavonoid contents was also confirmed which are one of the most essential phytochemicals to possess the activity.

In-vitro antioxidant activity using DPPH as standard

Absorbance of DPPH at 517 nm was 1.4908 and percentage inhibition was calculated on this absorbance by comparing extract with ascorbic acid. ANOVA was applied to the results by using Graphpad prizm 6 Software. P value is < 0.0001 which shows that the results are significant to that of control.

Table 1: Free radical scavenging activity of ascorbic acid and extract

Concentration (µg/ml)	Absorbance for Ascorbic Acid	% Inhibition by Ascorbic acid	Absorbance for Extract	% Inhibition by Extract
5	0.4458	70.09	0.3321	37.48
10	0.3983	73.28	0.2142	45.38
50	0.2898	80.56	0.1532	49.48
100	0.1498	89.52	0.0993	63.15
200	0.0987	93.38	0.0332	70.94
500	0.0505	96.61	0.0133	74.29

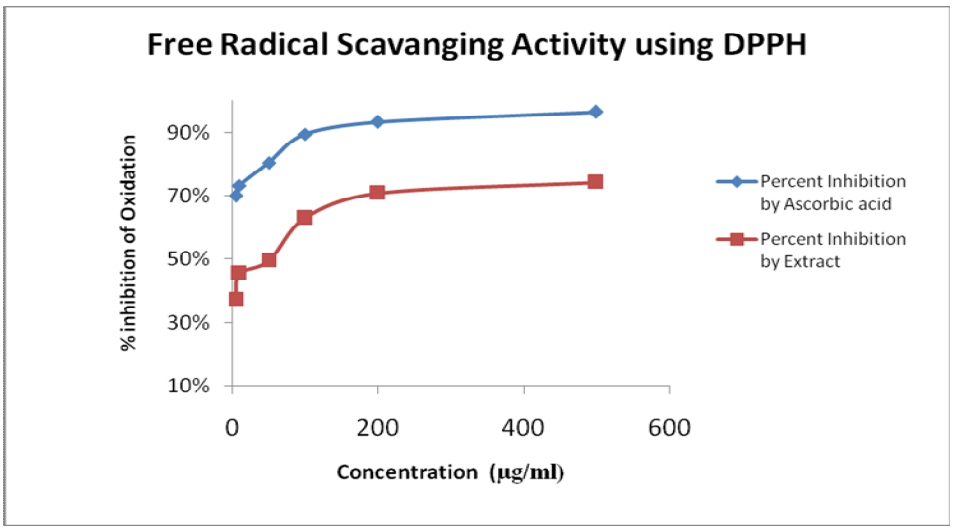

Figure 1: Free radical scavenging activity of ascorbic acid and ethanolic extract of *Helianthus annuus* L

Castor oil induced diarrhea model

Table 2: Effect of *Helianthus annuus* extract on castor oil induced diarrhea in experimental mice, where n = 4

Group	Dose (mg/kg)	Onset of time (minutes)	Total weight of fecal matter (g)	% Reduction
Group I (Control)	-	32.95 ± 2.20	0.357 ± 0.03	0.00
Group II (Standard)	3	89.79 ± 2.55	0.104 ± 0.01	76.71
Group III (Extract)	250	40.29 ± 1.54	0.139 ± 0.03	42.15
Group IV (Extract)	500	74.75 ± 2.12	0.137 ± 0.01	67.01

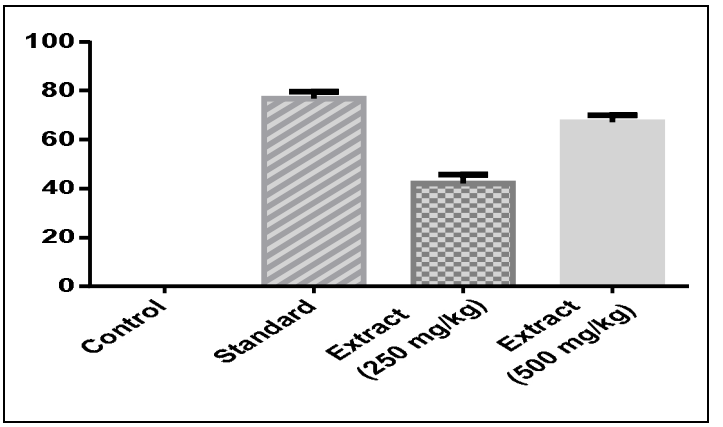

Figure 2: Graphical presentation of one way ANOVA using Graphpad prizm 6 software

