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ANTI-NOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITY OF *TRAPA BISPINOSA, CHENOPODIUM ALBUM* AND *CUSCUTA REFLEXA*

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ABSTRACT

Objective: The aim of this study was to assess analgesic and anti-inflammatory action of aqueous ethanolic extract of *Trapa bispinosa* (fruits)*, Chenopodium album* (leaves) *and Cuscuta reflexa* (stem). **Methodology:** Aqueous ethanolic extracts of *Trapa bispinosa* (fruits)*, Chenopodium album* (leaves) *and Cuscutta reflexa* (stem) were prepared by simple maceration process which yielded 0.8%, 8.3% and 3.0% semi solid extracts respectively. Albino rats were divided into twelve groups (n=6). Group I & II were only given normal saline and formaldehyde respectively. Group III was given diclofenac sodium while groups IV to XII were given plant extracts in different doses one hour prior to administration of formaldehyde in sub planter region of left hind paw of rats. Paw sizes of rats of all the groups were measured with vernier caliper. Readings were taken at the intervals of 00hour, 01hour, 02hours, 03hours and 04hours after treatment. Analgesic activity was performed on mice by using hot plate methods in which animals were arranged in eleven groups (n=6). Group A and B were given normal saline and diclofenac sodium, respectively. Groups C-K were administered with plant of each mouse was

observed by placing the animal on hot plate while the temperature of hotplate was controlled at 55 to 56°C. Observations were made at 00, 30, 60, 90 and 120 minutes. **Results:** Phytochemical investigations confirmed the presence of alkaloids, steroids, phenols, saponins, tannins, flavonoids and terpenoids. *Chenopodium album* (400mg/kg) represented maximum %age inhibition (64%) in edema, while *Trapa bispinosa* (400mg/kg) and *Cuscutta reflexa* (400mg/kg) showed 56% and 38% inhibition in edema, respectively as compared to diclofenac sodium (10mg/kg) which inhibited 72%. Hot plate activity indicated that all three plant extracts significantly (P<0.001) increased the latency time. **Conclusion:** Results indicated that all three plants can be used to treat signs of inflammation and pain.

Keywords: Analgesia, Inflammation, Diclofenac sodium, Latency time, Paw edema

INTRODUCTION

Inflammation is protective response of body against noxious stimuli which is characterized by vasodilatation, redness, itching, pain, edema and recruitment of immune cells to the target cite **[1]**. Inflammatory disorders are routinely encountered on daily basis by the whole population all over the World. Some of these heal automatically if the intensity of inflammation is low but if severity is beyond a certain limit then anti-inflammatory agents are usually used **[2]**. NSAIDs used to relieve pain and inflammation are most often causing peptic ulcer. Thus, it is difficult to manage noxious stimuli of pain and inflammation without aggravation of stomach acidity **[3, 4]**. However use of herbal extracts and preparation for the management of pain and inflammation are considered safe in this context **[5]**. Focus is particularly to discover an agent which inhibits inflammation and pain stimuli without causing marked effect on stomach pH. For this purpose, conventional use of herbs and medicinal plants are encouraged. **[6]**. There is a huge list of plants and herbs which are effectively used in the management of pain and inflammation and their analgesic and anti-inflammatory activities are scientifically proved **[7]**. In the past few decades, several attempts have been made to evaluate the anti-inflammatory and analgesic activities of different plants and compounds derived from plants **[8-10]**. In this research work we specially focused the evaluation of three plants for analgesic and anti-inflammatory actions. These three plants are locally available and used conventionally for these

purposes but no analgesic and anti-inflammatory activities of these plants are pharmacologically proved before that.

MATERIALS AND METHODS

Chemicals and drugs: Formaldehyde, distilled water, ethanol, normal saline, Dimethyl sulfoxide (DMSO), sulphuric acid, hydrochloric acid, chloroform and diclofenac sodium.

Instruments/Equipments: Electronic balance (Galvano), electric grinder (National, Japan), vortex mixer (Galvano), beakers, stirrer, funnel, tripod stand, filter papers, air tight containers, jars, burner, water bath, refrigerator (Orient), rotary evaporator (Yamato), test tubes, vernier caliper and stop watch.

