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Thrombolytic (*in vitro*) and analgesic (*in vivo*) effect of methanolic extract of *Cucumis sativus*

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Abstract
The present study was designed to investigate thrombolytic and analgesic activity of methanolic crude extract of ***Cucumis sativus.*** The screening for the thrombolytic activity of methanolic extract showed moderate activity (45.15%) in clot lysis. In applying on mice analgesic activity of selected extract compared with standard Aspirin and Pentazocine showed activity against pain and significantly reducing the sensation of pain.

Keywords: *Cucumis sativus*, Analgesic, Thrombolytic.

1. Introduction

1.1 Medicinal plants

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal plants". Although there are no apparent morphological characteristics in the medicinal plants that make them distinct from other growing with them, yet they possess some special qualities or virtues that make them medicinally important. The plants which naturally synthesize and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatile oils and contain minerals and vitamins possess medicinal properties. Accordingly, the WHO consultative group on medicinal plants has formulated a definition of medicinal plants in the following way: "A medicinal plants is any plant which, in one or more of its organs, contain substances that can be used for therapeutic purposes or which is a precursor for synthesis of useful drugs".

1.2 Thrombolytic Activity

Thrombolytic therapy consisting of the administration of a pharmacological agent to cause thrombolysis of an abnormal blood clot. Thrombolytic therapy is also used to dissolve blood clots that form in catheters or tubes put into people's bodies for medical treatments, such as dialysis or chemotherapy. When a blood clot forms in a blood vessel, it may cut off or severely reduce blood flow to parts of the body that are served by that blood vessel. This event can cause serious damage to those parts of the body. If the clot forms in an artery that supplies blood to the heart, for example, it can cause a heart attack. A clot that cuts off blood to the brain can cause a stroke. A blood clot (thrombus) developed in the circulatory system due to failure of hemostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic diseases such as myocardial or cerebral infarction, at times leading to death. Thrombolytic therapy is used to dissolve blood clots that could cause serious, and possibly life-threatening, damage if they are not removed. Thrombolytic agents that include tissue plasminogen activator (t-PA), Urokinas (UK), streptokinase (SK) etc. are used all over the world for the treatment of these diseases. In India, though SK and UK are widely used due to lower cost, as compared to other thrombolytic drugs, their use is associated with hyper risk of hemorrhage severe anaphylactic reaction and lacks specificity. Moreover, as a result of immunogenicity multiple treatments with SK in a given patient are restricted. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs. Throughout history, plants have been used as a major medicinal source, with interest in herbal formulations increasing globally over the past decade. The leaves and/or twigs, stem, bark and underground parts of plants are most often used for traditional medicines. Herbal products are often perceived as safe because they are "natural". In India, in recent years, there is increased research on traditional ayurvedic herbal medicines on the basis of their known effectiveness in the treatment of ailments for which they have been traditionally applied. Considerable efforts have been directed towards

the discovery and development of natural products from various plant and animal sources which have antiplatelet, anticoagulant, antithrombotic, and thrombolytic activity. Epidemiologic studies have provided evidence that foods with experimentally proved antithrombotic effect could reduce risk of thrombosis. Earlier worked on artificial clots and used ultrasound methods to measure the thrombolytic activity of streptokinase. Several other models have been reported which either uses complicated mathematical or computing skills, but all these methods are very costly and not affordable in developing countries. The above-mentioned problems demand a need of simple and cost effective clot lytic model for measurement of clot lysis activity of thrombolytic drugs. In the present study an attempt has been made to develop an in-vitro clot lytic model using a known thrombolytic drug, streptokinase.

1.2.1 List of thrombolytic agents and sources

Thrombolytic therapy uses drugs called thrombolytic agents, such as alteplase (Activase), anistreplase (Eminase), streptokinase (Streptase, Kabikinase), urokinase (Abbokinase), and tissue plasminogen activator (TPA) to dissolve clots. These drugs are given as injections, and given only under a physician's supervision.

1.2.2 Pharmacological properties

1.2.2.1 Streptokinase

Streptokinase is a protein produced by Beta-hemolytic streptococci as a component of that organism tissue destroying machinery.

