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Comparison of Anti-inflammatory Activity of a Bright Red Carotenoid Hydrocarbon Lycopene and Aceclofenac in Rats

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Abstract

There are numerous plants which are used in Ayurveda and other traditional system of medicine but their claim is not yet evaluated scientifically in Laboratory Animals. Present study was intended for the " Comparison of Anti-inflammatory Activity of a Bright Red Carotenoid Hydrocarbon Lycopene & Aceclofenac in Rats" to confirm Ethnomedical claim made by traditional health practitioners. Lycopene was obtained from the tomatoes from local market of Indore. Fruits (Tomatoes) were processed for juice extraction in ethanol. Ethanolic extract was evaporated using rota evaporator and stored at cool and dry place. Extract was Orally Administered in rats (400/600 mg/kg) and evaluated using Plethysmometer. Aceclofenac (10 mg/kg) was taken As A standard drug. Significant anti-inflammatory activity comparable with standard dose of aceclofenac was observed. This confirms ethnomedical claim of some workers regarding anti-inflammatory activity of the plant.

Key-words: Anti-inflammatory activity, *Lycopenes*, Aceclofenac, Plethysmometer, Tomatoes, Carrageenan induced rat paw edema

Introduction

Inflammation is a major threat to human health and plays an important role in the development of various infectious and non-infectious diseases such as Alzheimer's, heart disease, asthma, rheumatoid arthritis, etc. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever. Clinical treatment of inflammatory diseases is dependent on drugs, whichbelong either to the non-steroidal or to the steroidal chemical groups. The use of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of diseases associated with inflammatory reactions has potent activity, but long term uses of these drugs have various and severe adverse effects on liver, gastrointestinal tract, etc. Hence, new anti-inflammatory and analgesic drugs lacking such effects are being searchedfor as alternatives to NSAIDs.

Owing to safety concerns associated with the use of synthetic anti-inflammatory and analgesic agents, generally the people prefer to take natural anti- inflammatory and analgesic treatments from edible materials such as fruits, spices, herbs and vegetables. Therefore, the development and utilization of more effective anti-inflammatory and analgesic agents with fewer side effects from natural origin are desired.

In the present study, the prime objective was to select a medicinal plant which can be used as anti-inflammatory agent with fewer/without side effects.

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On the basis of literature survey, medicinal uses and availability of the plant, *Solanum lycopersicum* fruits were selected for evaluation of anti-inflammatory properties.

The result of physicochemical analysis showed 8% loss on drying. It contained 5.45% total ash, 0.57% acid insoluble ash and 2.47% water soluble ash. The maximum percentage of extractive value was obtained in water (41.59%) followed by methanol (32.77%). The extract was acidic in nature and maximum solubility was in DMF, methanol and DMSO. Lead, chromium and cadmium were not detected in the SLM The preliminary qualitative phytochemical analysis of SLM revealed the presence of alkaloids, flavonoids, phlobotannins, saponins, tannins and triterpenes. Tannins and alkaloids were present in more amounts as compared to other phytoconstituents. Quantitatively estimated total phenol content was higher than that of flavonoid content in the ethanol extract.

Anti-inflammatory studies of SLM was also done using carrageenand cotton pellet induced granuloma chronic model. Among these models, carrageenan induced rat paw edema is most widely used for evaluation of anti-inflammatory properties of medicinal plants. SLM inhibited the carrageenan induced paw edema at both early and late phase. SLM showed promising activity in all the studied anti-inflammatory models. Aceclofenac is one of the most widely used NSAID for the treatment of inflammatory diseases and analgesia. However, long term use of causes hepatotoxicity.

Overall it can be concluded that ethanol extract of *Solanum lycopersicum* fruits hadgood potential as anti-inflammatory and analgesic agent and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals.

Material and Methods

Collection and Identification of Plant Material

The fresh Fruits of *Solanum lycopersicum* were collected from Indore, Madhya Pradesh in the month of March 2020.

Pharmacognostic Studies

Macroscopic Characteristics

Fruits where physically examined for shape, size, surface characteristics, texture, color, consistency, odour, taste, etc.