Collection of plants and authentication: *Trapa bispinosa*, *Chenopodium album* and *Cuscuta reflexa* were used in this research. Fresh green leaves of *Chenopodium album*, stems of *Cuscuta reflexa* and fresh fruit of *Trapa bispinosa* were collected from local fields of Lahore, Pakistan. All three plant materials were identified by botanist and specimen were preserved in herbarium of Riphah Institute of Pharmaceutical Sciences (RIPS), Riphah International University Lahore. *Trapa bispinosa*, *Chenopodium album* and *Cuscuta reflexa* were given voucher numbers RIU-02-06-35, RIU-02-06-36 and RIU-02-06-37, respectively.

Preparation of crude extracts: Plant materials were separately washed with water and dried properly. Dried materials were then grinded separately into coarse powder by using electric grinder. 550g of *Cuscuta reflex* was soaked in 1100ml of 70% aqueous ethanol, while 272g of *Chenopodium album* and 550g of *Trapa bispinosa* were soaked separately in 70% aqueous ethanol (700ml and 100ml respectively). The process of maceration was performed for five days during which soaked materials were thoroughly stirred thrice daily. At the end of fifth day of maceration, soaked materials were first filtered using muslin cloth and then through filter papers. Residues were again macerated for five days individually in 500ml of 70% aqueous ethanol to get more filtrates. At the end of filtration the filtrates so obtained were individually subjected to rotary evaporator, to evaporate the solvents **[11]**. Semi solid pastes of thick consistency of all three extracts were obtained. Newly formed pastes were weighed to find the exact percentage yields which were 8.3% of *Chenopodium album*, 3.0% of *Cuscuta reflexa* and 0.8% of *Trapa bispinosa*. The extract pastes were individually packed in air

tight containers and put into refrigerator (8°C) after proper labeling for further use.

Solubility testing: For solubility testing the solvents used were water, ethanol, normal saline and DMSO. All three selected plant extracts were tested for solubility in the ratio of 1:1 and 1:5 in above mentioned solvents.

Phytochemical Screening: The extracts were then tested for various phytochemical studies. Following methods were used.

Test for Saponins: 500 mg of extract was dissolved in boiling water in test tube. Then it was cooled down and vigorously shacked. Presence of stable foam indicated saponins **[11]**.

***Test for Proteins (Ninhydrin Test)*:** Extract was boiled with 2ml of 0.2% ninhydrin solution, violet color indicated the presence of aminoacids and proteins **[12]**.

Test for Carbohydrates (Molisch's Test): Extract was blended with 2ml of Molisch's reagent and mixture was shaken. 2ml of concentrated sulfuric acid was added carefully along the side of test tube. Appearance of violet ring at inter-phase, indicated the presence of carbohydrates **[12]**.

Test for Tannins: 5ml of crude extract and few drops of 1% lead acetate were mixed together. A yellow precipitate was formed which indicated the presence of tannins **[13]**.

Test for Phenols: Crude extract was mixed with 2ml of 2% ferric chloride solution. Blue green or black coloration indicated the presence of phenols **[12]**.

Tests for Alkaloids: 500mg of crude extract was treated with 8ml of 1% hydrochloric acid warmed on water bath and then filtered and divided into four test tubes **[14]**.

a) ***Hager's Test:*** 2ml of filtrate was mixed with few drops of Hager's reagent. Appearance of turbidity or yellow precipitates indicated the presence of alkaloids.
b) ***Wagner's Test:*** 2ml of filtrate was mixed with few drops of Wagner's reagent. Appearance of reddish brown precipitates indicated the presence of alkaloids **[15]**.
c) ***Dragendroff's Test:*** 2ml of filtrate was mixed with Dragendroff's reagent. Appearance of turbidity or precipitates indicated the presence of alkaloids **[14]**.
d) ***Mayer's Test:*** 2ml of filtrate was mixed with Mayer's reagent. Appearance of turbidity or precipitates indicated the presence of alkaloids **[15]**.

