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DIURETIC ACTIVITY OF UNRIPE PULP EXTRACTS OF CUCUMIS SATIVUS L

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ABSTRACT

Objective of the study is the evaluation of Diuretic activity of aqueous, ethanolic and ethyl acetate, unripe pulp extract of *Cucumis sativus* (Linn.) in validated experimental animal models. It is belonging to the family Cucurbitaceae. It is a non-toxic natural therapeutic agent. The phytochemical investigations of extracts of *Cucumis sativus* L unripe pulp was performed for the identification of alkaloids, proteins, saponins, tannins, glycosides carbohydrates and triterpenes etc. An ethanol, ethyl acetate, aqueous extracts of unripe pulp of *Cucumis sativus* L was assessed in male albino rats using *in-vivo* Lipschitz test model. The volume of urine, electrolytes concentration, specific gravity and pH were the parameters of the study. The preliminary phytochemical screening showed the presence of carbohydrates, proteins, alkaloids, triterpenoids, saponins, glycosides. The interpretation of the result was done after subjecting the data obtained from study to appropriate statistical analysis which included one-way ANOVA followed by Dunnett's Multiple Comparison Test. The result obtained from *in-vivo* studies showed that the extracts have significant diuretic activity. The result indicated that aqueous and ethanol extracts at 200, 500 mg/kg body weight shows significant ($p<0.05$) and electrolyte excretion ($p<0.001$) when compared to control. The result of the study have shown diuretic activity with the extracts of *Cucumis sativus* (Linn.). Further, experimental and clinical studies are required to elucidate the chemical constituents of the extract and mechanism responsible for the diuretic activity.

KEYWORDS: *Cucumis sativus*. Diuretic activity. Lipschitz test model.

INTRODUCTION

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. Health is no longer defined simply inphysical terms, as the absence of disease or disability, but now includes mental and social dimensions.[1] Approximately 90% of herbal raw drugs used in the manufacture of *Ayurveda, Siddha, Unani and Homeopathy* systems of medicines largely for the wild species.[2]

While traditional medicine has long used herbal extracts on patients, reverse pharmacology is aimed at validating such extracts through rigorous science.[3] Revival of herbal medicines and their usage in new light will be the next medical evolution or medivolution.[4] The plants are indispensable to man for his life.[5] Nature has provided a complete storehouse of remedies to cure all ailments of mankind. In those days, people collected information on herbs methodically and scientifically and developed well-defined herbal pharmacopoeias.[6] In recent times, focus onplant research has increased all over world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period.[7]

In view of all these, the present investigations have been

carried out to the conserve and multiply the species *Cucumis sativus* Linn as a medicinally important plant. The species contain a number of pharmacologically potential active compounds including the saponine, alkaloids, glycosides, carbohydrates, triterpienoides and the extract of the plants are reported to be useful in the treatment of wound healing antacid, carminative and anti-inflammatory etc. Due to its ethno medicinal importance the present investigation have been under taken.

MATERIALS AND METHOD

Collection of plant material: Fresh *Cucumis sativus* was collected and botanical authentification was carried. Fresh fruit pulp was homogenized, dried under shade, mass obtained was powdered & subjected to phytochemical analysis.

Extraction:[8] Dry powder was extracted first with ethyl acetate. The marc left after the extract was dried and subsequently extracted with ethanol. The marc left after the extract was dried and subsequently extracted with water. All the extract was concentrated and dried.

Qualitative phytochemical screening: Phytochemical analysis was done by the standard methods of Trease and Evans, Kokate C.K., Purohit A. P. and Gokhale S.B.[9]

Acute oral toxicity studies: Acute oral toxicity test was performed as per Organization for Economic Co-operation & Development guidelines 423[10], using healthy young adult female[10] Swiss albino mice weighing 25-30 g.

Animals were randomly assigned to five groups of three (n=3) each. Doses of aqueous, ethanolic and ethyl acetate extract of *Cucumis sativus* Linn (CSE) was given orally. Animals were fasted prior to the experiment and administered single dose of CSE dissolved in water and observed for mortality up to 48 hrs.