Microscopic characteristics

Free hand sections of pedicel, calyx, sepal and ovary of fresh Fruit of *Solanum lycopersicum*were taken. The section cleaned with chloral hydrate and then stained with phloroglucinol and hydrochloric acid and placed with glycerin. Same procedure was followed for microscopic characteristics of ethanolic extract of dryed material of *Solanum lycopersicum* Fruit

Preparation of the Extract

Ethanolic Lycopene extract from tomato is a lycopene-rich source prepared from the fresh fruits of tomato .The dried powder is prepared by crushing tomatoes, to produce crude tomato juice that is then isolated the serum and pulp. The pulp is then extracted using ethanol as a solvent. The final extract consists of lycopene together with a number of other constituents that occur naturally in tomato.

Ethanolic Lycopene extract from tomato is produced from a tomato variety with very high lycopene content, within the range of 150 to 250 mg/kg. This variety is not generally marketed for direct consumption, but is used generally used in the production of this lycopene extract. The extract is produced by crushing tomatoes into crude tomato juice that is then separated into serum and pulp. The tomato pulp is then extracted with ethanol. The final product is obtained by vacuum drying at 40-60°C.

Physicochemical Analysis

The following physicochemical parameters were carried out in ethanol extract of *Solanum lycopersicum* Fruits.

Loss on drying

Two grams of powder of *Solanum lycopersicum* fruits was taken in an evaporating dish and then dried in an oven at 105ºC till constant weight was obtained. The difference in weight after drying was noted and loss on drying was calculated. The % loss was calculated on the basis of sample taken wihich was taken initially

Total ash

Two grams of dried powder of *ethanolic extract of lycopene* was taken in a crucible and ignited it by gradually increasing the heat to 500°C until it was white, indicating the absence of carbon. The obtained ash was then cooled in a desiccator and weighed immediately .The % of total ash was

calculated on the basis of the % loss of sample during heating which was taken initially.

Acid insoluble ash

To the crucible containing total ash, 25 ml of hydrochloric acid was added; it was covered with a cover and heated to boil for 5 minutes. The the glass cover was rinsed with 5 ml of hot water and this part was added to the crucible. The insoluble substance was collected on a filter paper and it was washed with water until the filtrate was neutral to litmus paper. The ashless filter paper containing the insoluble substance was transferred to the original crucible; it was dried on a oven and dryed to constant weight. The obtained residue was allowed to cool and then weighed immediately. The percentage of acid insoluble ash was calculated on the basis of % loss sample taken initially.

Water soluble ash

To the crucible containing the total ash, 10 ml of ditsiled water was added and heated for 5 minutes. The insoluble substance was collected on an ashless filter paper. It was cleaned with water and heated in a crucible for 30 minutes. The residue was allowed to cool and then weighed immediately. Weight of insoluble matter was calculated from the weight of total ash. The % of water soluble ash was calculated on the basis of % loss of sample taken initially.

Determination of petroleum ether soluble extractive

Five grams of dried powder ethanolic extract of lycopene was taken in 150 ml of hexane in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 12 h. Then, it was filtered and the filtrate was then evaporatedto dryness at 105°C till constant weight was obtained. The % of extractable matter was calculated by % loss with reference to the sample taken initially.

Determination of acetone soluble extractive

5 grams of dried extract of lycopene was taken in 150 ml of acetone in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 12 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The % of extractable matter was calculated with reference to the % loss of sample taken initially.

Determination of methanol soluble extractive

5 grams of dried ethanolic extract of Lycopeene was taken in 150 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shakerat 120 rpm for 12h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the % loss of sample taken initially.

Determination of water soluble extractive

Five grams of dried powder of *of ethanolic extract of lycopene* was taken in 100 ml of water in a conical flask, plugged with cotton wool and then kept on a shaker at 120 rpm for 24 h. Tthen it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the % loss of sample taken initially.

Determination of pH

The dried ethanolic extract of *lycopene* was dissolved in distilled waterand was kept in a water bath for 20 min. It was then filtered and the pH of the filtrate was measured with the help of pH meter.

Determination of melting point

The melting point of dried ethanolic extract of *lycopeene* was perfoemed

Determination of solubility

The solubility test of ethanolic extract of *lycopeene* was determined in different solvents. 5 mg of extract was weighed for solubility test for different solvents. The extract was added in each solvent until saturated solution developed. Solubility was calculated in mg/ml.

