ABSTRACT

The present study aims to evaluate the diuretic activity of the petroleum ether extract of Kaempferia galanga Linn in animal models using Lipschitz method. Kaempferia galanga Linn from Zingiberaceae family is often used in manufacture of traditional medicine. The leaves and rizomes of Kaempferia galanga is reported to have traditional use as aromatic, carminative, stimulant, diuretic and expectorant. There are no previous references in literature about any studies done on diuretic activity. So the present work was aimed to investigate the diuretic activity of Kaempferia galanga rhizome extract. The petroleum ether extract of Kaempferia galanga rhizomes were prepared by soxhlet extraction. Preliminary phytochemical investigation showed the presence of triterpinoids, sterols, resins, glycosides and volatile oil. The pharmacological evaluation of the drug for diuretic activity was performed on healthy wistar rat of either sex by Lipschitz method, using Furosemide as the standard drug. Here 4 groups, each containing 6 rats were selected and each group received control (CMC1%), standard (Furosemide 10mg/kg), tests of 200mg/kg and 400mg/kg respectively. The total volume of urine excreted in 24 hrs was collected for the assessment of diuretic activity. Sodium and potassium level of the urine sample were also estimated. The results are expressed as “Lipschitz value” (diuretic index) and the value of 1 or more suggested that, the extract shows dose dependent increase in urine volume. Therefore the results obtained in this study provide a quantitative basis to explain the traditional folkloric use of Kaempferia galanga as a diuretic agent.

KEYWORDS: Kaempferia galanga, Diuretic activity. Lipschitz value.
INTRODUCTION
Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. These drugs are made from renewable resources of raw materials by ecofriendly processes and will bring economic prosperity to the masses growing these raw materials. Although modern medicine may exists side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons.[1]

Urinary system is a group of organs in body concerned with filtering out excess fluid and other substances from blood stream. Kidneys are main organs of homeostasis because they maintain acid base balance and water salt balance of body. Diuretics are drugs that increase the rate of urine flow, sodium excretion and used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug-induced diuresis is beneficial in many life threatening disease conditions such as cognitive heart failure, hypertension and pregnancy toxemia, odema. These also play an important role in hypertensive patients, pulmonary congestion, this decreases cardiac work load, oxygen demand, plasma volume, thus decreasing blood pressure and also treat the acute and chronic renal failure, hypercalciuria, cirrhosis of liver. Most diuretic drugs have adverse effect on quality of life including impotence, fatigue and weakness. Naturally occurring diuretics include caffeine in coffee, tea and cola, which inhibit Na⁺ reabsorption and alcohol in beer, wine and mixed drinks, which inhibit secretion of Anti-Diuretic Hormones.[2] Kaempferia galanga Linn from Zingiberaceae family is often used in manufacture of traditional medicine. The leaves and rhizomes of Kaempferia galanga is reported to have traditional use as aromatic, carminative, stimulant, diuretic and expectorant. There are no previous references in literature about any studies done on diuretic activity. So the present work was aimed to investigate the diuretic activity of Kaempferia galanga rhizome extract.

MATERIAL AND METHODS
PLANT MATERIAL
The whole plant of Kaempferia galanga Linn were collected from Koothattukulam, Pala, Kottayam district Kerala, India in October 2013. The plant was identified and authenticated
by Gokul. G. Nair, Head of the Department of Botany, Baselius college, Kottayam. The rhizome was collected from whole plant and it was cleaned. It was dried under shade and cut into small pieces. About 50g of the rhizome was extracted using 300ml of petroleum ether in a soxhlet apparatus for about 48 hours. After 48 hours the crude extract was evaporated at room temperature to obtain concentrated petroleum ether extract, it is stored under 4°C and used for various studies.

**PHYTOCHEMICAL ANALYSIS**

For the identification of active principles in the petroleum ether extract of *Kaempferia galanga* rhizome various tests for carbohydrates [molish test], proteins [biuret test], sterols [salkowski and libermanns test], glycosides, alkaloids [dragandorrffs test], resins and volatile oil were done. 