Diuretic activity

Albino male rats of weighing between 150-200g were procured, acclimatized under laboratory conditions. The rats were fed with rat pellet diet. Water was allowed *ad libitum* under strict hygienic conditions. Studies were performed in accordance the CPCSEA guidelines. Acute and sub acute toxicity studies did not detect any changes in vital organ function. Hence two doses are selected in the range of 200 mg/kg and 500 mg/kg. Lipschitz et al. test is based on water and sodium excretion in test animals and compared to rats treated with a high dose of control.[11,12,13,14]

Table 1: Grouping of animals for diuretic activity.

Groups	No. of Rats	Drug	Dose (orally)
Group-I	6	Distilled water	-
Group-II	6	Frusemide	20mg/kg
Group-III	6	Test ACSELD	200mg/kg
Group-IV	6	Test ACSEHD	500mg/kg
Group-V	6	Test ECSELD	200mg/kg
Group-VI	6	Test ECSEHD	500mg/kg
Group-VII	6	Test EACSELD	200mg/kg
Group-VIII	6	Test EACSELD	500mg/kg

ACSELD, ACSEHD=Aqueous *Cucumis sativus* Linn low & high dose extract.
ECSELD, ECSEHD=Ethanol *Cucumis sativus* Linn low & high dose extract.
EACSELD, EACSEHD= Ethyl acetate *Cucumis sativus* Linn low & high dose extract.

Six animals per group are placed in metabolic cages, fed with standard diet and water *ad libitum*. Prior to the experiment food and water are withdrawn. For screening procedures one group of six animals are used for one dose of the compound. Group-I vehicle control, group-II standard drug Frusemide, group-III test drug aqueous extract low dose, group-IV test drug aqueous extract high dose, group-V test drug ethanol extract low dose, group-VI test drug ethanol extract high dose, group-VII test drug ethyl acetate extract low dose, group-VIII test drug ethyl acetate extract high dose. Urine excretion is recorded, the sodium, potassium and chloride content in mEq/l, pH values of the urine is determined by auto electrolyte analyzer.[11,12,13,14]

The results data was expressed as mean value ± SEM, N=6. Statistical comparison analysis carried out by one way ANOVA and the difference between mean of treated groups and the non treated control group was evaluated by the Dunnett's multiple comparison test. The result was considered statistically significant when *represents P<0.05, **represents P<0.01,*** represents P<0.001 when compared with Control.

RESULTS

Extraction: Yield of ethyl acetate, ethanol and aqueous obtained was 20, 100, 50gm respectively.

Phytochemical constituents: The extracts were subjected to qualitative chemical analysis for the identification of various phytoconstituents. The results was recorded in the following table.

Table 2: Details of qualitative phytochemical tests.

Phytoconstituents	ACSE	ECSE	EACSE
Carbohydrates			
Molish's Test	+	+	+
Fehling's test	+	+	+
Benedict's Test	+	+	+
Proteins			
Millon's Test	+	+	+
Ninhydrin test	+	+	+
Alkaloids			
Mayer's Test	+	+	+
Wagner's Test	+	+	+
Dragendroff's Test	+	+	+
Tannins and Phenols			
5% Ferric chloride solution	_	_	_
Lead acetate test	_	_	_
Flavonoids			
Aq. Sodium hydroxide test	--	--	--
Sterols			
Salkowski's test	--	--	--
Saponins			
Froth test	+	+	+
Glycosides			
Legal's test	+	+	+
Modified Borntrager's test	+	+	+
Triterpenoids			
Salkowski	+	+	+

Presence (+), Absence (-)

The results of phytochemical screening showed the presence of carbohydrates, proteins, alkaloids, triterpenoids, saponins, and glycosides.

Acute oral toxicity: All the parameters observed were normal even at highest dosage. So the therapeutic dose for the pharmacological evaluation was 1/10th of the maximum tolerated dose of the experimental animal for the study.

Diuretic activity

Table 3: Urine output volumes in different hours.