In castor oil induced diarrheal model, the ethanolic extract of leaves of *Helianthus annuus* L. at the doses of 250 mg/kg and 500 mg/kg has showed the significant inhibition in the fecal matter in experimental mice with p value is < 0.001. The ethanolic extract of *Helianthus annuus* L. at dose of 500 mg/kg has showed the significant decrease in the fecal matter in comparison with the standard drug loperamide (3 mg/kg). The corresponding decrease in fecal matter was 42.15 % and 67.01 % respectively for 250 mg/kg and 500 mg/kg body weight.

Small intestinal transit model

Table 3: Small intestinal transit model in experimental mice, where n = 4

Group	Dose (mg/kg)	Mean distance (cm ± SD)	% Reduction
Group I (Control)	-	27.90 ± 1.49	0.00 %
Group II (Standard)	5	7.58 ± 0.80	72.40 %
Group III (Extract)	250	21.18 ± 1.47	27.59 %
Group IV (Extract)	500	14.33 ± 1.28	48.62 %

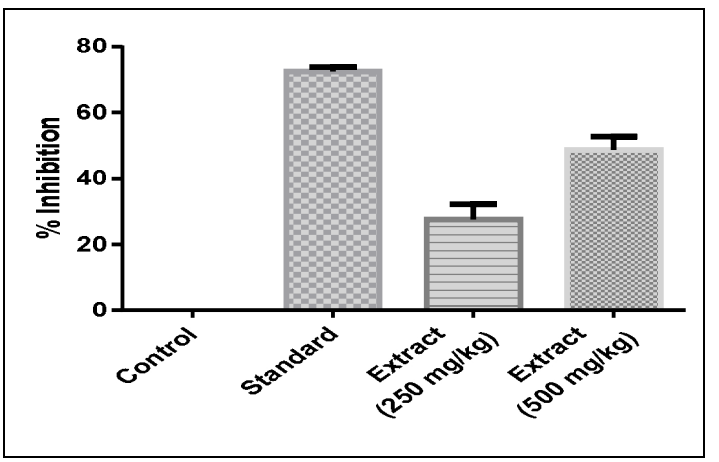

Figure 3: Graphical presentation of one way ANOVA using Graphpad prizm 6 software

In small intestinal transit model, the ethanolic extract of *Helianthus annuus* L. at the dose of 250 mg/kg and 500 mg/kg decreased the intestinal transit. The greater inhibitory effect against the charcoal meal was seen at the dose of 500 mg/kg of body weight in comparison to that of control with p value < 0.001 which is considered to be significant. The effect was compared with the 5 mg/kg dose of atropine sulphate. The corresponding reduction was 27.59 % and 48.62 % respectively for the 250 mg/kg and 500 mg/kg body weight.

Histamine induced bronchocontriction in guinea pigs

Table 4: Histamine induced bronchocontriction in guinea pigs, where n = 4

Group	Dose (mg/kg)	Pre convulsive dyspnea	
		4 h	24 h
Group I (Control)	-	55.10 ± 1.06	57.26 ± 0.97
Group II (Standard)	2	188.26 ± 1.47	143.26 ± 0.88
Group III (Extract)	250	102.79 ± 1.32	94.58 ± 1.69
Group IV (Extract)	500	144.77 ± 1.22	114.29 ± 0.86

Table 5: Percentage protection against histamine induced bronchocontriction in guinea pigs at different time interval, where n = 4

Group	Dose (mg/kg)	Percentage protection	
		4 h	24 h
Group I (Control)	-	-	-
Group II (Standard)	2	70.73 %	60.02 %
Group III (Extract)	250	46.54 %	39.55 %
Group IV (Extract)	500	62.15 %	49.88 %