There are two drawbacks for streptokinase therapy:

- Previous administration of the drug is a contraindication, because of the risk of anaphylaxis.
- The thrombolytic actions are relatively nonspecific and can result in systemic fibrinolysis.

1.2.2.2 Mechanism of action:

Streptokinase from complex with circulatory plasminogen. (streptokinase – plasminogen complex). This complex then converts uncomplexed plasminogen to active enzyme plasmin. Plasmin causes hydrolysis of fibrin plugs (i.e. Formation of fibrin degrading product)**.**The complex also catalyses the degradation of fibrinogen as well as clotting factors 7.

1.3 Analgesic Activity

An analgesic, or painkiller, is any member of the group of drugs used to achieve analgesia relief from pain. The word analgesic derives from Greek αν - ("without") and άλγος - ("pain")

Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anesthetics, which reversibly eliminate sensation, and include paracetamol (known in the US as acetaminophen or simply APAP), the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, and opioid drugs such as morphine and opium.

1.3.1 Analgesia

Analgesia, loss of sensation of pain that results from an interruption in the nervous system pathway between sense organ and brain. Different forms of sensation (e.g., touch, temperature, and pain) stimulating an area of skin travel to the spinal cord by different nerve fibres in the same nerve bundle.

1.3.2 Pain

Pain is an unpleasant feeling often caused by intense or damaging stimuli, such as stubbing a toe, burning a finger, putting alcohol on a cut, and bumping the "funny bone". The International Association for the Study of Pain's widely used definition states: "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.

1.3.2.1 Classification of pain

- **Nociceptive:** represents the normal response to noxious insult or injury of tissues such as skin, muscles, visceral organs, joints, tendons, or bones.
- **Neuropathic:** pain initiated or caused by a primary lesion or disease in the somatosensory nervous system.
- **Inflammatory:** a result of activation and sensitization of the nociceptive pain pathway by a variety of mediators released at a site of tissue inflammation.

1.3.3 Classification of Analgesic

- **Narcotic**-The narcotic analgesics are the agents that cause sleep or loss of consciousness (narcosis) in conjunction with their analgesic effect. In other words, drugs that directly act on central nervous system (CNS) to relieve pain are termed as narcotic analgesics.
- **The non-narcotic** analgesics act peripherally on the nervous system to reduce pain. Excluding the analgesic effect, the non-narcotic analgesics usually have two other properties (antipyretic and anti-inflammatory effects). Unlike narcotic analgesics, drugs of this class do not cause physical dependencies and narcosis. However, most of the drugs in this class are gastric irritant.
- **NSIDS (Non-steroidal anti-inflammatory drugs)** - Another class of analgesic drug is the NSAIDs or the non-steroidal anti-inflammatory drugs. Drugs of this class not only show chemical dissimilarities but also vary in their analgesic, antipyretic and anti-inflammatory properties. These drugs work principally by inhibiting the COX1 and COX2 enzymes. However, they do not act on the lipoxygenase enzymes. Aspirin, the most widely used analgesic, is a prototype of this class.

1.3.4 Current Analgesics Options

- NSAIDs: 1970’s
- opioids: 1970’s
- tramadol: 1980’s & 1990’s
- COX-2 inhibitors: 1990’s
- acetaminophen: unknown
- combinations
- adjuncts

2. Method and Materials

2.1 Thrombolytic activity

2.1.1 Principle

Plasmin is produced in the blood to break down fibrin, the major constituent of blood thrombi, therefore dissolving clots once they have fulfilled their purpose in stopping bleeding. Extra production of plasmin caused by streptokinase breaks down unwanted blood clots, for example, in the lungs (pulmonary embolism). The usual activation of Plasminogen (Plgn) is by proteolysis of the Arg561—Val562 bond. The amino group of Val562 then forms a salt-bridge with Asp740,

which triggers a conformational change producing the active protease Plasmin (Pm).

2.1.2 Method

2.1.2.1 Plant sample preparation

1. 100 mg of extract was suspended in 10 ml of distilled water.
2. Shaken in a vortex mixer.
3. Kept over-night. The soluble supernatant was decanted and filtered.
4. 100 µl of this aqueous preparation was added to each micro-centrifuge tubes.