Groups	Urine Volume in ml (Mean ± Sem)					
	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	24 hrs
Control	0.5±0.5	0.5±0.5	0.5±0.5	1±1	1.8±0.84	5.15 ± 0.05
Frusemide	4.75±1.25	6.5±1.25	8.5±1.89	8.5±1.8	8.75±1.79***	18.25±0.62***
ACSELD	1.07±0.41	1.07±0.41	1.45±0.21	1.95±0.69	1.95±0.69	12.2 ± 1.9**
ACSEHD	1.5±0.86	2.25±0.62	2.25±0.62	3±0.57	3±0.57	16 ± 0.81***
ECSELD	0.25±0.25	1±0.57	1±0.57	2.75±0.47	3±0.57	10.5 ± 2.5*
ECSEHD	0.75±0.75	1±1	1.25±0.94	2.75±0.47	3±0.40	13.5 ± 1.5**
EACSELD	0.5±0.5	1±0.408	1.5±0.28	2±0	2.5±0.28	8.5 ± 0.45
EACSEHD	0.75±0.75	1±1	1±0.94	2±0.47	3±0.40	11.63 ±0.37*

The values were expressed in Mean± SEM. N=6. * represents P<0.05, **represents P<0.01, *** represents P<0.001 when compared with Control.

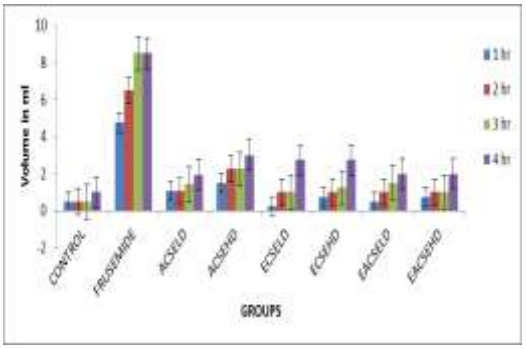

Fig. 1: Effect of various extracts of *C. sativus* in Urine output.
Each bar represents urine volume per hour

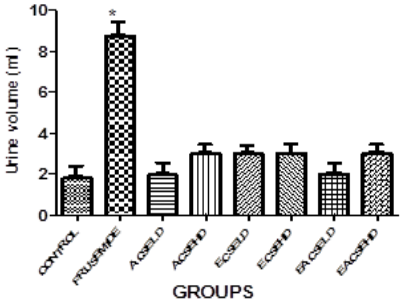

Fig. 2: Effect of extracts *C. sativus* in 5 hrs.
Each bar represents urine volume in 5 hrs.

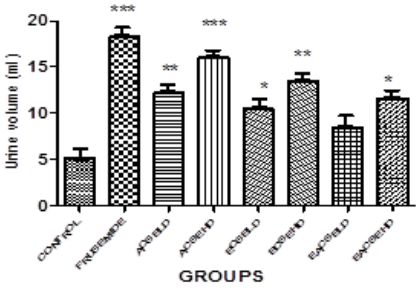

Fig. 3: Effect of extracts *C.satius* in 24 hrs.
Each bar represents urine volume in 24 hrs

The values were expressed in Mean± SEM. N=6. * represents P<0.05, ** represents P<0.01,*** represents P<0.001 when compared with Control.

Table 4: Urinary Excretion, Diuretic Action & Diuretic Activity in 24 hrs.

Groups	Average Volume of Water input / rat (V_0 in ml)	Average volume Urine output in 24hr (V_1 in ml)	Urinary excretion $V_0/V_1 \times 100$	Diureticaction	Diuretic activity
Control	5.6	5.15	91.96	0	-
Frusemide	5.2	18.25	350.96	381.6	100
ACSELD	5.4	12.25	226.85	246.68	64.6
ACSEHD	5.2	16	307.69	334.6	87.7
ECSELD	5.3	10.5	198.1	215.4	56.4
ECSEHD	5.2	13.5	159.6	173.55	45.5
EACSELD	5.2	8.5	163.46	177.75	46.6
EACSEHD	5.2	11.63	223.65	243.2	63.73

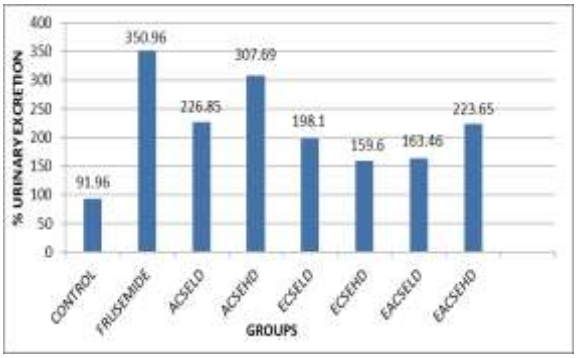

Fig. 4: Effect of extracts *C. sativus* in % Urinary Excretion.
Each bar represents % urinary excretion.

