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PHARMACOLOGICAL EVALUATION FOR THE ETHANOLIC EXTRACT OF EVOLVULUS ALSINODES RHIZOME FOR IT'S ANTI - ASTHMATIC, ANTIOXIDANT AND ANTI – INFLAMMATORY ACTIVITIES

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ABSTRACT
Asthma is a chronic inflammatory lung disease that can cause repeated episodes of cough, wheezing and breathing difficulty. During an acute asthma episode, the airway lining in the lungs becomes inflamed and swollen. In addition, mucus production occurs in the airway and muscles surrounding the airway spasm. Combined, these cause a reduction in air flow. [1] Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. In recent year there has been tremendous increase in demand for herbal drugs because of its safety, efficacy and better therapeutic results. Due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic compilations. *Evolvulus Alsinodes* is also considered to be therapeutically important in traditional system of medicine. Aim of the study to evaluate the anti-asthmatic, antioxidant and anti-inflammatory activities of ethanolic extract of *Evolvulus Alsinodes* rhizome.

Keywords: Anti - Asthmatic, Antioxidant and Anti – Inflammatory Activities, *Evolvulus Alsinodes*, Phytoconstituents.

INTRODUCTION
The term "**medicinal plant**" include various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses. The word "**herb**" has been derived from the Latin word, *"herba"* and an old French word *"herbe"*. Now days, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non- woody plant. Earlier, the term "herb" was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities (Jamshidi-Kia, 2017).

Plants have been used for medicinal purposes long before prehistoric period. Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically (Hussain *et al*., 2011).

Asthma is a chronic inflammatory lung disease that can cause repeated episodes of cough, wheezing and breathing difficulty. During an acute asthma episode, the airway lining in the lungs becomes inflamed and swollen. In addition, mucus production occurs in the airway and muscles surrounding the airway spasm. Combined, these cause a reduction in air flow. The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function (Holgate, 2008; Busse and Rosenwasser, 2003).

Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating (Kay, 1991).

In recent year there has been tremendous increase in demand for herbal drugs because of its safety, efficacy and better therapeutic results. Due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic compilations. *Evolvulus Alsinodes* is also considered to be therapeutically important in traditional system of medicine (Singh, 2008). Aim of the study to evaluate the anti-asthmatic, antioxidant and anti-inflammatory activities of ethanolic extract of *Evolvulus Alsinodes* rhizome.

MATERIALS & METHODS

Collection and authentication of *Evolvulus Alsinodes*

The dried rhizomes of the *Evolvulus Alsinodes* were collected. The rhizomes were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

Preparation of plant extract

The powdered rhizomes were extracted using ethanol by soxhlet extractor. In this process the powdered drug is placed into the extractor with ethanol as solvent. After extraction the extract was concentrated by evaporation then it was kept in a refrigerator for further use (Gupta *et al*., 2012).

Preliminary phytochemical screening

The ethanolic extract of *Evolvulus Alsinodes* rhizomes were subjected for the following chemical tests for the identification of various active constituents (Yadav *et al.*, 2014)

Acute toxicity studies

Acute toxicity of *Evolvulus Alsinodes* was done as per OECD guidelines 423. The substance was administered in a single dose by gavage using specially designed mice oral tube. Animals were fasted prior to dosing with food but not water withheld overnight. Following the period of fasting, the animals were weighed and the test substance was orally at a dose of 5, 50, 300 and 2000 mg/kg. The animals are observed continuously for first three hours, four any toxic manifestations like increased motor activity, salivation, acute convulsion, coma and death. Changes in the

animal behavior should be noted before and after administration for 24hours. Treated animals are to be further observed for 14 days. If the extract does not produce mortality at the highest dose, then the $1/10^{th}$ or $1/20^{th}$ of the dose was selected for experiment (Akhila *et al*., 2007; Asare *et al*., 2011).