Test for Glycosides (Keller Kiliani test): The extract was blended with 2ml of glacial

acetic acid containing one to two drops of 2% ferric-chloride solution. Then mixture was poured into another test tube which was containing concentrated sulfuric acid of 2ml. Brown ring at the inter-phase indicated the presence of glycosides **[12]**.

Test for Flavonoids (Alkaline Reagent test): Crude extract was mixed with 2ml of 2% sodium hydro-oxide solution. An intense yellow color was formed which turned colorless on addition of few drops of dilute acid, this indicated the presence of flavonoids **[12]**.

Test for Steroids: The extract was blended with 2ml of chloroform and concentrated sulfuric acid was added side wise. Red color produced in lower chloroform layer indicated the presence of steroids **[12]**.

Test for Terpenoids: Extract was dissolved in 1ml of chloroform and 1ml of acetic anhydride. 2ml of concentrated sulfuric acid was then added. Reddish violet color indicated the presence of terpenoids **[13]**.

Animals and experimental design: Anti-inflammatory study was conducted on albino male rats weighing between 160 to 190g. Ethical clearance for the use of animals was obtained from research and ethical committee of institute with protocol no RIU-AEC-110. The animals were kept in cages with grouping of six albino male rats in each cage. All animals were placed in animal house of Riphah Institute of pharmaceutical Sciences Lahore, where they had free access of water, animal diet and controlled room temperature of 25°C. The analgesic study was carried out on thirty six albino mice weighing between 30 to 40g. The animals were kept in cages with grouping of six albino mice in each cage. All animals were placed where they had free access of water, animal diet and controlled room temperature of 25°C.

Formaldehyde induced rat paw edema: The activity was started with twelve groups with six rats in each group. Group I received normal saline (10ml/kg/p.o.). Group II was only administered with formaldehyde (0.1ml/kg) subcutaneously in paw, Group III was given the standard drug diclofenac sodium (10mg/kg/i.p), Groups IV-VI received extract of *Trapa bispinosa* at dose 100, 200 and 400mg/kg/p.o, respectively. Groups VII-IX received extract of *Chenopodium* album at dose 100, 200 and 400mg/kg/p.o, respectively. Groups X-XII received extract of *Cuscuta reflexa* at dose 100, 200 and 400mg/kg/p.o, respectively. Formaldehyde dose of 0.1ml/kg was injected in sub planter region of left hind paw of rats to all experimental groups from group II to

XI. Treatment to standard control and experimental groups will be given according to above mentioned doses, one hour prior to administration of formaldehyde. Paw sizes of rats of all the groups were measured with vernier caliper. Vernier readings were observed at the intervals of 00hour, 01hour, 02hours, 03hours and 04hours after treatment **[16]**.

Hotplate method in mice for analgesic activity: The activity started with eleven groups of six albino mice in each group. Group-A was given distilled water (5ml/kg), Group-B was injected with standard drug diclofenac sodium (10mg/kg/i.p), Groups-C, D and E were given extract dose of *Trapa bispinosa* (100, 200 and 400mg/kg/i.p). Groups-F, G and H were given extract of *Chenopodium album* at dose (100, 200 and 400mg/kg/i.p) and Groups-I, J and K were given extract of *Cuscuta reflexa* in dose (100, 200 and 400mg/kg/i.p). Normal group latency time of each mouse was observed by placing the animal on hot plate while the temperature of hotplate was controlled at 55 to 56°C. Treated group animals were placed on hotplate and time until either licking or jumping occurs was recorded by a stop watch. Readings were taken at 00minute, 30minutes, 60minutes, 90minutes and 120 minutes **[17]**.

RESULTS

Solubility studies of all three extracts: Solubility of all three extracts was determined by dissolving in water, ethanol, normal saline and DMSO in ratio of 1:1 and 1:5. Solubility results are shown in table 1.

Phytochemical testing of Plants: Different phytochemical tests were performed to determine the various phytochemical constituents of all three plant extracts. The results of these phytochemical tests were categorized as either positive or negative and referred as +ve and –ve respectively as shown in table 2.