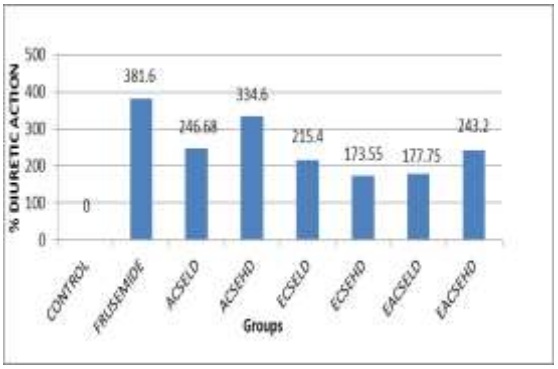

Fig. 5: Effect of extracts *C. sativus* in % Diuretic Action.
Each bar represents % diuretic action.

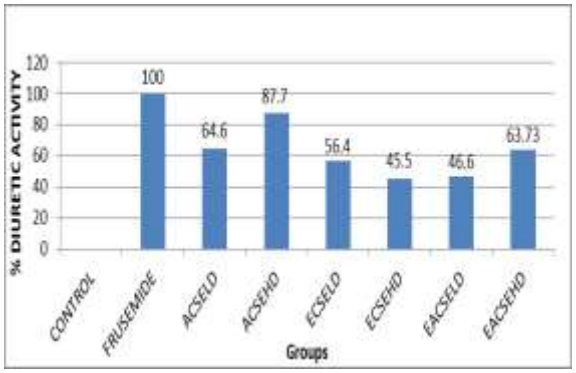

Fig. 6: Effect of extracts *C. sativus* in % Diuretic Activity.
Each bar represents % diuretic activity.

Table 5: Sodium, Potassium and Chloride levels in 24 hrs.

Groups	Na^+ mEq/l	K^+ mEq/l	Cl^- mEq/l
CONTROL	145± 2.38	97.5± 5.23	110.8± 5.18
FRUSEMIDE	291.5± 4.57***	187.6± 6.66***	223.5± 2.75***
ACSELD	235.5± 10.81***	159.2± 14.37***	170± 6.86***
ACSEHD	264± 6.37***	187.4± 11.01***	196± 13.34***
ECSELD	206.3± 10.51**	135.9± 12.81***	137± 5.94
ECSEHD	243.8± 24.35***	170.6± 19.1***	176.5± 17.73***
EACSELD	180.4± 5.7	119.6± 10.16**	132.3± 4.36
EACSEHD	241.2± 16.62***	168.9± 7.08***	186.7± 8.15***

The values were expressed in Mean± SEM. N=6. ** represents P<0.01, *** represents P<0.001 when compared with Control.

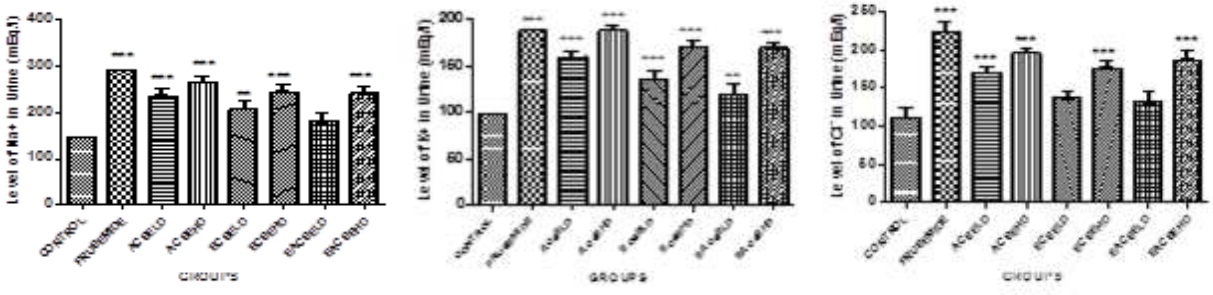

Fig. 7,8 & 9: Effect of extracts *C.sativus* in Na^+, K^+ & Cl^- excretion respectively.
Each bar represents level of Na+, K^+ & Cl^- ions in urine.
The values were expressed in Mean± SEM. N=6. * represents P<0.05,** represents P<0.01,*** represents P<0.001 when compared with Control.