2.1.2.2 Blood specimen preparation

1. 10 pieces micro centrifuge tubes were taken, sterilized and weighed (let n=1).
2. 5 ml of blood was drawn from volunteer.
3. The blood was distributed in 10 different per weighed (W_1) micro-centrifuge tube, each tube .05 ml.
4. The blood specimen was centrifuged at 2500 rpm for 5 minutes.
5. Incubated the blood for 45 minutes at 37 ^{0}C.
6. After clot formation i.e. incubation, the serum was completely removed by decantation, capillary absorption and by removing the serum from the inner surface of the tube carefully by cotton bar or by use of cotton bound at top of a glass rod without disturbing the clot and ensure complete removal of serum or the result will be erroneous.
7. Kept the tubes at lying position on a tray for 6 minutes after first removal of serum and then removed the liquids of the tube surface by the cotton rod.
8. Each tube was weighed (W_2) again.
9. Weight of clot was found as,
10. Weight of clot = weight of containing tube (W_2) – weight of tube alone (W_1).
11. Finally weighed very carefully, because result varies for inappropriate weighing, checked the balance before weighing.
12. To each micro-centrifuge tube containing pre-weighed clot, 100 µl of organic solvent extract of plant (*Cucumis sativus*) was added separately.
13. As a positive control, 100 µl of streptokinase was added to clot of tube no. 5 (standard).
14. As a negative control, 100 µl water is added to clot of tube no. 4 (blank).
15. All the tubes were incubated at 37 ^{0}C for 90 minutes and observed if clot lysis has occurred.
16. After 90 minutes of incubation, the released fluid was completely removed by decanted clot containing liquid from the inner surface of the tube carefully by cotton bar or by use of cotton tightly bound at top of a glass rod without disrupting the clot. The tubes were then weighed again and ensured complete removal of released fluid or the result will be erroneous. Kept the tubes at lying position on a tray for 6 minutes after first removal of released clot and then removed the liquids of the tube surface by the cotton rod.
17. Weighed the tubes (W_3) very carefully, because result varies for inappropriate weighing.
18. The difference obtained in weight taken before and after clot lysis is expressed as percentage of clot lysis.

2.1.3 Carefulness

1. Collect sufficient test tubes.
2. Label the tubes appropriately and work accordingly.
3. Cheek the temperature of incubation chamber before starting to work.
4. Remove the serum and the released dissolved clot completely; keep the tubes at lying position on a tray for 6 minutes after first removal of serum.

2.2 Analgesic Activity

2.2.1 Principle

Since time immemorial, indigenous plants have been a major source of medicine. In folk medicine, they are used, in single or in combined forms for treating different types of inflammatory and arthritic conditions. Prolonged administrations of steroidal and non-steroidal anti-inflammatory drugs are known to be associated for their adverse effects.

Herbal drugs have lesser side effects and are largely replaced by synthetic drugs no reports on its analgesic and anti-inflammatory activity.

2.2.2 Method

2.2.2.1 Chemicals and drugs

Aspirin and Pentazocine were used in the study; Aspirin and Pentazocine were dissolved in water for injection before intraperitoneal administration.

2.2.2.2 Experimental Design

Twenty five experimental animals were randomly selected and divided into nine groups denoted as group-I, group-II, group-III, group- IV and group V consisting of 5 mice in each group. Each group received a particular treatment i.e. control, standard and the dose of the methanolic extract of *Cucumis sativus*. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly.

2.2.2.3 Method of Identification of Animals

Each group consisted of five mice. As it was difficult to observe the biologic response of five mice at a time receiving same treatment, it was quite necessary to identify individual animal of a group during the treatment. The animals were individualized in the following way and marked as M-1=Mice 1, M-2=Mice 2, M- 3=Mice 3, M-4=Mice 4 and M-5=Mice 5.

2.2.3 Herbal extracts

Cucumis sativus methanolic extract 100 mg/kg, 200 mg/kg and 300 mg/kg.

