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HEPATO PROTECTIVE AND ANTIULCER PROPERTIES OF ISOLATED COMPOUND FROM *Cucumis sativus* L.

Vaijayanthimala Palanisamy[2]*, Sureshkumar Shanmugam[1], Sangameswaran Balakrishnan[2].

1. Department of Pharmaceutical Chemistry, J. K. K. Nattraja College of Pharmacy, Komarapalayam Namakkal-638183, Tamilnadu, India.

2. Department of Pharmaceutical Chemistry, SSM College of Pharmacy, Erode, Tamilnadu-638312, India.

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***Corresponding author:**

Vaijayanthimala Palanisamy

Email:

vaijsiva@gmail.com

Tel.:+91-9488376917.

ABSTRACT

***Aim*:** In the present investigation triterpenoid isolated from an ethanol extract of *Cucumis sativus* L. (EECS) leaves were carried out by column chromatography method. The column chromatography system is broadly used for separation, isolation, and purification of the natural products.

Methods: The fundamental principal followed for separation of the compounds is adsorption at the solid liquid interface. In EECS the fractions of 231-248 eluted with ethyl acetate: n-hexane (20:80) gave a light green residue and showed one major spot with minor impurities on TLC. Repeated recrystallisation with chloroform: methanol (90:10) gave colorless compound. The color less compound showed a single spot in n-hexane: ethyl acetate (8:2) and was further proved for its homogeneity with different solvent system by TLC and designated as compound 2 (ISO-2).

Result and conclusion: From preliminary characterization using IR, NMR and MASS spectra it was identified as triterpenoid. The compound was evolved to show moderate hepatoprotective and antiulcer activity at 150mg/kg than compared with control. It was concluded that an isolated compound may be triterpenoid which shows moderate activity against hepatoprotective and anti ulcer effect and further investigation is needed to isolate the potent compound from the drug.

Key words: *Cucumis sativus* L.., Hepatoprotective, Column chromatography and Antiulcer.

INTRODUCTION

Cucumbers (***Cucumis sativus***) are botanically categorized as berries, which are available in different sizes shapes and colors. They range from thick, stubby little fruits (10 - 12 cm long) to Dutch greenhouse varieties (of up to 50 cm long). The most popular variety is the long, smooth salad, cucumber which has a smooth, dark-green skin [1].

They were already used in ancient times to dissolve stones caused by uric acid. Their cleansing effect on the intestines, kidneys, lung and skins was already known. People suffering from stomach or liver diseases also benefit from the consumption of cucumbers. It is also known to cure some headaches, bleeding, dizziness, and pale skin. Cucumber juice contains substances, which promotes blood circulation of the skin [2]. For this reason it is widely used in cosmetics. Fruits are used as laxative, astringent, anthelmintic and antipyretic also useful in hepatitis, bronchitis, asthma, dyspepsia, piles, diarrhoea, coughs hoarseness of voice, eye diseases and scorpion sting and hair tonic. A decoction of the green fruit is used for cough. The pulp of the fruit is useful in dysentery-diarrhea, dropsy, piles and leprosy. Half ripe fruit is used as purgative. The kernel of the fruit is narcotic. Fruits are used in menstrual disorder in Khagrachari. Seed oil is used in rheumatism. The gum of the bark is demulcent and purgative. The Triterpenoid present in the fruits

possesses significant antimicrobial activity. Kernel oil has purgative action and its prolonged use was well tolerated in mice.

The extensive survey of literature revealed that ***Cucumis sativus*** is an important medicinal plant with diverse pharmacological spectrum. ***Cucumis sativus*** is widely used in Ayurveda, Siddha, Chinese medicine, etc. [3]. The vast study done on the plant proved that the plant has many important phytoconstituents like Glycosides, flavones, terpinoids, phytosterol, saponins, anolignan B, Tannins, glucose, fructose. These compounds were found to be responsible for many of the pharmacological activities such as antibacterial, antifungal, antidiabetic, Cytotoxic , Antacid & Carminative activity , Hepatoprotective activity, Wound healing activities. Further the plant is used in the treatment of gastric ulcer, constipation, general debility, piles. Hence, this plant provides a significant role in the prevention and treatment of a disease [4]. So it was planned to isolate the compound from ***Cucumis sativus*** and carry out pharmacological screening for Hepatoprotective and Antiulcer effect of the isolated compound.