Table 6: Saluretic action, Natriuretic action & Carbonic anhydrase inhibition.

GROUPS	Saluretic activity $Na^+ + Cl^-$	Natriuretic activity Na^+/K^+	Carbonic anhydrase inhibition $Cl^-/Na^+ + K^+$
CONTROL	255.8±4.55	1.176±0.37	0.5769±0.03
FRUSEMIDE	515±3.69***	1.559±0.05***	0.4670±0.01
ACSELD	405.5±15.76***	1.524±0.17***	0.4329±0.02*
ACSEHD	460±13.49***	1.421±0.07***	0.4346±0.02*
ECSELD	343.3±5.44**	1.579±0.23***	0.4019±0.02**
ECSEHD	420.3±24.94***	1.486±0.23***	0.4407±0.06*
EACSELD	312.7±7.32	1.544±0.14***	0.4427±0.02*
EACSEHD	427.8±22.2***	1.429±0.08***	0.4577±0.02

The values were expressed in Mean± SEM. N=6. *represents P<0.05, **represents P<0.01, *** represents P<0.001 when compared with Control.

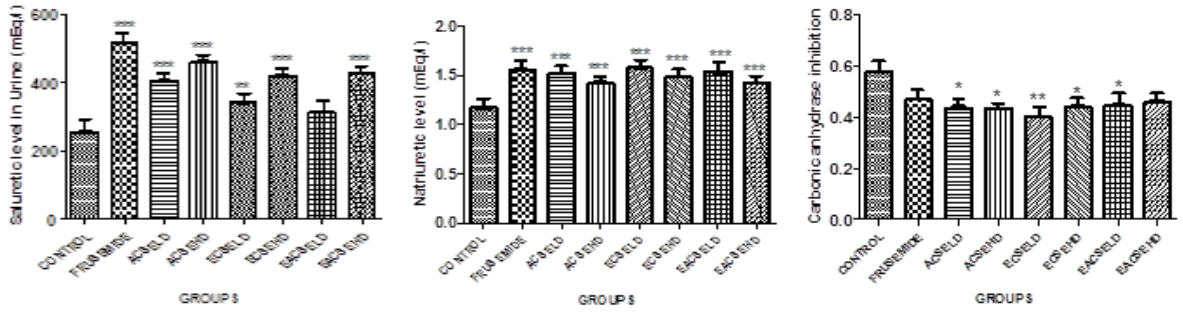

Fig.10,11 & 12: Saluretic actions, Natriuretic actions & Carbonic anhydrase inhibition respectively.

Each bar represents level of saluretic, natriuretic & Carbonic anhydrase inhibition

The values were expressed in Mean± SEM. N=6. *represents P<0.05, **represents P<0.01, ***represents P<0.001 when compared with Control.

Table 7: Urine pH & Specific gravity.

Groups	Control	Frusemide	ACSELD	ACSEHD	ECSELD	ECSEHD	EACSELD	EACSEHD
Urine pH	6.375 ± 0.12	7.75 ± 0.25*	7.5 ± 0.28	6.75 ± 0.43	7.5 ± 0.28	7.375 ± 0.37	8.25 ± 0.25***	8.75 ± 0.25***
Specific gravity	1.014± 0.001	1.01 ± 0.002	1.008 ± 0.001*	1.013 ± 0.001	1.011 ± 0.001	1.011 ± 0.001	1.005 ± 0***	1.005 ± 0***

The values were expressed in Mean± SEM. N=6. *represents P<0.05, **represents P<0.01, *** represents P<0.001 when compared with Control.

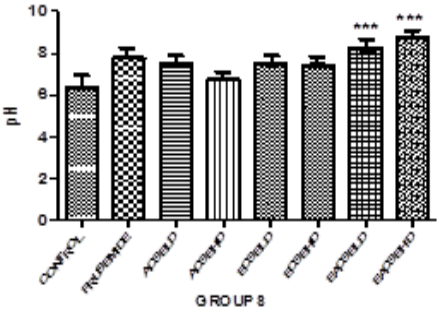

Fig. 13: Urine pH.