2.2.3.1 Acetic acid induced writhing method

In this method, mice in groups of five each were treated with methanol extract of *Cucumis sativus* (100, 200, 300 mg/kg). Analgesic activity of *Cucumis sativus* (100, 200, 300 mg/kg) was assessed by counting the number of writhes induced by 0.6% acetic acid (10 ml/kg). Number of writhes per animal was counted in the following 20 min. Aspirin (20 mg/kg) was used as a positive standard. Extracts solution and aspirin were administered 1 h prior to intra-peritoneal administration of 0.6% acetic acid. Percentage protection against writhing was taken as an index of analgesia. It is calculated as:

(Number of writhing in control group –Number of writhing in treated group)/ Number of writhing in control group × 100.

2.2.4 Hot plate method

Mice in groups of five each were treated with vehicle pentazocine (17.5 mg/kg,) and *Cucumis sativus* (100, 200 mg/kg). They were placed on a hot plate maintained at a temperature of 55 ± 0.5 ºC. The latency to lick the paw or jump from the hot plate was noted as the reaction time. The reaction time was noted at 0, 15, 30, 45, 60, 90 and 120 min. The cut off time was considered as 15 s. The cut off time is determined by taking the average reaction time plus 3 times the standard deviation of the combined latencies of the control mice at all time periods.

2.2.5 Thrombolytic activity test of *Cucumis sativus*

The percentage of weight loss of clot after application of extract solution was taken as the functional indication of thrombolytic activity. The average value of percentage of weight loss was calculated and shown in the bellow data analysis. the value of standard deviation is also calculated to examine the variation of biological system.

3.1 Results of Clot lysis work

Table 3.1.1: Determination of the different value of clot before adding Methanol extracts of *Cucumis sativus* (Before Clotlysis).

No. of tube	Empty alpine tube weight (gm)	Weight of tube with clot (gm)	Weight of clot (gm)	Weight of tube with clot after lysis (gm)	Weight of lysis clot (gm)
01	.7924	1.2222	.4298	1.0922	0.13
02	.8023	1.3047	.5024	1.0852	0.2195
03	.8251	1.2798	.4547	1.0522	0.2276
04	.8030	1.2301	.4271	1.1987	0.0314
05	.8365	1.2652	.4296	0.9139	0.3513
06	.8261	1.2829	.4568	1.0366	0.2463
07	.8160	1.2651	.4491	1.0304	0.2347
08	.8141	1.2947	.4806	1.9850	0.3097
09	.8088	1.2240	.4152	1.1108	0.1132
10	.8071	1.2339	.4268	1.0666	0.1673

Table 3.1.2: Determination of the different percentage (%) of clot after adding Methanol extracts of *Cucumis sativus*

No. of tube	Weight of clot (gm), A	Weight of lysis clot (gm), B	Difference	% of lysis $\left(\frac{B \times 100}{A}\right)$
01	.4298	0.13	.3628	30.24
02	.5024	0.2195	.2829	43.7
03	.4547	0.2276	.2271	50.1
04	.4271	0.0314	.3957	7.35
05	.4296	0.3513	.0783	81.7
06	.4568	0.2463	.2105	53.92
07	.4491	0.2347	.2144	52.3
08	.4806	0.3097	.1709	64.4
09	.4152	0.1132	.302	27.3
10	.4268	0.1673	.2595	39.2

3.1.1 Statistical analysis

Average clot analysis: 45.15%
% clotlysis of Streptokinase: 81.7 (tube no. 5)
% clotlysis of water (control): 7.35 (tube no. 4)
The significance between % clot lysis by methanol extract by means of weight difference

Standard Deviation (SD) = 12.54
Standard Error (SE) = 4.75
Degrees of freedom (n-1) = 9

3.1.2 Discussion

Addition of 100-µl Streptokinase, a positive control to the clots along with 90 minutes of incubation at 37 °C, showed 81.7% clotlysis.

Clots when treated with 100 µl methanol extract of *Cucumis sativus* showed only clot lysis 45.15%. After treatment of clots with 100 µl of methanol extract of *Cucumis sativus* shows average clot lysis 45.15%. From compare with Streptokinase it revealed that the plant has got a moderate clotlysis effect.