MATERIAL AND METHODS

Drugs and chemicals

All reagents used in the procedure were analytical grade. Paracetamol tablet (Sun Pharmaceuticals Ltd) purchased from a drugstore. Total Bilirubin, Direct Bilirubin, Total Proteins, SGOT, SGPT, Alkaline Phosphate was assayed by using kits from Ranbaxy diagnostic, New Delhi. Ranitidine (Ranbaxy Pharmaceuticals Ltd) purchased from the local drug store for antiulcer properties.

Plant collection

Fresh leaves of C. *sativum* L. Were collected from field of Komarapalayam and authenticated by Dr. P. Satyanarayana, Scientist D & Head office in charge, Southern Regional Centre, TNAU campus, Coimbatore. Voucher specimen (No: JKKNCP 0102/13) has been deposited in the Department of Pharmacognosy, JKK Nataraja College of Pharmacy, Komarapalayam, Tamilnadu, India

Ethanol extract of *Cucumis sativum* Linn (EECS)

Fine powdered Leaves of *C. sativum* L. were extracted successively with petroleum ether and ethanol (60-80° C) using soxhlet apparatus. The extract was filtered and evaporated to separate solvent and residue. The semisolid residue thus obtained was stored in desiccator until for using isolation. 5 gms of above residue were mixed with solvent and introduced into silica gel column chromatography for isolation purpose.

The fractions were collected in 50 ml portions and monitored on TLC and the fractions showing similar sports were collected. The fractions of 231-248 eluted with Ethyl acetate: n-hexane (20:80) gave light green residue and showed one major spot with minor impurities on TLC. Repeated recrystallisation with chloroform: methanol (90:10) gave colorless compound. The colorless compound showed a single spot in n-hexane: ethyl acetate (8:2) and was further proved for its homogeneity with different solvent system by TLC and designated as compound 2 (ISO-2). The gradient isolation technique was followed; the result was given in Table 1.

Animals:

Albino rats either sex weighing between 175 ± 25gm was used in this evaluation. These rats aged between 2 -2.5 months were procured from animal house located in JKK Nataraja College of Pharmacy, Komara palayam. They were housed in well ventilated stainless-steel cages at room temperature (24±2°C) in hygienic condition under natural light and dark schedule and were fed on a standard laboratory diet. Food and water were given ad libitum.

EXPERIMENTAL PROTOCOL

Acute oral toxicity study.

The acute oral toxicity study was followed by using OECD guidelines - 423 (Organization of Economic Co-operation and Development) – Fixed dose procedure (FDP)

Acute toxicity study was performed for EECS according to the acute toxic classic method as per OECD (423) guidelines [5], albino rats were used for acute toxicity study. The animals were kept in fasting condition for overnight providing only water, and then the extracts were administered orally at the doses of 5, 50, 300 and 2000 mg/kg and observed for 16 days. If death was observed in 2 out of 3 animals, then the dose administered was concluded as toxic dose. Animals aren't shown signs of toxicity including mortality; nature, severity, and duration of effects up to the dose level of 2000 mg/kg for all the three extracts.

Hepatoprotective activity

Hepatoprotective active is carried out as follows [6].

Group 1 - Normal control rats, which received 0.5% Carboxy methyl cellulose (CMC)Solution (1ml/kg) one time daily for 7 Days.

Group 2 - Received Hepato toxic agent Paracetmol (3gm/kg) a single dose on day 7.

Group 3 - Received Standard drug control, Silymarin (100mg/kg) once daily for 7days (Std).