Each bar represents pH of urine

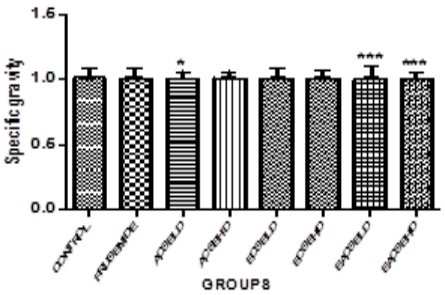

Fig. 14: Specific gravity.

Each bar represents specific gravity of urine.

The values were expressed in Mean± SEM. N=6. * represents P<0.05, ** represents P<0.01,*** represents P<0.001 when compared with Control.

DISCUSSION

Non-toxic nature of extracts of *Cucumis Sativus* Linn is evident by the absence of mortality of the test animals at oral treatment.

Phytochemical screening revealed the presence of alkaloids, triterpenoids, carbohydrates, proteins, saponins and glycosides. Diuretic activity observed in the crude extract might be due to the presence of these phytochemicals.

A method for testing diuretic activity in ratswas designed as described by Lipchitz *et al*. The mean urine output in 5th hrs the results when compared with control group and standard group, p value were 0.001 showed statistically significant. The mean urine output in 24th hrs the results when compared with control group and trial group ACSELD, ECSEHD **'p'** value was 0.01 showed statistically significant and when compared with control and standard, **'p'** value was 0.001 showed statistically highly significant and when compared control group and trial group ACSEHD, **'p'** value was 0.001 showed statistically highly significant like standard. Control group and trial group ECSELD, EACSEHD **'p'** value was 0.01 showed statistically significant. After the 24th hrs ACSEHD produce more urine output compare to other all extract CSE. Out of all the group standard Frusemide produce more urine output and also the onset of action is more in Frusemide compared to test extracts of CSE .after the treatment of 1 hrs Frusemide produce 4.75 ml of urine output.

Urinary excretion in control group was 91.96%, standard (Frusemide) group 350.96%, ACSELD, ECSELD, EACSELD, ACSEHD, ECSEHD, EACSEHD was 226.85, 198.1, 163.46, 307.69, 159.6, 223.65%, respectively of loaded water orally. In the present study showed the ACSEHD produce more urinary excretion activity.

Diuretic actions of groups were estimated by ratio of urinary excretion of treated group by urinary excretion of control group. Diuretic action of standard (Frusemide) group being 381.6% more than control group, ACSELD, ECSELD, EACSELD, ACSEHD, ECSEHD, EACSEHD was 246.68, 215.4, 117.75, 334.6, 215.4, 243.2% more than control respectively. In the present study showed the ACSEHD produce more diuretic action.

Diuretic activity of standard (Frusemide) group being 100%, ACSELD, ECSELD, EACSELD, ACSEHD, ECSEHD, EACSEHD was 64.6, 56.4, 46.6, 87.7, 45.5, 63.73% compared with standard respectively. In the present study showed the ACSEHD produce more diuretic activity compared with standard.

The mean Na^+ output in 24th hrs results when compared with control group and trial groups ECSELD **p<** 0.01 showed statistically significant when compared with control and standard, **'p'** value was 0.001 showed statistically highly significant when with compared with control group and trial groups ACSELD, ACSEHD, ECSEHD, EACSEHD showed **'p'** value was 0.001 statistically highly significant like standard.

The mean K^+ output in 24th hrs results when compared with control group and standard, **'p'** value was 0.001 showed statistically highly significant when compared with control group and trial groups ACSELD, ACSEHD, ECSELD, ECSEHD and EACSEHD showed **'p'** value was 0.001 statistically highly significant like standard.

The mean Cl^- output in 24th hrs results when compared with control group and standard, **'p'** value was 0.001 showed statistically highly significant when compared with control group and trial groups ACSELD, ACSEHD, ECSEHD, EACSEHD showed **'p'** value was 0.001 statistically highly significant like standard.

The mean saluretic activity in 24th hrs results when compared with control group and trial groups ECSELD **p<** 0.01 showed statistically significant when compared with control and standard, **'p'** value was 0.001 showed statistically highly significant when compared control group and trial groups ACSELD, ACSEHD, ECSEHD, EACSEHD showed **'p'** value was 0.001 statistically highly significant like standard.