Phytochemical Analysis

Qualitative Phytochemical Analysis

Chemical tests were carried out for ethanolic extract to identify different phyto-constituents

Alkaloids

The ethanol extract of *lycopenee* was dissolved in 2 N Hydrochloric acid. The solution was filtered and the filtrate was divided into 3 equal portions. One part was treated with few drops of Mayer's reagent; one part was treated with equal amount of Dragondroff's reagent and the other part was treated with equal amount of Wagner's reagent. The observatopn of creamish precipitate, orange precipitate and brown precipitate was noted the

presence of respective alkaloids. A (+) score is noted if the reagent produced only a slight opaqueness; a (++) score was recorded if a definite turbidity but no flocculation was observed and a (+++) score was note if heavy precipitate was observed

Flavonoids

Shinoda test

The presence of flavonoids was estimated by Shinoda test. The ethanolic extract of *lycopne* was treated with few drops of concentrated HCl. The presence of pink or tomato red colour in short time indicated the presence of flavonoids

Alkaline reagent test

The ethanoic extract of *dried lycopene* was treated with few drops of diluted sodium hydroxide (NaOH) separately. Formation of intense yellow color which turned colorless on addition of few drops of diluted HCl indicated presence of flavonoids.

Cardiac glycosides

The test of Keller-kiliani was performed for the presence of cardiac glycosides. The ethanol extract of lycopene was treated with 1 ml mixture of 5% $FeCl_3$ and glacial acetic acid (1:99 v/v). To this solution, few ml of concentrated H_2SO_4 were added. Preseence of greenish blue color within few minutes indicated the presence of cardiac glycosides

Plobotannins

The ethanoilc extract of Lycopenee was boiled with 1% aqueous HCl. The preseencee of red precipitate was taken as evidence for the presence of phlobatanins

Saponins

The cotent of saponins was determined by Frothing test. The ethanolic extract of lycopene was vigorously shaken with clean water and was allowed to stand for 10 min and classified for saponin content as follows: no froth indicates absence of saponins and stable froth for more than 1.5 cm indicated the content of saponins

Steroids

Liebermann-Burchard reaction was doen for the presence of steroids. A chloroform solution of the dried powder of ethanolic extract of lycopenee was treated with acetic anhydride and few drops of concentrated H_2SO_4 were added down the sides of test tube. A blue green ring indicated the presence of steroids.

Tannins

The the dried powder of ethanolic extract of lycopene was treated with alcoholic ferric chloride ($FeCl_3$) reagent. Blue color was obtained which shows the presence of tannins

Triterpenes

Chloroform extract of the ethanolic extartc of lycopene was treated with concentrated sulphuric acid (H_2SO_4). Appearance of reddish brown ring was noted which indicated the presence of triterpenes.

Animals

We used Wistar albino rats of both sexes (180-220 g) for the study. The animals were collected from the animal house of Swami Vivekanand College of Pharmacy, Indore. All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature (25 ± 2°C). Theywere provided with commercial rat and mice feed (Sheetal Agro Industries Ltd., Indore. Swacch Brand rat & mice pellet feed) and water given ad libitum. The use of these animals and the study protocols were approved by CPCSEA recognized local ethical committee.

Selection of the doses for animal study

The dose considered for the experiment on rats was obtained from conversion of human dose of *dried ethanolic extract of lycopeene (*3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats .Hence the calculated dose for the rats (considering human dose 5 g/kg) is 450 mg/kg. Thus, anti-inflammatory activity was done at two different doses 400 mg/kg and 600 mg/kg of body weight.

Anti-inflammatory studies

The animals were divided into four groups (six animals in each group) for anti- inflammatory studies.

Group I: Vehicle treated control (distilled water)

Group II: Dried Ethanolic extract of *Solanum lycopersicum*- 400 mg/kg body weight(SLM-400)

Group III: Dried Ethanolic extract of *Solanum lycopersicum*- 600 mg/kg body weight(SLM-600)

Group IV: Aceclofenac- 10 mg/kg body weight (-10)

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Carrageenan induced rat paw edema

Carrageenan induced rat paw edema was done by the method of Winter et al. (1962). Inflammation was induced by injection of 0.1 ml of freshly prepared carrageenan (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw of rats. The different groups of rats were administered with SLM (400 and 600 mg/kg, p.o.) and (10 mg/kg, p.o.). The control group was administed with vehicle (distilled water, 10 ml/kg, p.o.).

