INTRODUCTION

Cassava also known as Manihot esculenta Crantz is categorized under the family of phorbiaceae; this plant is a heterozygous, vegetative propagated root crop with a wide variety of uses. Cassava was introduced in Africa and Asia, as a consequence of the Portuguese trading activities in the southern hemisphere. Today, Africa produces more cassava than the rest of the world combined. Cassava is to African peasant farmers as rice is to Asian farmers, or wheat and potatoes are to European farmers [1,2,3]. Cassava is a staple food for at least 500 million people and animal feed in tropical and subtropical Africa, Asia, and Latin America, with an estimated total cultivated area greater than 13 million hectares, of which more than 70% is in Africa and Asia. Cassava is the third most important food source after rice and maize [4]. It is one of the plants that is believed to protect itself; it does so by producing poisonous latex which is mainly found in its leaves. The main reason this plant is grown is because of its roots which are highly rich in starch. Cassava may be a useful source of starch for people who are suffering from celiac disease (gluten intolerance) as it does not contain any gluten [4]. The leaves are very rich source of protein (up to 30%, compared to only 1-3% in the roots), but must be cooked thoroughly in order to render the prussic acid harmless. The young leaves are rich in vitamin B, C, Carotene, Calcium and Iron.

METHODS

The leaves of cassava were gotten from the local market in Kuala Lumpur. They were identified by a botanist.

ETHANOL EXTRACTION

Grinded powder leaves of 895.97g were weighed accurately using an analytical balance (EL-2000S, Setra, USA) and poured into a large conical flask. The sample was extracted by adding ethanol to the flask. The sample was left for three days for maximal extraction after which the solution was evaporated to dryness under reduced pressure and controlled temperature of 40°C water bath by using Buchi rotary evaporator model R-200. The extraction process was repeated until the extraction medium turned pale green. The extract was stored in a refrigerator at 4°C in a flask covered with aluminum foil, for further use.

PHYTOCHEMICALS SCREENING

Alkaloids

Alkaloids were measured by accurately weighing 0.5g of the (ECLE). The extract was prepared with ethanol filter and rotary evaporated and stored in desiccator. Preliminary phytochemical screening terpenoids, tannins, flavonoids, anthraquinones, alkaloids, and cardiac glycosides and carotenoids were carried out. A different concentration (100, 250, 500mg/kg) of the leaf extract was evaluated for their anti-inflammatory, analgesic and anti-pyretic effect using carrageenan and histamine induced oedema, acetic acid induced writhing and yeast induced pyrexia in rats respectively.

Keywords: cassava leaves, anti-inflammatory, anti pyretic, analgesic, phytochemicals
A solution of 100ml of the ECLE was made in chloroform, and concentrated sulphuric acid was added. A deep blue color indicates the presence of carotenoids [6].

Preparation of animals
The animals were purchased at the international medical institute in Kuala Lumpur. The rats were kept in groups of five in a standard condition in animal holding units, UCSI University, Kuala Lumpur Malaysia. The animals were fed with standard pellet diet and water ad libitum and left for 2 weeks before starting the experiments. The Project has ethical committee approval code no. ETUCSI 10012.

Carrageenan induced Paw Oedema
The test was carried out as described [6]. Saline (1ml/kg) was used as a negative control ECLE (100, 250, 500 ml/kg) as a test compound while indomethacin (10mg/kg), was used as a positive control. Saline 1ml test compound and drug were orally administrated 1 hour before injection into the sub plantar side of right hind paw of the rats to induce paw oedema. The paw diameter of swelling (mm) was measured after carrageenan injection at 1 hour interval for 5 hours. The percentage inhibition for each group was calculated according to the formula:

\[ \text{Equation 1} \]

\[ \% \text{ inhibition}=100- \left( \frac{\text{oedema volume in the control} - \text{oedema volume in the treated}}{\text{oedema volume in the control}} \right) \times 100 \]

Histamine induced Paw Oedema
Adopting the method described [7]; the paw oedema was produced by sub plantar administration of 0.1% freshly prepared solution of histamine into the right hind paw of the rats. The paw volume was recorded before the histamine injection. Rats five per group were orally administered with 0.5 ml of the ECLE at 100, 250 and 500 mg/kg body weight, indomethacin 10mg/kg (positive control) and saline (negative control) and histamine was administered 1 hour after the administration of the saline, saline and indomethacin. The right hind paw volume was measured at 1, 2, 3, 4 and 5 h. The anti-inflammatory activity was calculated as described earlier for carrageenan induced oedema.