Evaluation Of Anti Asthmatic Activity

***In vivo* anti-asthmatic activity**

Histamine aerosol induced bronchoconstriction in guinea pigs

Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Experimentally bronchial asthma was induced in guinea pigs by exposing histamine aerosol by a nebulizer in an aerosol chamber. The required time for appearance of preconvulsive dyspnoea produced by the histamine was noted for each animal. Each animal was placed in the histamine chamber and exposed to 0.2% histamine aerosol. The preconvulsion time (PCT), i.e. the time of aerosol exposure to the start of dyspnoea leading to the appearance of convulsion, was noted. As quickly as the preconvulsion dyspnoea (PCD) was recorded, the animals were removed from the chamber and positioned in fresh air for recover. This time for preconvulsive dyspnoea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnoea for 2 days. After that, the animals were allotted to four different groups of 4-5 animals per group. Animals in group 1 served as control and received carboxy methyl cellulose. The animals of group 2 and 3 were given, by oral intubation, 100 and 200 mg/kg of the plant extract, respectively, while group 4 received the standard drug - Chlorpheniramine maleate, intraperitoneally. After receiving the drugs, all the animals were again exposed to histamine aerosol in the chamber, one hour, four hours and 24 hours, to determine pre convulsive time (PCT) (Sekizawa *et al*., 1993; Umeno *et al*., 1992)

Percentage protection was calculated using the formula.

$$\text{Percentage protection} = \frac{E^{ta} - E^{tb}}{E^{ta}} \times 100$$

Where *Eta* is the preconvulsion time after administration of drug and *Etb* is the preconvulsion time before administration of drug.

Milk induced leukocytosis and eosinophilia

Mice were divided into 4 groups with six in each group. Blood samples were collected from retro-orbital plexus. Group 1 served as control and received carboxy methyl cellulose solution, groups 2-3 received plant extract at (100-200 mg/kg) group 4 received dexamethasone at 50 mg/kg i.p. All the groups injected boiled and cooled milk (4 ml/kg, s.c.) 30 min after treatments. Total leukocyte and eosinophile count was done in each group before administration of test compound and 24 hours after milk injection. Difference in total leukocytes and eosinophile count before and after 24-hour drug administration was calculated (Mali and Dhake, 2011; Khatri *et al*., 2021).

***Ex vivo* anti-asthmatic activity**

Isolated guinea pig tracheal preparation

Isolated guinea pig tracheal tissue was obtained by, Animals were sacrificed by cervical dislocation and carotid bleeding. The trachea was dissected out and transferred into a dish containing Krebs solution and cut crosswise between the section of the cartilage of the trachea and continuously ventilated and maintained at 37±0.5°C. The adjourned

trachea was allowed to make steady for at least
40 minutes. On equilibrium, the bath was supplied with Krebs solution for every 15 minutes Dose response curve of histamine (10 µg/ml) in plane Krebs solution and in 1 mg/ml of plant extract act in Krebs solution was taken. Percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and presence of plant extract (Boskabady and Sheiravi, 2002; Pedersen *et al*., 1993).

***In vitro* antioxidant activity**

Hydrogen peroxide scavenging

Hydrogen peroxide solution (20 Mm) was prepared with standard phosphate buffer (pH 7.4). Extract samples (25, 50, 100, 200 and 400 µg/ml) in distilled water were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the reference standard. The percentage scavenging of hydrogen peroxide of plant extract was calculated using the formula (Sroka and Cisowski, 2003).

$$\% \text{ Scavenged} = \frac{A^C - A^C}{A^C} \times 100$$

Where, *Ac* = Absorbance of control
As = Absorbance of sample
The experiments were performed in triplicates, and the results were expressed as Mean± S.E.M

Reducing power assay

The reducing power of the extract was determined by the method. 1 ml of the extract solution (25, 50, 100, 200 and 400 µg/ml) was mixed with 2.5 ml phosphate buffer (0.2 M, Ph 6.6) and 2.5 ml of potassium ferricyanide ($[K_2 Fe (CN)_6]$ (10g/l)), then the mixture was incubated at 50^0 C for 20 minutes. A portion (2.5ml) of trichloroacetic acid (TCA) (15%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5ml ferric chloride ($FeCl_3$. 0.1%) and absorbance was measured at 700 nm in UV- visible spectrophotometer.