Table 1: Column chromatography of EECS

Fraction No	Eluent	Residue on evaporation
1-5	n-Hexane	No Residue
6-10	n-Hexane: Petroleum ether(80:20)	No Residue
11-15	n-Hexane: Petroleum ether(60:40)	No Residue
16-20	n-Hexane: Petroleum ether(20:80)	No Residue
21-25	Petroleum ether	No Residue
26-36	Petroleum ether: Chloroform(80:20)	Light Yellow Residue
37-45	Petroleum ether: Chloroform(70:30)	Light Yellow Residue
46-54	Petroleum ether: Chloroform(60:40)	Light Yellow Residue
55-61	Petroleum ether: Chloroform(50:50)	Yellow Residue
62-72	Petroleum ether: Chloroform(40:60)	Yellow Residue
73-82	Petroleum ether: Chloroform(30:70)	Yellow Residue
83-95	Petroleum ether: Chloroform(20:80)	Yellow Residue
96-126	Petroleum ether: Chloroform(10:90)	Yellow Residue
127-132	Chloroform	Yellow Residue
133-142	Chloroform: Ethyl Acetate(80:20)	White Residue
143-155	Chloroform: Ethyl Acetate(60:40)	White Residue
156-170	Chloroform: Ethyl Acetate(40:60)	White Residue
171-185	Chloroform: Ethyl Acetate(20:80)	Yellow Residue
186-206	Ethyl acetate	Light Brown Residue
207-215	Ethyl acetate: n-hexane(80:20)	Light Brown Residue
216-224	Ethyl acetate: n-hexane(60:40)	Light Brown Residue
225-230	Ethyl acetate: n-hexane(40:60)	Light Brown Residue
231-248	Ethyl acetate: n-hexane (20:80)	Light green Residue
249-277	Methanol	Brown Residue
278-290	Acetone	Brown Residue

Group 4 - Receives EECS (150mg/kg) for 7 days.

Group 5- Receives ISO 2 for 7 days (150mg/kg)

Group-3 to Group-5 receives Paracetmol (3gm/kg) as a single dose on 7 th days, after thirty

Minute's administration of drug extract and Silymarin respectively.

Assessment of hepatoprotective activity

In the present study the hepatoprotective activity was evaluated biochemically and histopathologically. After 24 hours of drug treatment, the animals were dissected under ether anesthesia. From each rat blood sample was withdrawn from the carotid artery in the neck and collected in previously labelled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum from the blood was separated by centrifugation at 7000 RPM for 10 minutes. The separated serum was used for the estimation of some biochemical parameters like SGPT, SGOT, bilirubin and protein.

For the histopathological study [Fig 1-5], liver from each animal was removed after dissection and preserved in 10% formalin. Then representative blocks of liver tissues from each lobe were taken and processed for paraffin embedding using the standard micro technique. Sections (5 µm) of livers stained with eosin and hemotoxylin, observed microscopically for histopathological studies. The result was given Table no.2.

Antiulcer activity:

Group 1-Normal control rats, which received distilled, water (1ml/kg) orally.

Group 2-Receives Indomethacine (25mg/kg) as a single dose for 3days

Group 3-Receives Ranitidine (100mg/kg) as a standard reference drug (Std).

Group 4-Receives EECS (150mg/kg) once daily.

Group 5-Receives ISO-2 (150mg/kg) once daily

Group 2 and Group 5 receive Indomethacine (25mg/kg) as a single dose for 3days as an ulcerative agent 1 hour before the ulcerogenic procedures.

The animals were sacrificed 6 hr. after the administration of necrotizing agent. The stomachs were removed and opened along with the greater curvature of the stomach; the ulcer index was evaluated according to severity and scored microscopically with the help of a hand lens (10 xs) as follows [7, 8, and 9].

Scoring of ulcer [10]

0 = Normal colored stomach,

0.5 = Red coloration,

1 = Spot ulcers,

1.5 = Hemorrhagic streaks,

2 = Ulcer > 3mm but < 5mm,

3 = Ulcers > 5mm

Calculation of ulcer Index [11]

UI = UN + US + UP x 10-1

UI = Ulcer Index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animals with an ulcer

Determination of Acidity:

$$\text{Acidity} = \frac{\text{VolumeofNaoH} \times \text{NormalityofNaoH}}{0.1} \times 100\text{mEq/L}$$

Determination of Percentage Protection:

$$\%\,\text{Protection} = \frac{\text{Controlmeanulcerindex} - \text{Testmeanulcerindex}}{\text{Controlmeanulcerindex}} \times 100$$

In the present study, evaluated for ISO -2 for anti-ulcer activity against Indomethacine induced gastric ulcer model. The results of study are tabulated in Table-3.