**PHARMACOLOGICAL EVALUATION**

**Experimental animals**

Healthy wistar albino rats of either sex weighing 150-200g were procured from Animal house, Department of Pharmacology, DPS, MGU, RIMSR, Puthuppally. The animal were housed under standard laboratory condition of light and dark cycle, temperature of 25°C ± 2°C and 30 - 60% relative humidity. They were maintained with standard diet and water ad libitum and housed in clean polypropylene cages.

**Acute oral toxicity**

Acute toxicity studies of the plant extract was already done and reported as per OECD423 guidelines. The study suggested that, petroleum ether extract of *Kaempferia galanga* was found be safe up to dose of 5000 mg/kg.

**Evaluation of diuretic activity**

Diuretic activity was evaluated on petroleum ether extracts of rhizome of plant *Kaempferia galanga* using Lipschitz method. Healthy wistar rats of either sex were divided into four groups of six animals each. Furosemide [10mg/kg] was used as standard reference drug. Before the experiment, the rats were fasted for 18 hours. On the day of experiment Group 1 which serves as control received carboxy methyl cellulose orally. Group 2 served as positive control and received furosemide [10mg/kg] orally. Group 3 and Group 4 received petroleum ether extract, orally at a dose of 200mg/kg, p.o and 400mg/kg, p.o respectively suspended in 1% CMC.
Immediately after administration, the rats [one in each cage] were placed in metabolic cages specially designed to separate urine and feces and kept at room temperature of 25 ± 0.5ºC. The metabolic cage is provided with wire mesh bottom and a funnel to collect the urine. The urine was collected in measuring cylinder up to 8 hour for all control and drug treated groups. During this period no food or water was made available to the animals. The volume of urine was estimated for assessment of diuretic activity and concentration of sodium and potassium were determined by flame photometer.\[^5\]

**Diuretic index**

Urine volume excreted per 100g body weight is calculated for each group. The results are expressed as “Lipschitz value (Diuretic index)” that is ratio T/U in which T is response of test compound and U is that of standard. Indices of 1 or more are regarded as positive effect.\[^6\]

**RESULTS**

In the present study Petroleum ether extract of *Kaempferia galanga* Linn was screened for Diuretic activity. The extract was subjected to Phytochemical and Pharmacological investigations. The present study revealed the following data.

**Phytochemical investigation**

**Preparation of Extract and Properties**

The yield of Petroleum ether extract of rhizome of *Kaempferia galanga* obtained by soxhlet extraction was found to be 5.32g with dark yellow colour of the extract.

**Preliminary Phytochemical Screening**

The Qualitative chemical investigation of the extract was carried out to check the presence of various phytoconstituents in the extract. It is observed from phytochemical study that volatile oil, sterols, triterpenoids and resins are present in petroleum ether extract of rhizome of *Kaempferia galanga*.

<table>
<thead>
<tr>
<th>SL.NO</th>
<th>TEST</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates: Molisch test</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Proteins: Biuret test</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>Triterpenoids</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>Sterols &amp; triterpinoids</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>: Salkowski test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>: Libermann Buchnard test</td>
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</tr>
</tbody>
</table>
PHYSIOLOGICAL INVESTIGATION