Anti inflammatory activity of formaldehyde induced rat paw edema: The normal control group did not show any change in paw sizes with respect to time scale observation. The disease control showed visible increase in paw edema while the standard drug controlled the edema at certain level. The paw sizes of all these experimental groups were observed and measured with vernier caliper. The readings were taken on time scale in millimeters.

Trapa bispinosa showed some decrease in volume of edema in certain intervals when applied at dose 100mg/kg on time scale. At the dose of 200mg/kg, extract showed major

decrease in volume of edema on 03hrs and04 hrs of time scale but the dose 400mg/kg showed maximum control and reduction in volume of edema.

The extract of *Chenopodium album* also showed some decrease in volume of edema in certain intervals at the dose of 100mg/kg on time interval. Moreover, at the dose of 200mg/kg, extract showed major control and decrease in volume of edema on 03hrs and 04hrs of time scale. At dose 400mg/kg, extract showed maximum control and reduction in volume of edema at 03hrs and 04hrs on time scale. Extract of *Cuscuta reflexa,* which did not showed any visible control and decrease in volume of edema in certain intervals at the oral dose of 100mg/kg. On the given dose of 200mg/kg, the extract showed minimal decrease in volume of edema on 03hrs and 04hrs of time scale while in dose of 400mg/kg, extract showed some control and reduction in volume of edema at 03hrs and 04hrs on time scale. Detailed results are shown in table 3.

Table 3 represents various paw sizes measured on different time interval after applying different doses. However, values were expressed as Mean±S.D with n=6. One way Anova followed by multiple comparison "t"test was applied. $P<0.05$ was considered as significant.

%age inhibition of edema by plant extracts: Percentage inhibition of edema of standard drug group, and all nine groups treated with three extracts i.e. *Trapa bispinosa,* (at doses of 100, 200 and 400mg/kg), *Chenopodium album,* (at doses of 100, 200 and 400mg/kg) and *Cuscuta reflexa* (at doses of 100, 200 and 400mg/kg) were observed at time intervals of 00hr, 01hr, 02hrs, 03hrs and 04hrs.

The standard drug inhibited edema from 25% to 72% at time intervals of 00hr, 01hr, 02hrs, 03hrs and 04hrs. *Trapa bispinosa* at 100mg/kg dose reduced edema from 16% to 53% and at 200mg/kg it inhibited edema from 11% to 52 % at dose of 400mg/kg, inhibition of edema was 11% to 56%. Extract of *Chenopodium album* at 100mg/kg reduced edema from 24% to 60% while at dose 200 mg/kg inhibition of edema was 32% to 63% and at dose 400mg/kg the inhibition percentage was 33% to 64%.

The plant extract of *Cuscuta reflexa* at 100mg/kg reduced edema from 2% to 15% and at increased dose of 200mg/kg reduction of edema was 3 % to 16%. When applying the maximum dose of 400 mg/kg, percentage inhibition was 4% to 38% at time intervals.

Analgesic activity in mice: *Trapa bispinosa* showed some increase in latency in certain intervals at the applied dose of 100mg/kg on time scale. At the dose of 200mg/kg, extract showed slightly better increase in latency time between 120 to 180 min. While the dose of 400mg/kg, extract showed significant ($P < 0.001$) increase in latency time between 90min to 180min. *Chenopodium album* also showed some increase in latency time at certain intervals at the intraperitoneally applied dose of 100 mg/kg on time scale. Moreover, at the dose of 200mg/kg, it showed marked increase in latency between 90 to 180 min on time scale and at the dose of 400mg/kg, extract showed most significant ($P < 0.001$) increase in latency. Extract of *Cuscuta reflexa* moderately increased latency time at certain intervals at the applied dose of 100mg/kg on time scale. At dose 200mg/kg, the extract showed moderate increase in latency time on time scale and at dose of 400mg/kg, extract showed significant ($P < 0.001$) increased latency between 60 to 180min on time scale. The standard drug diclofenac sodium showed significant latency time.