RESULT AND DISCUSSION:

Histopathological studies of rats treated with Paracetmol illustrate severe necrosis and the disappearance of nuclei. This could may be because of the formation of highly reactive metabolites NAPQI. The histopathological modification was considerably reduced in rats treated with EECS and ISO-2.

The study of serum markers viz SGOT, SGPT, ALP, bilirubin and total protein was charged due to clinical and experimental liver damage [12]. In the present investigation, the rats are undergoing major hepatic damage from treatment with Paracetmol, was pointed out by elevated levels of serum markers (Table 2). The Hepatoprotective properties of *Cucumis sativus* are due to the presence of triterpinoid, saponins, flavonoids and tannins [13]. ISO-2 provided the moderate hepatoprotective than compared to control.

Antiulcer Activity:

Indomethacin is normally used for inducing ulcer in experimental rats [14]. Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) that reduces prostaglandin production which results in increase acid production and decrease in cytoprotective mucus formation that can lead to induce gastrointestinal ulcer [15].

In the present study, confirmed that ISO-2 can significantly enhance gastric mucus secretion though reducing the acidity of the gastric juice in rats. Gastric

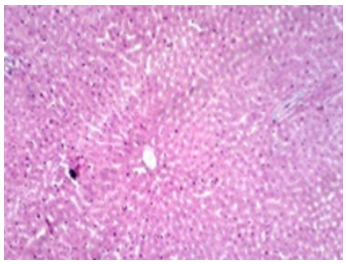

Fig No1: Control

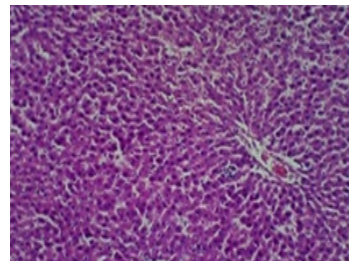

Fig No 2: Paracetmol induced

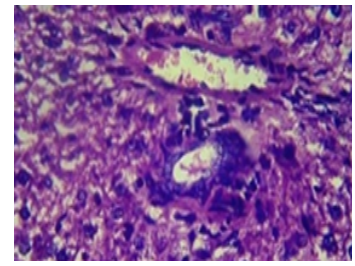

Fig No3: Silymarin

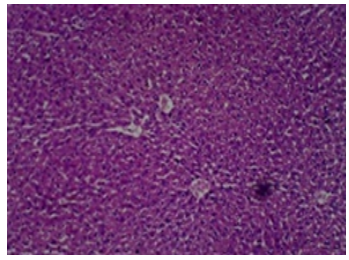

Fig No 4: EECS

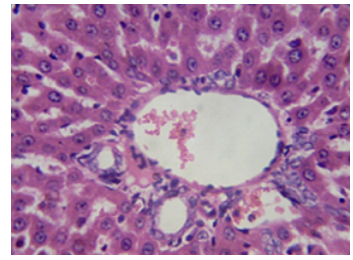

Fig No 5: ISO-2

Table 2: Evaluation of serum parameters of ISO 2 and standard drug on hepatotoxic rats

S.No.	Particulars	SGPT (U/ml)	SGOT (U/ml)	ALP (U/ml)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total Protein (mg/dl)
1.	Control	63.73±5.33	135.03± 12.21	170.33±22.22	0.653±.13	0.31±0.17	6.98±0.19
2.	Paracetamol (3gm/kg)	282.58±10.13 *	419.65±25.93 *	436.51±27.07*	2.14±.90*	1.89±0.12*	2.56±0.24*
3.	Sylimarin (100 mg/kg)	68.31±7.44†	171.06±17.75 †	175.88±22.84†	0.94±.17†	0.34±0.16†	5.87±0.25†
5.	EECS (150 mg/kg)	174.08±20.92 †	222.7±14.06†	206.30±17.47†	1.45±.31 ^{ns}	0.85+0.10†	4.73±0.29†
9.	ISO 2 (150 g/kg)	156.93±12.88 †	207.6±6.72†	246.05±21.82†	1.23±.61†	0.77±0.17†	3.79±0.16†