After 1 hr of drug treatment, paw edema was induced by the injection of carrageenan (an edematogenic agent). The paw volume was measured by a Plethysmometer.

Cotton pellet induced granuloma in rats

The effect of ethanol extract of Solanum lycopersicum Fruits on the chronic phases of inflammation was assessed in the cotton pellet induced granuloma rat model, as described by Swingle and Shideman (1972). Autoclaved cotton pellets weighing 100 mg each were implanted subcutaneously. One on each side of the abdomen of the animal, through a small ventral incision of rats anesthetized with ether. The different groups of rats were administered with SLM (400 and 600 mg/kg, p.o.) and (10 mg/kg. p.o.) once daily for 7 consecutive days from the day of cotton pellet insertion. The control group received vehicle (distilled water, 10 ml/kg, p.o.). On the eighth day the animals were sacrificed and the cotton pellets were removed, dried at 60°C for 24 h and their mass was determined. The results are expressed as mg granulation tissue formed per 100 g body weight.

Statistical analysis

The data obtained from animal experiments are expressed as mean ± SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA)

Values are considered statistically significant as P value is <0.05. F value >F crit indicates, the effect of the drug is comparable with the standard

Results and Discussion

The result of proximate analysis of crude powder of *Solanum lycopersicum* Fruit is shown in Table . The average values are expressed as percentage of air-dried material. The loss on drying was 8%. Total ash was 5.45%, acid insoluble ash was 0.57% and water soluble ash was 2.47%. The extractive value of crude powder was maximum in water (41.59%), followed by methanol (32.77%) and minimum was in hexane (0.71%). pH and melting point of ethanol extract was 3.5 and 114°C respectively.

Table 1: Proxmate parameters of crude powder of *Solanum lycopersicum* Fruits

Parameters	Value (w/w)
Loss on drying	8.00%
Total ash	5.45%
Acid insoluble ash	0.57%
Water soluble ash	2.47%
Petroleum ether soluble extractive	0.74%
Hexane soluble extractive	0.71%
Ethyl acetate soluble extractive	1.80%
Acetone soluble extractive	8.08%
Methanol soluble extractive	32.77%
Water soluble extractive	41.59%
pH of ethanol extract	3.5
Melting point ethanol extract	114°C

The ethanol extract of *Solanum lycopersicum*Fruits was evaluated for solubility in 10 solvents with varied polarities. The extract was highly soluble in dimethylformamide, methanol and dimethylsulphoxide but insoluble in hexane, petroleum ether and toluene solvents.

Table: Table 2: Solubility of ethanol extract of *Solanum lycopersicum* Fruits indifferent solvents

Solvent	Solubility (mg/ml)
Acetone	6.0
Chloroform	3.0
Dimethylformamide (DMF)	119.0
Dimethylsulphoxide (DMSO)	109.8
Distilled water	34.2

Ethyl acetate	3.0
Hexane	-
Methanol	113.1
Petroleum ether	-
Toluene	-

(-): Not soluble

The results of qualitative phytochemical analysis theethanol extract of *Solanum lycopersicum* Fruits is shown in Table.

Table 3: Preliminary qualitative phytochemical analysis of *Solanum lycopersicum* Fruits

Phytochemical	**Test**		**Ethanol extract**
Alkaloids	Dragondroffs test		+
	Mayers test		+
Flavonoids	Shinoda test		+
	Alkaline reagent test		+
glycosides	Keller-kilianni test		+
Phlobotannins	HCl test		-
Saponins	Frothing test		-
Steroids	Liebermann-Burchard reaction		-
Lycopenes	$FeCl_3$ test		+++
Triterpenes	H_2SO_4 test		+

(-): No presence, (+): Less presence, (++): Moderate presence, (+++): High presence

The results of anti-inflammatory activity of ethanol extract of *Solanum lycopersicum* Fruits on carrageenan induced paw edema is shown in Table 10. The lower dose i.e. SLM-400 showed inhibition at both early and late phase; though maximum inhibition was at late phase (36.04%,).

The higher dose i.e. SLM-600 also showed maximum anti-inflammatory activity at late phase (46.45%) The standard -10 showed maximum activity at early phase (46.97 %,).