Table 1: Solubily studies of aqueous ethanolic extracts of *Trapa bispinosa, Chenopodium album* and *Cuscuta reflexa.*

Solvents used and Ratio		*Trapa bispinosa*	*Chenopodium album*	*Cuscuta reflexa*
Water	1:1	Soluble	Soluble	Soluble
	1:5	Soluble	Soluble	Soluble
Ethanol	1:1	Not soluble	Not soluble	Not soluble
	1:5	Soluble	Soluble	Not soluble
Normal saline	1:1	Soluble	Soluble	Not soluble
	1:5	Soluble	Soluble	Soluble
DMSO	1:1	Soluble	Soluble	Soluble
	1:5	Soluble	Soluble	Soluble

Table 2: Phytochemical studies of aqueous ethanolic extracts of *Trapa bispinosa, Chenopodium album* and *Cuscuta reflexa*

No	Phytochemical Tests		*Trapa bispinosa*	*Chenopodium album*	*Cuscuta reflexa*
1	Saponins		+ve	+ve	+ve
2	Proteins (Ninhydrin test)		+ve	+ve	-ve
3	Carbohydrates (Molisch test)		+ve	+ve	+ve
4	Tannins		+ve	+ve	+ve
5	Phenols test		+ve	+ve	+ve
6	Alkaloids	Hager's test	+ve	+ve	+ve
7		Wagner's test	+ve	+ve	+ve
8		Dragendroff's test	-ve	-ve	+ve
9		Mayer's test	-ve	-ve	+ve
10	Glycosides (Keller Kiliani test)		+ve	+ve	+ve
11	Flavonoids (Alkaline reagent test)		+ve	+ve	+ve
12	Steroids		+ve	+ve	+ve
13	Terpenoids		-ve	-ve	-ve

Table 3: Paw sizes with respect to doses and time interval.

Experimental Group	Paw sizes in (mm)				
	00 hr	01 hr	02 hr	03 hr	04 hr
Group-I (Normal saline 10ml/kg)	3.96±0.39	3.96±0.39	3.96±0.39	3.96±0.39	3.96±0.39
Group-II (Formaldehyde 0.1mg/kg)	6.60±0.41	7.60±0.35	9.18±0.43	10.50±0.52	12.58±0.85
Group-III (Diclofenac sodium 10mg/kg)	4.90±0.21*	5.00±0.34*	4.83±0.25**	4.41±0.38***	3.51±0.41***
Group-IV (Tb 100mg/kg)	5.50±0.51*	6.15±0.56*	6.13±0.63**	6.15±0.22**	5.83±0.31***
Group-V (Tb 200mg/kg)	5.90±0.24*	6.10±0.41**	6.05±0.14**	6.05±0.58***	5.96±0.30***
Group-VI (Tb 400mg/kg)	5.90±0.25**	6.13±0.65**	5.98±0.48**	6.78±0.56***	5.45±0.45***
Group-VII (Ca 100mg/kg)	5.00±0.59*	4.65±0.40*	4.63±0.50*	4.58±0.49**	4.56±0.45**
Group-VIII (Ca 200 mg/kg)	4.60±0.44*	4.40±0.32**	5.33±0.34*	4.75±0.42**	4.93±0.49**
Group-IX (Ca 400 mg/kg)	4.40±0.41**	4.30±0.36***	5.63±0.44**	4.19±0.35***	4.47±0.37**
Group-X (Cr 100 mg/kg)	6.50±0.24	6.73±0.45*	8.06±0.66	9.20±0.32	10.66±0.56
Group-XI (Cr 200mg/kg)	6.42±0.48*	6.63±0.40*	8.08±0.66*	7.15±0.27**	10.46±0.50*
Group-XII (Cr 400mg/kg)	6.31±0.39**	6.50±0.29**	7.39±0.33**	7.88±0.44*	7.81±0.46**