The natriuretic activity in 24th hrs results when compared with control group and standard, **'p'** value was 0.001 showed statistically highly significant when compared with control group and trial groups ACSELD, ACSEHD, ECSELD, ECSEHD, EACSELD, EACSEHD showed **'p'** value was 0.001 statistically highly significant like standard. The ratio of Na^+/K^+ was calculated for natriuretic activity. Values greater than 2.0 indicate a favorable natriuretic effect. So *Cucumis sativus* L pulp extract may not produce natriuretic activity. But it increased the urine output.

The carbonic anhydrase inhibition results when compared with control group and trial groups ECSELD **p<** 0.01 showed statistically significant when compared with control and trial groups ACSELD, ACSEHD, ECSEHD, EACSELD, showed **'p'** value was 0.05 slightly significant when compared with control. The ratio Cl^-/Na^+ & K+ (ion quotient) was calculated to estimate carbonic anhydrase inhibition. Carbonic anhydrase inhibition was excluded at ratios between 1.0 and 0.8. With decreasing ratios slight to strong carbonic anhydrase inhibition can be assumed. So *Cucumis sativus* L produces slight carbonic anhydrase inhibition.

The mean pH change results when compared with control group and trial groups ECSELD **p<** 0.01 showed statistically significant and when compared with control and standard, **'p'** value was 0.05 showed statistically significant when compared with control group and trial groups EACSELD, EACSEHD showed **'p'** value was 0.001 statistically highly significant compared with control. Urine excreted in group standard tends to exhibit alkaline pH value being and urine excreted in group EACSELD, EACSEHD tends to exhibit alkaline pH valve being 8.25, 8.75, respectively .The other groups do not show much change in their urine pH valve compared

with control.

The mean specific gravity change results when compared with control group and trial groups ECSELD **p<** 0.01 showed statistically significant when compared with control and standard, **'p'** value was 0.05 showed statistically significant when compared with control group and trial groups EACSELD, EACSEHD showed **'p'** value was 0.001 statistically highly significant compared with control. Except trial group EACSELD, EACSEHD, standard the specific gravity of urine was unaltered in all groups.

In the study there is an increase in urine output and urinary pH. In the study the treatment with extract improved urinary pH slightly and also urinary volume. Thisincrease in pH might be due to electrolyte excretion. An increase was observed in the 24^{th} hrs urine volume in the rats treated with extract when compared with control. This improvement in the urinary volume and pH may indicate diuretic activity.

Cucumis sativus L had diuretic activity, from above discussion it is apparent that the glycoside, triterpenoid, saponin complex play a major role in mechanism of action of all the groups. In crude powder this complex had major role in diuretic activity regulated by Arachidic acid or Eicosanoids induced prostaglandins renin pathway, again glycosides becomes prominent and show increased carboxyl inhibition predominance and low saluretic, carbonic anhydrase inhibition activity. Phytochemical screening revealed the presence of alkaloids, triterpenoids, carbohydrates, proteins, saponins, and glycosides. Therefore the reason for diuretic activity might alkaloids, saponins, glycoside and triterpenoids. All the results obtained were support the diuretic activity of unripe pulp of CSE.

CONCLUSION

On preliminary phytochemical screening showed the presence of carbohydrates, proteins, alkaloids, triterpenoids, saponinsand glycosides. These compounds offers diuretic activity. The non-toxic nature of extracts was evident from the acute oral toxicity conducted as per OECD guidelines. Normal behaviour of test animals suggests the non-toxic nature of the foresaid extracts. Hence *Cucumis. sativus* Linn could be safe. Further studies are warranted for determining chronic toxic symptoms.

Cucumis.sativus Linn extracts produce low ceiling diuretic effect. Mode of action of drug is demonstrated carbonic anhydrase effect and also showed activity by regulation of Renin–Angiotensin pathway. The experimental data should be validated further in the clinical model ascertain its exact mode of activity in human subjects. Use of other low ceiling diuretics should be used to validate further the mode of action of the drug. However, the contribution of alkaloid, saponin and glycoside compounds to diuretic activity cannot be ruled out. Further studies like isolation and characterization of the diuretic principle from the extract of *Cucumis. sativus* L is needed to conform the activity from the present study it may be concluded that the claim of the native practitioners that, *Cucumis.sativus* L possesses diuretic activity is justifiable.

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