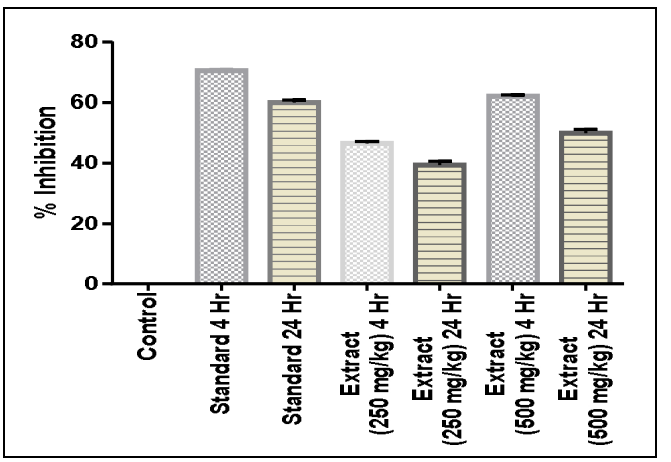

Figure 4: Graphical presentation of one way ANOVA using Graphpad prizm 6 software

In the present study, the guinea pigs were exposed to the 0.1 % of histamine aerosol which showed the sign of progressive dyspnoea leading to convulsion. The chlorphenamine maleate significantly prolong the latent period of convulsions. The ethanolic extract showed significant results when compare to the standard ($p < 0.0001$). The percent protection was found to be 46.54 % and 62.15 % after 4 hours at the doses of 250 mg/kg and 500 mg/kg respectively.

Micro shock method on rabbit

Table 6: Histamine induced bronchoconstriction in rabbits, where n = 4

Group	Dose (mg/kg)	Pre convulsive dyspnea	
		4 h	24 h
Group I (Control)	-	55.10 ± 1.06	57.26 ± 0.97
Group II (Standard)	2	188.26 ± 1.47	143.26 ± 0.88
Group III (Extract)	250	102.79 ± 1.32	94.58 ± 1.69
Group IV (Extract)	500	144.77 ± 1.22	114.29 ± 0.86

Table 7: Percentage protection against histamine induced bronchoconstriction in rabbits at different, where n = 4

Group	Dose (mg/kg)	Percentage protection	
		4 h	24 h
Group I (Control)	-	-	-
Group II (Standard)	2	72.14 %	53.22 %
Group III (Extract)	250	52.55 %	25.43 %
Group IV (Extract)	500	70.69 %	41.65 %

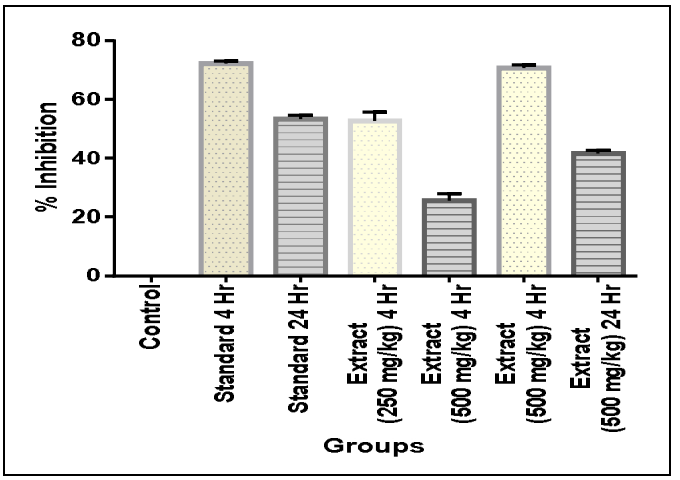

Figure 5: Graphical presentation of one way ANOVA using Graphpad prizm 6 software

In the present study, the rabbit were exposed to the 0.2 % of histamine aerosol which showed the sign of progressive dyspnoea leading to convulsion. The chlorphenamine maleate significantly protected prolonged the latent period of convulsions. The ethanolic extract showed significant protection ($p < 0.001$). The percent protection was found to be 52.55 % and 70.69 % after 4 h at the doses of 250 mg/kg and 500 mg/kg respectively.

CONCLUSION

In present study, the leaves part of *Helianthus annuus* L. were evaluated for its anti diarrheal activity by castor oil induced diarrheal model, small intestinal transit model and antihistaminic activity by histamine induced bronchoconstriction in guinea pigs and rabbits. The significant results were observed in which ethanolic extract showed the marked decrease in the total volume of the fecal matter and acted by increasing the reabsorption of electrolyte and water for antidiarrheal activity and significantly prolonged the pre convulsive time as compared to the control in guinea pigs and rabbits. Leaves were also confirmed for its antioxidant activity. The presence of phenols and flavonoids are responsible for these activities. Sunflower, the heliotropism plant can further be evaluated for active constituents responsible for these activities and utilized for different asthmatic or inflammatory disease treatment and as antidiarrheal herb.

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