Table 4: Percentage inhibition of edema

Experimental Groups	Percentage inhibition				
	00 hr	01 hr	02 hr	03 hr	04 hr
Group-III (Diclofenac sodium 10mg/kg)	25%	34.20%	47.30%	58%	72%
Group-IV (Tb 100mg/kg)	16%	19%	33%	41%	53%
Group-V (Tb 200mg/kg)	11%	19%	34%	42%	52%
Group-VI (Tb 400mg/kg)	11%	19%	34%	44%	56%
Group-VII (Ca 100mg/kg)	24%	38%	49%	56%	60%
Group-VIII (Ca 200 mg/kg)	30%	42%	41%	54%	63%
Group-IX (Ca 400 mg/kg)	33%	43%	38%	60%	64%
Group-X (Cr 100 mg/kg)	2%	11%	12%	38%	15%
Group-XI (Cr 200mg/kg)	3%	12%	12%	31%	16%
Group-XII (Cr 400mg/kg)	4%	15%	20%	25%	38%

Percentage inhibition of paw edema was calculated by using following formula; Percentage inhibition =(C-T/C)*100; C=Increase in paw volume of control group. T=Increase in paw volume after treatment.

Table 5: Latency in experimental groups on standard and extract doses

Experimental Groups	Latency time (Minutes)					
	00 minutes	30 Minutes	60 Minutes	90 Minutes	120 Minutes	180 Minutes
Group-A (Normal saline 5ml/kg)	11.00±0.37	11.30±0.50	11.00±0.38	11.30±0.50	11.30±0.50	11.58±0.43
Group-B (Diclofenac sodium 10mg/kg)	13.00±0.52*	13.83±0.48*	15.50±0.43**	16.60±0.49**	20.33±1.11***	24.30±0.67***
Group-C (Tb 100mg/kg)	12.30±0.42*	12.00±0.58*	12.83±0.60*	13.30±0.52**	13.83±0.31**	13.83±0.48**
Group-D (Tb 200mg/kg)	13.33±0.49**	14.33±0.33**	14.50±0.43**	14.83±0.31**	14.50±0.43**	15.10±0.31***
Group-E (Tb 400mg/kg)	12.83±0.60*	15.00±0.37**	15.00±0.37**	19.33±1.08***	20.10±0.65***	23.33±0.49***
Group-F (Ca 100mg/kg)	12.83±0.60*	14.33±0.56**	14.00±0.37**	14.83±0.48**	14.33±0.67**	15.00±0.37**
Group-G (Ca 200 mg/kg)	13.83±0.48*	15.83±0.31**	16.00±0.37**	16.16±0.48**	16.83±0.31**	18.16±0.48***
Group-H (Ca 400 mg/kg)	14.16±0.31**	15.33±0.33**	19.66±0.56***	23.00±0.37***	21.33±0.49***	21.66±0.56***
Group-I (Cr 100 mg/kg)	15.16±0.31**	16.16±0.31**	17.50±0.43***	18.16±0.31***	17.16±0.48***	17.83±0.48***
Group-J (Cr 200mg/kg)	15.33±0.33**	16.50±0.43**	18.16±0.31***	19.00±0.37***	19.66±0.56***	17.00±0.37***
Group-K (Cr 400mg/kg)	15.16±0.31**	17.83±0.31***	18.83±0.31***	18.66±0.33***	18.00±0.37***	17.83±0.48***

Values were expressed as Mean±S.D and n=6. One way Anova followed by multiple comparison t test was applied. P<0.05 was considered significant.

DISCUSSION

Edema caused by inflammation is the result of synergism among variety of inflammatory mediators that results in increased vascular permeability and blood flow **[18]**. Leucocytes play an important role in the development of inflammation while neutrophils have important source free radicals at the inflamed area. When there is decrease in population of neutrophils this mechanism gets involved in reducing inflammation **[19]**. It has been observed that formaldehyde produces inflammation through proliferation and migration of fibroblasts which are involved in formation of connective tissue. Acute inflammation produced by formaldehyde results from the cell damage. Inhibition of edema produced by formaldehyde is one of the best procedures adopted for sub acute inflammatory agents and closely resembles with human inflammation **[20]**. In this study inflammation was induced by the use of formaldehyde by understanding above described severity over paw tissues of rats. Diclofenac sodium was used as standard drug in assessment of anti-inflammatory and analgesic activities. It showed remarkable results on the given dose to rats and mice. Diclofenac sodium belongs to non-steroidal anti-inflammatory class which is widely used in relieving pain and swelling. Its mechanism is to block enzymes like COX which results in cordon the prostaglandins fusion. By this way pain and inflammation tends to be inhibited.