Protein denaturation

A solution of 0.2% of bovine serum albumin (BSA) was prepared in tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Test drug of different concentration (25, 50, 100, 200 and 400 µg/ml) was prepared using ethanol as solvent. 50 µl of each test drug was transformed to test tubes using micropipette. 5 ml of 0.2% w/v of BSA was added to the test tubes. The control consists of 5 ml of 0.2% w/v of BSA solution and 5µl alcohol. The test tubes were heated at 72^0 C for 5 min and then cooled for 10 min. The absorbance of these solution was determined using UV–visible spectrophotometer at 660nm. Diclofenac sodium was used as standard and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated using the following formula (Kauzmann, 1959; Xiong *et al*., 1997).

The rabbit red blood cell membrane stabilization method

Preparation of red blood cell suspension (RBCs suspension)

The fresh whole rabbit blood (5 ml) was collected from marginal ear vein to syringes containing sodium citrate to prevent clotting.

The tubes were centrifuged at 3000 rpm for 10 minutes and were washed 3 times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

Membrane stabilization test by hypotonicity induced haemolysis

The reaction mixture consists of 1 ml of test sample of different concentration (25, 50, 100, 200 and 400 µg/ml) in normal saline and 0.5 ml of 10% RBC suspension, 1 ml of 0.2 M phosphate buffer, 1 ml hypo saline were incubated at 37^0C for 30 minutes and centrifuged.

Evaluation of anti asthmatic activity

***In vivo* anti asthmatic activity**

Histamine aerosol induced bronchoconstriction in guinea pigs

Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Experimentally bronchial asthma was induced in guinea pigs by exposing histamine aerosol by a nebulizer in an aerosol chamber. The required time for appearance of pre convulsive dyspnoea produced by the histamine was noted for each animal. Each animal was placed in the histamine chamber and exposed to 0.2% histamine aerosol. The preconvulsion time (PCT), i.e. the time of aerosol exposure to the start of dyspnoea leading to the appearance of convulsion, was noted. As quickly as the preconvulsion dyspnoea (PCD) was recorded, the animals were removed from the chamber and positioned in fresh air for recover. This time for preconvulsive dyspnoea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnoea for 2 days. After that, the animals were allotted to four different groups of 4-5 animals per group. Animals in group 1 served as control and received carboxy methyl cellulose. The animals of group 2 and 3 were given, by oral intubation, 100 and 200 mg/kg of the plant extract, respectively, while group 4 received the standard drug - Chlorpheniramine maleate, intraperitoneally. After receiving the drugs, all the animals were again exposed to histamine aerosol in the chamber, one hour, four hours and 24 hours, to determine pre convulsive time (PCT) (Breschi *et al*., 2002; Ibulubo, 2012).

Percentage protection was calculated using the formula.

$$\text{Percentage protection} = \frac{Et^{a} - Et^{b}}{Et^{a}} \times 100$$

Where *Eta* is the preconvulsion time after administration of drug and *Etb* is the preconvulsion time before administration of drug.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of ethanolic extract of

***Evolvulus Alsinodes* rhizome.**

The ethanolic extract of *Evolvulus Alsinodes* rhizomes were subjected to phytochemical screening. The result indicated that, rhizome extract shows the presence of carbohydrate, proteins, cardiac glycosides, flavonoid, tannins and phenol. The knowledge of chemical constituents of plant is desirable because such information will be valuable for synthesis of complex chemical substances and to screen for biological activities.