3.2 Result of Analgesic activity

3.2.1 Acetic acid induced writhing method

Cucumis sativus (100, 200, 300 mg/kg) significantly reduced the number of writhing induced by acetic acid. Maximum percentage of inhibition of writhing response was observed with *Cucumis sativus* (300 mg/kg). Aspirin showed a maximum inhibition of writhing response as 50.625%. The observations are given below-

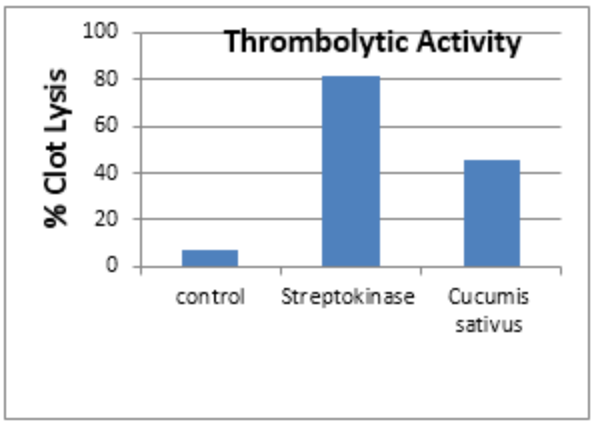

Fig 3.1.1: % Clot lysis by Streptokinase and methanol extract of *Cucumis sativus*

Table 3.2.1.1: Observation of Acetic acid induced writhing inhibition for 100 mg *Cucumis sativus* dose

	No. of writhing		
Mice no.	**M.E 100**	**control**	**Aspirin**
M1	49	70	33
M2	56	65	35
M3	50	60	30
M4	57	58	32
M5	55	67	28
AVERAGE	53.4	64	31.6
SD	3.6469165	4.949747	2.701851
SE	0.4558646	0.618718	0.337731
%inhibition	16.5625		50.625

M.E = Methanolic extract of *Cucumis sativus*
SD = Standard Deviation
SE = Standard Error

Table 3.2.1.2: Observation of Acetic acid induced writhing inhibition for 200 mg *Cucumis sativus* dose

	No. of writhing		
Mice no.	**M.E 200**	**control**	**Aspirin**
M1	46	70	33
M2	45	65	35
M3	48	60	30
M4	38	58	32
M5	45	67	28
AVERAGE	44.4	64	31.6
STDEV	3.781534	4.949747	2.701851
SE	0.472692	0.618718	0.337731
%inhibition	30.625		50.625

Table 3.2.1.3: Observation of Acetic acid induced writhing inhibition for 300 mg *Cucumis sativus* dose

	No. of writhing		
Mice no.	**M.E 300**	**control**	**Aspirin**
M1	40	70	33
M2	41	65	35
M3	48	60	30
M4	37	58	32
M5	45	67	28
AVERAGE	42.2	64	31.6
STDEV	4.32435	4.949747	2.701851
SE	0.540544	0.618718	0.337731
%inhibition	34.0625		50.625

Table 3.2.1.4: Final observation of Acetic acid induced writhing inhibition for *Cucumis sativus*

Groups	**No. of writhing (mean)**	**% Protection**
Control	64 ± 0.61	
Aspirin	31.6 ± 0.33***	50.625
Dose 100	53.4 ± 0.45**	16.56
Dose 200	44.4 ± 0.47***	30.62
Dose 300	42.2 ± 0.54***	34.06

*P < 0.05, ** P < 0.01, ***P<.001 vs. control.
Values are mean ± SE from groups of 5 animals

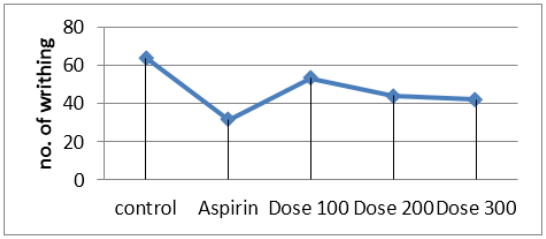

Fig 3.2.1.1: Graphical representation of data of Acetic acid induced writhing inhibition for methanolic extract of *Cucumis sativus*