Diuretic Activity of Kaempferia galanga Linn

Table: 2.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TREATMENTS</th>
<th>URINE VOLUME</th>
<th>SODIUM EXCRETION [ ppm ]</th>
<th>POTASSIUM EXCRETION [ ppm ]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Control (1% CMC, p.o)</td>
<td>0.4666 ± 0.0614</td>
<td>1823 ± 20.41</td>
<td>441.16 ± 11.97</td>
</tr>
<tr>
<td>Group 2</td>
<td>Furosemide (10 mg/kg, p.o)</td>
<td>1.2666 ± 0.0494**</td>
<td>2034 ± 26.88**</td>
<td>639.59 ± 11.22**</td>
</tr>
<tr>
<td>Group 3</td>
<td>KGPEE (200 mg/kg, p.o)</td>
<td>0.7333 ± 0.0666*</td>
<td>2436.16 ± 24.48**</td>
<td>677 ± 14.97**</td>
</tr>
<tr>
<td>Group 4</td>
<td>KGPEE (400 mg/kg, p.o)</td>
<td>1.2833 ± 0.05426**</td>
<td>2234.50 ± 21.64**</td>
<td>699.50 ± 15.94**</td>
</tr>
</tbody>
</table>

24 Hour test. Values are mean ± SEM of 6 animals in each group.*p < 0.05, **p < 0.01. (one way ANOVA followed by Dunnett’s test as compared to control group.).

Effect of Kampferia galanga petroleum ether extract [KGPEE] on diuretic activity in rat.

Values are mean ±SEM of 6 animals in a group. *p<0.05, **p<0.01 as compared with control.
DIURETIC INDEX
Diuretic index of 200mg/kg and 400 mg/kg KGPEE was found to be 0.76 and 1.62 respectively. Diuretic index of 1 and more than 1 shows positive effect. Thus here in this study high dose of extract (400 mg/kg) shows significant diuretic activity.

DISCUSSION
The present study was undertaken to evaluate the Diuretic activity of Petroleum ether extract of *Kaempferia galanga* rhizomes in animal models using Lipschitz method.

*Kaempferia galanga* Linn from Zingiberaceae family is often used in manufacture of traditional medicine, or more commonly referred to as herbs. It is used as a base for traditional medicines for hundreds of years and also finds uses in ayurvedic medicines. Its roots, leaves and rhizomes are used in various ailments mainly abdominal pain, headache, tooth ache and skin protection. Essential oil of rhizome possesses anti-fungal properties.

In the present study extract at doses of 200 mg/kg p.o. (p< 0.05) and 400 mg/kg p.o. (p< 0.01) significantly shows diuretic activity. The extract shows dose-dependent increase in urine volume and Na⁺, K⁺ excretion. High dose (400 mg/kg) p.o. of extract showed significant diuretic activity comparable to standard drug Furosemide (10 mg/kg) p.o. It is also evident from diuretic index evaluation, the value of 1 and more than 1 shows positive effect. In this study diuretic index value of high dose (400 mg/kg) of KGPEE was found to possess significant effect. Plant extract has been tested for various phytochemical constituents. It contains Volatile oils, Steroids, Triterpenoids and Resins. The volatile oils and triterpenes are capable of increasing the urine volume, the mechanism of which is still to be explored. Limonene, which is a major component of Essential oils of the plant, is responsible for diuretic activity of Grape fruit oil.[⁷] Diuretic activity of *Acorus calamus Linn* is due to presence of steroids and triterpinoids.[⁸] Thus there is a possibility that diuretic activity may be due to the constituents present in petroleum ether extract of *Kaempferia galanga* rhizome. Therefore the results obtained in this study provide a quantitative basis to explain the traditional folkloric use of *Kaempferia galanga* as a Diuretic agent.

CONCLUSION
In present study, pharmacological effect of *Kaempferia galangal Linn* Rhizome Petroleum ether extract were investigated in animal model and it characterized diuretic effect.
*Kaempferia galanga* rhizome extract was studied on diuretic activity by Lipschitz method in rat. Extract shows significant increase in urine volume and also increased level of sodium and potassium in urine which proves as a strong diuretic agent, but active constituent responsible for diuretic effect and increased electrolyte excretion cannot be concluded on the basis of this study. The preliminary phytochemical investigation reveals the presence of volatile oil, steroids, triterpinoids and resins in petroleum ether extract which may be responsible for diuretic activity. However to know the exact mechanism of action of *Kaempferia galanga* Linn rhizome extract diuresis, further study with purified fractions or bioactive compounds are warranted.

**REFERENCES**