Phytochemicals are natural bioactive compounds found in plants. They are mainly two groups, which are primary and secondary metabolites. Primary metabolites are sugars, amino acids, proteins and chlorophyll while

secondary metabolites consist of alkaloids, terpenoids and phenolic compounds. The beneficial medicinal effect of plant material results from the combination of secondary metabolites present in the plant. So the systematic screening of plant species is necessary for the purpose of discovering new bioactive compounds.

The phenolic compounds and flavonoids are secondary metabolites in plants having antioxidant activity. They have wide range of biological activities as cardio protection, cell proliferation and anti-aging. Tannins are potential toxic agents to fungi, bacteria and viruses in plants. They are currently investigated for human medicinal use, in order to reduce the risk of coronary heart diseases.

Histamine aerosol induced bronchoconstriction in guinea pigs

Histamine is one of the major inflammatory mediators in the immediate phase of asthma, causing airway hyper responsiveness and bronchial airway inflammation. Histamine induced bronchodialators is the traditional immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to convulsion in guinea pigs and causes very strong smooth muscle contraction, profound hypotension and capillary dialation in the cardiovascular system. A prominent effect caused by histamine leads to severe bronchoconstriction in the guinea pigs that causes convulsion and leads to death. Bronchodialators can delay the occurrence of these symptoms. In this histamine induced bronchospasm the ethanolic extract of the plant *Evolvulus Alsinodes* rhizomes showed significant activity and increase in dose of extract increase % protection. The maximum % protection shown by the plant extract was 60.79 % observed at 200 mg/kg for bronchorelaxant study comparable to that of standard drug chlorpheniramine maleate 78.3 %.

The results of the study confirmed the bronchodialotor properties of the plant, justifying its traditional claim in the treatment of asthma.

Milk induced leukocytosis and eosinophilia

In the present investigation ethanolic extract of the plant *Evolvulus Alsinodes* rhizomes (100, 200 mg/kg) was evaluated for the management of asthma using milk induced leukocytosis and eosinophilia in mice. Asthma involves various types of mediator in pathology. It was demonstrated that potential administration of milk produces a marked increase in leukocytes and eosinophils count after 24 hours of its administration. Leukocytes during asthmatic inflammation release the inflammatory mediators like cytokines, histamine and major basic protein, which promote the ongoing of inflammation. The infiltration of leukocytes potentiates the inflammatory process by the release of reactive oxygen species into the surrounding tissue, resulting in increased oxidative stress and associated with many pathological features of asthma. In this study we observed that leukocyte count was decreased in mice treated with ethanolic extract of plant at doses 100 and 200 mg/kg significantly as compared to control group. Results suggests that the ethanolic extract of the plant *Evolvulus Alsinodes* rhizomes decreases milk induced leukocytes count by normalizing oxidative stress.

An abnormal increase in peripheral eosinophil to more than 4% of total leukocytes count is termed as eosinophil. In asthmatic patient there is an increase in eosinophilic count and mucus hypersecretion and airway hyper reactivity were stimulated. Eosinophils infiltrating the airway also have an effect on mucus secretion by epithelial goblet cell. In our study it was observed that the ethanolic extract of *Evolvulus Alsinodes* rhizomes at doses 100 and 200 mg/kg significantly decreased milk induced eosinophils count.

The ethanolic extract of *Evolvulus Alsinodes* rhizomes decrease the leukocyte count by normalizing oxidative stress and decrease in eosinophil count may reduce type I hypersensitivity in asthma. In conclusion ethanolic extract of the plant *Evolvulus Alsinodes* rhizomes is effective in management of asthma.

Isolated guniea pig tracheal preparation

It was observed that the ethanolic extract of *Evolvulus Alsinodes* rhizomes inhibits contraction produced by histamine in these tissue preparations. Histamine (10 µg/ml) was taken in different dose level and concentration response curve was plotted. Study revealed that the ethanolic extract of the plant *Evolvulus Alsinodes* rhizome exhibit significant percentage decreased contraction at concentration 0.8 mg/ml.