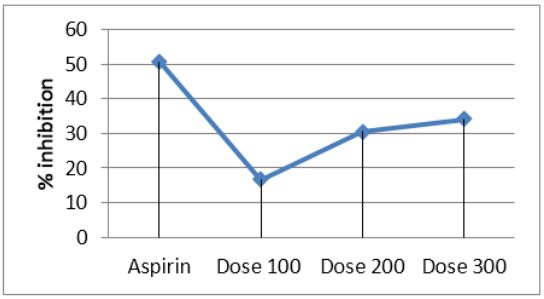

Fig 3.2.1.2: % protection against Acetic acid induced writhing for methanolic extract of *Cucumis sativus*

3.2.2 Hot plate method

Cucumis sativus (100, 200 mg/kg) significantly elevated the mean basal reaction time as compared to control group. The highest nociception inhibition was exhibited by *Cucumis sativus* (200 mg/kg) at 45 min. The observations are given below-

Table 3.2.2.1: Observation of hot plate method (0, 15, 30, 45, 60, 90, 120 min.) for 100 mg *Cucumis sativus* dose:

0 minute	**Basal reaction time (sec.)**		
	control	**Pentazocine**	**M.E**
Mice1	2	11	11
Mice2	3	9	14
Mice3	1	10	9
Mice4	2	8	8
Mice5	2	11	9
AVG.	2	9.8	10.2
SD	0.707107	1.30384	2.387467
SE	0.353553	0.65192	1.193734

15 minute	**Basal reaction time (sec.)**		
	control	**Pentazocine**	**M.E**
Mice1	2	14	12
Mice2	2	12	6
Mice3	1.9	13	7
Mice4	2	14	12
Mice5	2	14	11
AVG.	1.98	13.4	9.6
SD	0.044721	0.894427	2.880972
SE	0.022361	0.447214	1.440486
30 minute	**Basal reaction time (sec.)**		

	control	Pentazocine	M.E
Mice1	2	14	14
Mice2	3	15	13
Mice3	1	10	15
Mice4	1.9	14	14
Mice5	2	13	13
AVG.	1.98	13.2	13.8
SD	0.70852	1.923538	0.83666
SE	0.35426	0.961769	0.41833

45 minute	Basal reaction time (sec.)		
	control	Pentazocine	M.E
Mice1	3	11	15
Mice2	2	12	15
Mice3	1	12	15
Mice4	1.9	13	12
Mice5	2	12	14
AVG.	1.98	12	14.2
SD	0.70852	0.707107	1.30384
SE	0.35426	0.353553	0.65192

60 minute	Basal reaction time (sec.)		
	Control	Pentazocine	M.E
Mice1	2	11	10
Mice2	3	10	13
Mice3	1	9	9
Mice4	1.9	11	8
Mice5	2	11	9
AVG.	1.98	10.4	9.8
SD	0.70852	0.894427	1.923538
SE	0.35426	0.447214	0.961769

90 minute	Basal reaction time (sec.)		
	control	Pentazocine	M.E
Mice1	3	9	14
Mice2	3	10	7
Mice3	1	11	10
Mice4	1.9	10	9
Mice5	2	9	7
AVG.	2.18	9.8	9.4
SD	0.843801	0.83666	2.880972
SE	0.4219	0.41833	1.440486

120 minute	Basal reaction time (sec.)		
	control	Pentazocine	M.E
Mice1	2	8	10
Mice2	3	8	8
Mice3	3	9	11
Mice4	1.9	10	8
Mice5	2	9	8
AVG.	2.38	8.8	9
SD	0.56745	0.83666	1.414214
SE	0.283725	0.41833	0.707107

Table 3.2.2.2: Observation of hot plate method (0, 15, 30, 45, 60, 90, 120 min.) for 200 mg *Cucumis sativus* dose:

0 minute	Basal reaction time (sec.)		
	Control	Pentazocine	M.E
Mice1	2	11	14
Mice2	3	9	7
Mice3	1	10	12
Mice4	2	8	8
Mice5	2	11	9
AVG.	2	9.8	10
SD	0.707107	1.30384	2.915476
SE	0.353553	0.65192	1.457738