Values are expressed as mean ± S.D. of six animals in each group. Statistical analysis ANOVA followed by Dunnett *t*-test.N= 6

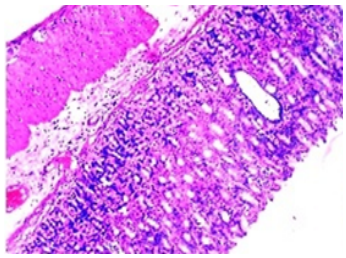

Fig No 6: Control

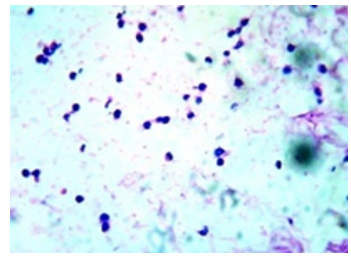

Fig No 7: Indomethacin

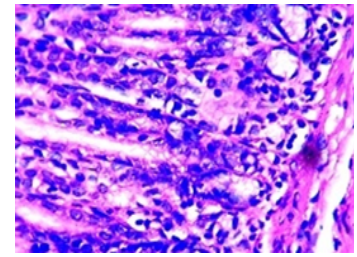

Fig No 8: Ranitidine

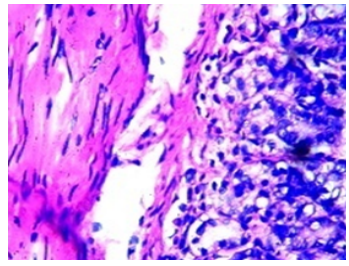

Fig No 9: EECS

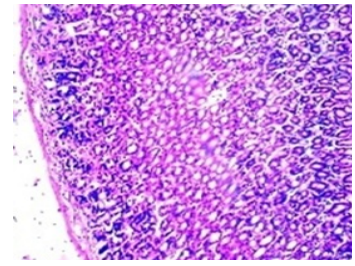

Fig No 10: ISO-2

Table 3. Antiulcer activities of rats treated with extracts and standard

S. No.	Body wt. gms	Treatment Group Control	Vol. of Gastric Juice (ml)	Free Acidity (Eq/l) 100gm	Total Acidity (Eq/l) 100gm	PH
1.	**175**±25	Control	1.2±0.71	33.66± 3.14	42.33±3.14	7.33±0.33
2.	**175**±25	Ulcer Control Indomethacin 25mg/kg	10.1±2.39**	83.16± .16**	104±6.16**	2.55±0.32**
3.	**175**±25	Ranitidine 100mg/kg	2.28±0.64†	36±7.63†	45±2.60†	7.4±0.36†
4.	**175**±25	EECS150 mg/kg	4.25±0.34†	49.±5.79†	60.5.±8.87†	4.75±0.77†
5.	**175**±25	ISO 2 150 mg/kg	4.5±0.19†	47.3±2.60†	50.5±5.54†	5.81±0.85†

Values are expressed as mean ± S.D. of six animals in each group. Statistical analysis ANOVA followed by Dunnett *t*-test, N= 6 ***P* < 0.01 as compared with control, †*P* < 0.01 as compared with standard

mucus is one of the protective factors for the gastric mucosa and it is able to acting as an antioxidant agent and reducing mucosal damage mediated by oxygen free radicals [16]. Another important involving in gastro protective effect is the inhibition of acid secretion, since when levels of acid overcome mucosal resistance mechanisms this lead to ulcer formation [17]. In our study, ISO -2 caused significant inhibition in the acidity (both total and free) with an elevation in gastric pH. A similar model was reported [18].

In the present study, indomethacin, one of NSAIDs family, caused a remarkably significant increase in ulcer index, gastric juice free and total acidity and PH. Oral administration of RAN significantly reduced the ulcer index, gastric juice free and total acidity and PH. The result was expressed in Table 3. ISO-2 provided a moderate effect in gastro protective activity than compared to control. Histopathology report also supported the same [Fig: 6-10].

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