Hydrogen peroxide scavenging

The ethanolic extract of *Evolvulus Alsinodes* rhizomes showed good dose dependent hydrogen peroxide scavenging activity. Hydrogen peroxide (H_2O_2), a biologically relevant, non- radical oxidizing species, may be formed in tissue through oxidative processes. Although hydrogen peroxide is a weak oxidizing agent it can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide itself is not reactive, but can generate hydroxyl radical (OH) (via fenton reaction) in the cells resulting in initiation and propagation of lipid peroxidation. Thus the removal of H_2O_2 is very important for antioxidant defence in cell or food systems. Hydrogen peroxide can cross cell membranes and may oxidize a number of compounds. The ability of the extracts to quench OH^- seems to be directly related to the prevention of the lipid peroxidation and appears to be moderate scavenger of active oxygen species, thus reducing rate of chain reaction.

As a conclusion the ethanolic extract of the *Evolvulus Alsinodes* rhizomes showed hydrogen peroxide activity as compared to standard ascorbic acid (100 µg/ml).

Reducing power assay

The reducing power of the extract, which may serve as a significant reflection of antioxidant activity, was determined using reducing power assay, where by the yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. The presence of radicals (ie, antioxidant) causes the conversion of the Fe^{3+}/ ferricyanide complex $[Fe^{3+}(CN^-)_6]$ used in this method to the ferrous form (Fe^{2+})/ ferrocyanide complex $[Fe^{2+}(CN^-)_6]$. Therefore, by measuring the formation of Pearls Prussian blue spectrophotometrically, the Fe^{2+}concentrationcan be monitored, a higher absorbance indicates a higher reducing power.

The increased reducing power in the extracts indicates that some components in the extract were electron donors that could react with the free radicals to convert them into more stable products to terminate radical chain reaction. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable unreactive species.

The reducing power of the ethanolic extract of *Evolvulus Alsinodes* rhizomes increased with increasing concentration.

Protein denaturation method

Denaturation of protein is a well-documented cause of inflammation. Production of auto antigens may be due to the denaturation of tissue protein. Agents that can prevent protein denaturation therefore would be worthwhile for anti- inflammatory drug development. The mechanism of denaturation probably involves the alteration electrostatic hydrogen, hydrophobic and disulphide bonding. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs in their ability to stabilize heat treated protein at physiological pH.

The ethanolic extract of the *Evolvulus Alsinodes* rhizomes exhibited concentration dependant inhibition of protein denaturation. Therefore, from the study it can be concluded that the rhizomes of the plant extract possess marked in vitro anti- inflammatory effect.

Rabbit red blood cell membrane stabilization method

The ethanolic extract of plant exhibit membrane stabilization property by inhibiting hypotonicity which effectively indicates the anti-inflammatory property. The viability of the cell depends up on the integrity of their membrane exposure of RBC to hypotonic medium, injurious substance results in the lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin. Compounds with membrane stabilizing properties are well known for their ability to their ability to interfere with the early phase of inflammatory reactions.

Table 1: Histamine aerosol induced bronchoconstriction in guinea pigs

Group	Latent period of convulsion			
	Before	**1 hour**	**4 hour**	**24 hour**
Control	16.3±2.23	18.36±0.183	18.63±0.186	18.4±0.12
Evolvulus Alsinodes Ethanolic extract (100 mg/kg)	16.71±1.31	29.65±.28	39.38±0.05*	28.2±0.23
Evolvulus Alsinodes Ethanolic extract (200 mg/kg)	15.71±0.77	30.5±3.08	40.36±1.04*	28.4±.35
Standard (CPM) (1 mg/kg)	18.46±0.89	60.25±0.03*	68.26±1.01**	36.5±0.55

Table 2: % Protection of the plant *Evolvulus Alsinodes* rhizomes against histamine induced bronchoconstriction in guinea pig