15 minute	Basal reaction time (sec.)		
	Control	Pentazocine	M.E
Mice1	2	14	13
Mice2	2	12	14
Mice3	1.9	13	4
Mice4	2	14	5
Mice5	2	14	6
AVG.	1.98	13.4	8.4
SD	0.044721	0.894427	4.722288
SE	0.022361	0.447214	2.361144

30 minute	Basal reaction time (sec.)		
	control	Pentazocine	M.E
Mice1	2	13	15
Mice2	3	12	4
Mice3	1	12	6
Mice4	1.9	14	14
Mice5	2	13	13
AVG.	1.98	12.8	10.4
SD	0.70852	0.83666	5.029911
SE	0.35426	0.41833	2.514955

45 minute	Basal reaction time (sec.)		
	control	Pentazocine	M.E
Mice1	3	11	15
Mice2	2	12	15
Mice3	1	12	15
Mice4	1.9	13	13
Mice5	2	12	14
AVG.	1.98	12	14.4
SD	0.70852	0.707107	0.894427
SE	0.35426	0.353553	0.447214

60 minute	Basal reaction time (sec.)		
	Control	Pentazocine	M.E
Mice1	2	11	14
Mice2	3	10	5
Mice3	1	9	9
Mice4	1.9	11	12
Mice5	2	11	12
AVG.	1.98	10.4	10.4
SD	0.70852	0.894427	3.507136
SE	0.35426	0.447214	1.753568

90 minute	Basal reaction time (sec.)		
	control	Pentazocine	M.E
Mice1	3	9	12
Mice2	3	10	7
Mice3	1	11	12
Mice4	1.9	10	9
Mice5	2	9	10
AVG.	2.18	9.8	10
SD	0.843801	0.83666	2.12132
SE	0.4219	0.41833	1.06066

120 minute	Basal reaction time (sec.)		
	Control	Pentazocine	M.E
Mice1	2	8	12
Mice2	3	8	6
Mice3	3	9	12
Mice4	1.9	10	9
Mice5	2	9	12
AVG.	2.38	8.8	10.2
SD	0.56745	0.83666	2.683282
SE	0.283725	0.41833	1.341641

Table 3.2.2.3: Final observation of hot plate method test for *Cucumis sativus*

	Mean basal reaction time (sec.)						
Groups	**0 minute**	**15 minute**	**30 minute**	**45 minute**	**60 minute**	**90 minute**	**120 minute**
Control	2	1.98	1.98	1.98	1.98	2.18	2.38
Pentazocine	9.8	13.4	12.8	12	10.4	9.8	8.8
M.E 100	10.2	9.6	13.8	14.2	9.8	9.4	9
M.E 200	10	8.4	10.4	14.4	10.4	10	10.2

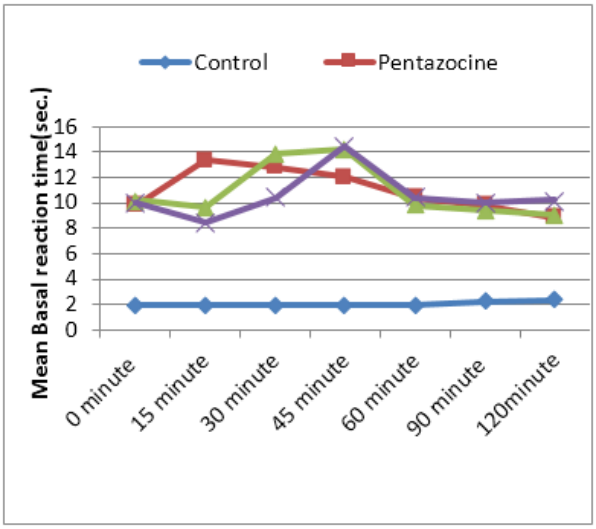

Fig 3.2.2.1: Graphical representation of data of Hot plate method test for *Cucumis sativus.*

4. Discussion

The preliminary phytochemical screening of *Cucumis sativus* showed the presence of alkaloids, saponins, steroids and flavonoids in our laboratory. These compounds have well known anti-inflammatory effects. The effects observed with Cucumis sativus could possibly be due to the synergistic actions of these compounds. In the present study, Cucumis sativus demonstrated a significant analgesic activity at different dose levels in animal models of pain.