Table 4: Anti-inflammatory activity of ethanol extracts of *Solanum lycopersicum fruits* in carrageenan induced rat paw edema
Values are expressed as mean ± SEM (n=6)

Groups	% Difference in paw size in ML									
	After 1 hr		After 2 hr		After 3 hr		After 4 hr		After 5 hr	
	ml	% change	ml	% Change	ml	% Change	ml	% Change	ml	% Change
Control	1.59 ± 0.12	-	2.64 ± 0.19	-	3.33 ± 0.25	-	3.23 ± 0.24	-	3.10 ± 0.16	-
SLM-400	1.76 ± 0.11	31.16 ↓	1.94 ± 0.15	26.75 ↓	2.42 ± 0.19	26.63 ↓	2.38 ± 0.24	25. 58 ↓	1.99 ± 0.25	36.04 ↓
SLM-600	1.29 ± 0.18	34.78 ↓	1.80 ± 0.16	30.11 ↓	2.11 ± 0.39	35.90 ↓	2.25 ±0.15	31.26 ↓	1.66 ±0.16	46.45 ↓
Aceclofenac-10	0.62 ± 0.18	59.45 ↓	1.24 ± 0.18	51.47 ↓	1.34 ±0.33	59.67 ↓	1.76 ±0.15	46.79 ↓	1.70 ±0.19	46.97 ↓

Values are considered statistically significant as P value is <0.05. F value > F crit indicates, the effect of the drug is comparable with the standard.

In this model, the *Solanum lycopersicum* Fruit extract at both dose levels and standard showed anti-inflammatory activity at late phase. A clear dose dependent inhibition of paw edema was observed. The percentage inhibition of SLM-400 was 36% while that of SLM-600 was 46%. The later was nearer to standard -10.

The results of anti-inflammatory activity of ethanol extract of *Solanum lycopersicum*Fruit in cotton pellet induced granuloma is shown in below Table. SLM-400 and SLM-600 groups showed 11.28% and 16.45% decrease in granuloma formation respectively as compared to control group, while standard -10 groupshowed significant decrease in granuloma formation.

Table 5: Anti-inflammatory activity of ethanol extract of *Solanum lycopersicum* Fruits in cotton pellet induced granuloma in rats

Groups	Pellet weight (g/100 g.b.w)	% Change
Control	0.105 ± 0.005	-
SLM-400	0.094 ± 0.005	11.28 ↓
SLM-600	0.090 ± 0.005	16..45 ↓
Aceclofenac-10	0.088 ± 0.004	17.15 ↓

Values are expressed as mean ± SEM (n=6)

Inflammation is a major threat to human health and plays an important role in the development of various infectious and non-infectious diseases such as Alzheimer's, heart disease, asthma, rheumatoid arthritis, etc. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever. Clinical treatment of inflammatory diseases is dependent on drugs, whichbelong either to the non-steroidal or to the steroidal chemical groups. The use of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of diseases associated with inflammatory reactions has potent activity, but long term uses of these drugs have various and severe adverse effects on liver, gastrointestinal tract, etc. Hence, new anti-inflammatory and analgesic drugs lacking such effects are being searchedfor as alternatives to NSAIDs.

Owing to safety concerns associated with the use of synthetic anti-inflammatory and analgesic agents, generally the people prefer to take natural anti- inflammatory and analgesic treatments from edible materials such as fruits, spices, herbs and vegetables. Therefore, the development and utilization of more effective anti-inflammatory and analgesic agents with fewer side effects from natural origin are desired.

In the present study, the prime objective was to select a medicinal plant which can be used as anti-inflammatory agent with fewer/without side effects. On the basis of literature survey, medicinal uses and availability of the plant, *Solanum lycopersicum* fruits were selected for comparison of anti-inflammatory.

The result of physicochemical analysis showed 8% loss on drying. It contained 5.45% total ash, 0.57% acid insoluble ash and 2.47% water soluble ash. The maximum percentage of extractive value was obtained in water (41.59%) followed by methanol (32.77%). The extract was acidic in nature and maximum solubility was in DMF, methanol and DMSO. Lead, chromium and cadmium were not detected in the SLM.

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Conclusion

From the study, it can be concluded that ethanol extract of *Solanum lycopersicum* fruits had good potential as anti-inflammatory and analgesic agent and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals.

It can be evident that lycopne can be used as synergistic anti-inflammatory durg along with

NSAID or can be used as alternative treatment for chronic conditions like rheumatoid arthritis therapy

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