Chenopodium album is widely used in herbal medicines since many years to cure inflammation and pain. It contains various phytochemical constituents like saponins, tannins, flavonoids, steroids, alkaloids, glycosides, phenols and lignan. Phenols and lignan contains many aromatic rings with multiple hydroxyl groups. *Chenopodium album* plant has number of curing effects that are found in its various parts like leaves, roots and stem. Keeping in view its contribution in treating many illnesses this study was carried out to investigate its effects particularly on swelling and pain. Plant extract was given to rats at various doses for the observation of its edema and pain reduction capabilities on different time intervals. It was observed that at certain volume and time interval, latency was increased in mice and swelling was reduced in rats. In comparison to different given doses of *Chenopodium album* to rats it was also observed that at the maximum applied dose of 400mg/kg the plant extract showed competitive result for treating swelling and pain. All the constituents are responsible for

anti-inflammatory and analgesic activites **[21]**.

There are many important constituents found in *Cuscuta reflexa* which are flavonoids, alkaloids, glycosides, saponins, tannins, phenols and other organic substances. It also contains chemical constituents like cucutine, stigmasterol, kaempferol, delcitol, myricetin and coumarin. *Cuscuta reflexa* stem is used in making medicines to cure various types of inflammation and relieves pain. *Cuscuta reflexa* extract was applied in different doses to rats and mice in order to observe its effects on inflammation and analgesia. At dose variations, it showed remarkable effects in analgesic activities however it also showed less marked anti-inflammatory effects. Its constituents play vital role in controlling inflammation and pain **[22, 23]**.

Trapa bipinosa contains alkaloids, anti-oxidants, saponins, tannins, phenols, glycosides. It is also used as a good source of enzymes like amylase, invertase, cellulose, lipase and protease. Damage of tissue in inflammation is due to release of reactive oxygen species from phagocytes which surround the area of inflammation. The fruit of *Trapa bipinosa* reduces arachidonic acid formation. As a result formation of prostaglandins gets inhibited and urge of pain and inflammation is remarkably reduced. This observation was noted at the maximum given dose of *Trapa bipinosa* at 400mg/kg which showed viable results. The antioxidants, enzymes and phytochemical constituents of the plant are responsible for reducing inflammation and analgesia **[23, 24]**. From all above discussion it is clear that all three plants are enriched with flavonoids. Flavonoids are actually responsible for analgesic and anti-inflammatory activity **[25]**. They cross the blood brain barrier and bind with various receptors like GABA-A, opioid and adrenergic receptors. They also inhibit enzymes involved in inflammations **[26]**. Several plants contain flavonoids and they inhibit cyclo-oxigenase enzymes **[25]**. Moreover, they may have analgesic action which is due to inhibition of release of nitric oxide which is produced upon administration of formaldehyde injection **[27]**. There is evidence that flavonoids inhibit prostaglandin production as well **[28]**. So possible mechanism of action of plant extracts in inhibition of pain and inflammation is the presence of flavonoids in extracts. Moreover, Tannins also inhibit pain stimulus by binding with pain receptors thereby reducing the intracellular movements of ions and thus reduce sensory action potential **[29]**. Phenols and tannins are responsible for restricting the inward

movements of calcium ion across the membranes hence decreasing pain stimulus **[30]**. Phytochemical studies indicated the presence of phenols, alkaloids, tannins, glycosides, terpenes and terpenoides in all three plant extracts. Thus, alkaloides, flavonoids, terpenes and terpenoids inhibit prostaglandin synthesis and may contribute in analgesic and anti-inflammatory action **[31]**.

CONCLUSION

On the basis of results it has been concluded that aqueous ethanolic extracts of *Trapa bispinosa, Chenopodium album and Cuscuta reflexa* possess strong anti-inflammatory and analgesic activity as compared to standard drug diclofenac sodium.

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