Acetic acid induced writhing is a sensitive method for screening peripheral analgesic effect of compounds. It causes an increase in concentration of PGE2 and PGF2a in the peritoneal fluid. In this method, the plant shows significant analgesic effect.At dose 300 mg of plant extract shows 34.06% and dose 200 mg shows 30.62% inhibition.

The hot plate method originally described by Wolfe and Mac Donald (1994) has been found to be suitable for the evaluation of centrally but not peripherally acting analgesics. The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PGs may be minimized in this model. In this study, *Cucumis sativus* (200,300 mg/kg) exhibited a significant analgesic effect in all above models of pain.

Thus, it is concluded that *Cucumis sativus* possess good analgesic properties which are probably mediated via inhibition of prostaglandin synthesis as well as central inhibitory mechanism and may have a potential benefit for the management of pain disorders.

5. References

1. De-Smet PA. The role of plant derived drugs and herbal medicines in healthcare, Drugs, 1997, 54, 108.
2. History_-scope-importance-medicinal-plants.html, 27 Jan, 2015.
3. Joy PP, Thomas J, Mathew S, Skaria BP. Medicinal Plants. In Tropical Horticulture. Aromatic and Medicinal Plants Research Station. 2001; 2:449-632.
4. Shaker AM. Anticoagulants, Antiplatelets, and Thrombolytics. Methods in Molecular medicine TM. 93.
5. Lee HS. How safe is the readministration of streptokinase. Drug Saf 1995; 13:76-80.
6. Nicolini FA, Nichols WW, Mehta JL, Saldeen TG, Schofield R, Ross M. Sustained reflow in dogs with coronary thrombosis with K2P, a novel mutant of tissue plasminogen activator. J Am Coll Cardiol 1992; 20:228–235.
7. Adams DS, Griffin LA, Nachajko WR, Reddy VB, Wei CM. A synthetic DNA encoding a modified human urokinase resistant to inhibition by serum plasminogen activator inhibitor. J Biol Chem 1991; 266:8476–8482.
8. Lijnen HR, Vanhoef B, DeCock F, Okada K, Ueshima S, Matsuo O. On the mechanism of fibrin-specific plasminogen activation by staphylokinase. J Biol Chem 1991; 266:11826–11832.
9. Yamamoto J, Yamada K, Naemura A, Yamashita T, Arai R. Testing various herbs for antithrombotic effect. Nutrition 2005; 21:580–587.
10. en.wikipedia.org (Coagulation - Wikipedia, the free encyclopedia)
11. en.wikipedia.org (Thrombosis - Wikipedia, the free encyclopedia)
12. Furie B, Furie BC. Mechanisms of thrombus formation. New England Journal of Medicine 2008; 359(9):938–949.
13. Handin RI. "Chapter 53: bleeding and thrombosis". in Kasper DL, Braunwald E, Fauci AS, *et al*. Harrison's Principles of Internal Medicine 2005, 16.
14. eMedicine Article on Internal Jugular Vein Thrombosis by Dr. Dale K. Mueller. http://www.emedicine.com/med/topic2762.htm, 27 Jan, 2015.
15. Harvey, Richard; Champe, Pamela (series editors). "Lippincott illustrated reviews: Pharmacology", Edn 4, LWW, 2009.
16. Lullmann H, Mohr K. Color Atlas of Pharmacology, Edn 2, Thieme, 1999.
17. Golan DE. Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy, Edn 2, LWW, 2008.
18. Harper D. Online Etymology Dictionary: Analgesia. 3 Dec, 2012.
19. The examples represent respectively the three classes of nociceptive pain - mechanical, thermal and chemical - and neuropathic pain.
20. International Association for the Study of Pain: Pain Definitions [Retrieved 10 Sep 2011]. "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" Derived from Bonica JJ. The need of a taxonomy. Pain 1979; 6(3):247–8.
21. Raffa RB. J Clin Pharm Ther. Pain and Analgesic pathways 2001; 26:257-64.
22. Koster R, Anderson M, De-Beer EJ. Acetic acid for analgesic screening. Fed Proc 1959; 18:418.