Group	% Protection		
	1 hour	**4 hour**	**24 hour**
Control (carboxy methyl cellulose)	10.9	12.3	11.4
Evolvulus Alsinodes ethanolic extract (100 mg/kg)	43.2	57.2	40.2
Evolvulus Alsinodes ethanolic extract (200 mg/kg)	48	60.79	44.3
Standard(CPM)	69.76	78.3	50.1

Table 3: Effect of ethanolic extract of *Evolvulus Alsinodes* rhizomes on histamine induced contraction on isolated guinea pig tracheal preparation

S. No.	Dose of histamine (10µg/ml) in ml	Control (Histamine 10 µg/ml) % maximum contraction	Test Histamine(10µg/ml)+EEAC(1mg/ml) % maximum contraction
1	0.1	38.46 ± 1.58	30.76 ± 1.32**
2	0.2	53.48 ± 4.23	46.15 ± 2.91**
3	0.4	61.5 ± 3.89	53.48 ± 3.31**
4	0.8	73.07 ± 2.32	65.3 ± 1.76**
5	1.6	84.6 ± 2.13	69.2 ± 1.09**
6	3.2	100 ± 1.07	76.92 ± 2.11*

Values are Mean± S.E.M., where n=6 in each group, P< 0.05 *, P< 0.01 ** (significant) compared with control. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

Table 4: Hydrogen peroxide scavenging activity of ethanolic extract of *Evolvulus Alsinodes* rhizomes

Sl no.	Concentration(µg/ml)	Absorbance [A]	% inhibition
1	25	0.632±0.0005	17.16
2	50	0.539±0.0052	29.5
3	100	0.474±0.0056	38.04
4	200	0.414±0.0005	46
5	400	0.357±0.0032	53.3
6	Ascorbic acid (100 µg/ml)	0.256±0.056	60.23

(Values are Mean±S.E.M., where n=6) in each group, P< 0.05 *, P< 0.01 **(significant) compared with control. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

Table 5: Reducing power activity of ethanolic extract of *Evolvulus Alsinodes* rhizomes

Sl no	Concentration (µg/ml)	Absorbance [A]
1	25	0.782±0.32
2	50	0.891±0.21
3	100	1.3±0.35
4	200	1.4±0.42
5	400	1.56±0.82

Table 6: Effect of ethanolic extract of *Evolvulus Alsinodes* on protein denaturation

Sl no	Concentration (µg/ml)	Absorbance [A]	% inhibition
1	25	1.28±0.05	14
2	50	0.578±0.03	61.6
3	100	0.382±0.002	74.63
4	200	0.189±0.01	87.4
5	400	0.172±0.002	88.57
6	Diclofenac sodium (100µg/ml)	0.165±0.005	89.43

(Values are Mean±S.E.M., where n=6) in each group, P< 0.05 *, P< 0.01 ** (significant) compared with control. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

Table 7: Effect of ethanolic extract of *Evolvulus Alsinodes* rhizomes on hypo tonicity induced RBC membrane stabilization

Sl no	Concentration (µg/ml)	Absorbance[A]	% Protection	% Haemolysis
1	25	0.61±0.03	41.4	58.6
2	50	0.58±0.002	44.3	55.7
3	100	0.382±0.004	63.3	36.7
4	200	0.36±0.009	65.3	34.7
5	400	0.32±0.007	69.82	30.18
6	Diclofenac sodium (100 µg/ml)	0.34±0.008	66.75	33.25

(Values are Mean±S.E.M., where n=6) in each group, P< 0.05 *, P< 0.01 **(significant) compared with control. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test

CONCLUSION

The result of the investigation showed that the ethanolic extract of *Evolvulus Alsinodes* rhizomes possess anti asthmatic activity. The antioxidant and anti-inflammatory property of the plant also supports its anti-asthmatic property. Drugs effective in asthma are mostly steroidal in nature. Phytochemical analysis showed presence of flavonoid and steroids. The anti-asthmatic property showed by the plant may be because of these chemical moieties. The results obtained in the study supports the traditional and also demands further research and to isolate and characterize active principles responsible for anti-asthmatic activity.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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