Acetic acid induced writhing
This study was performed according to the standard protocol [6]. Each rat was intraperitoneally injected with 0.6% aqueous solution of acetic acid (10ml/kg body weight);1 hour after receiving oral administration of the saline 1ml as a negative control ECLE at a concentration 100,250, 500ml/kg and indomethacin (10mg/kg) as a positive control. Immediately after the acetic acid injection, each animal was placed in a transparent observation cage and the number of writhes per rat was counted for 30 minutes. The writhing activity comprised of constriction of the abdominal muscles together with a stretching of the hind limbs .The percentage inhibition was calculated using the following formula:

\[ \text{Equation 2} \]

\[ \left( \frac{\text{control mean}-\text{treated mean}}{\text{control mean}} \right) \times 100 \]

Induction of Pyrexia
Yeast induced pyrexia was used to evaluate the antipyretic activity of the ECLE. Before conducting the experiment, the weight of the female rats were measured in order to determine the volume of drugs, extracts and yeast suspension to be given to each rat. The rats were divided into 5 groups of five and the basal rectal (anal) temperature of each rat was recorded by insertion of thermometer probe 3-4cm deep into the rectum. The animals were given a subcutaneous injection of 4ml/kg of 15% w/v Brewer’s yeast suspension to induce fever and the animals were returned to their cages. Twenty four hours after yeast injection, the rat’s rectal temperature was measured, only rats with elevated rectal temperature of at least 0.5°C were selected for the study [8].

Table 1: Effects of ethanol extract of cassava leaves and indomethacin on carrageenan-induced paw oedema in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
<th>5hr</th>
</tr>
</thead>
</table>
| ECLE Control     | 10           | 0.77±0.006 | 0.78±0.004 | 0.79±0.001 | 0.79±0.006 | 0.78±0.004 | 0.79±0.001
| ECLE 250mg/kg    | 0.72±0.0004 | 0.73±0.006 | 0.738±0.004 | 0.658±0.004 | 0.658±0.004 | 0.658±0.004
| ECLE 500mg/kg    | 0.72±0.0000 | 0.710±0.000 | 0.738±0.004 | 0.658±0.004 | 0.667±0.004 | 0.598±0.004
| ECLE 1000mg/kg   | 0.78±0.0006 | 0.720±0.000 | 0.738±0.004 | 0.658±0.004 | 0.667±0.004 | 0.672±0.004
| ECLE 5000mg/kg   | 0.78±0.0004 | 0.778±0.004 | 0.776±0.001 | 0.776±0.001 | 0.740±0.001 | 0.731±0.004

Table 2: Effects of ethanolic extract of cassava leaves and indomethacin on histamine-induced paw oedema in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
<th>5hr</th>
</tr>
</thead>
</table>
| ECLE Control  | 10           | 0.60±0.004 | 0.652±0.004 | 0.542±0.004 | 0.558±0.004 | 0.558±0.004
| ECLE 100mg/kg | 0.620±0.006 | 0.608±0.004 | 0.620±0.010 | 0.652±0.004 | 0.628±0.004 | 0.640±0.006

Anti-inflammatory activity of ECLE was evaluated using two very popular assay; carrageenan and histamine induced paw oedema. Carrageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drug [9]. Oedema induced by carrageenan is believed to be biphasic: the first phase (1 hour after the induction) involves the release of serotonin and histamine and the second phase which is determined (after the first 1 hour) is believed to be mediated by prostaglandins cyclooxygenase products leukotriene and kinin. ECLE was able to show a significantly (P<0.0001) decrease paw oedema induced by carrageenan by at the fifty hour compared to negative control saline. 100mg/kg exhibited the highest percentage inhibition 26.2%, which similar to indomethacin (21%), while 500mg/kg showed the lowest inhibition at the fifth hour (table1). The effects shown by ECLE may be through the inhibition 5-lipoxygenase and/or cyclooxygenase [10]. It may also be through the inhibition of the release or synthesis of histamine and serotonin [9].

RESULTS AND DISCUSSION

The data was expressed as mean ± standard deviation. The statistically significant differences between groups were measured using one way ANOVA (one way analysis of variance) followed by Dunnett's test. Statistical analysis was performed using Graph pad prism 6.0(Graph Pad software, San Diego, CA, USA). Values of *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 were considered statistically significant.

Histamine is an important inflammatory mediator as well as a potent vasodilator which increases vascular permeability [11]. ECLE was able to significantly (P<0.001) decrease paw edema induced by histamine at the fifty hour compared to negative control saline. 100 mg/kg showed the highest percentage 15.9%, which was higher than indomethacin (12.9%), while the lowest effect was showed by 250 and 500 mg/kg (table 2). The effects shown by ECLE may be through the inhibition of the release or synthesis of histamine. Although ECLE at 100 mg/kg showed a stronger inhibition of carrageenan induced paw edema compared to histamine induced paw oedema but the result correlate with the earlier assumption that ECLE inhibition the phase one by inhibiting histamine release or synthesis [9].

Table 3: Effects of ethanol cassava extract leaves and indomethacin on Acetic acid-induced writhing in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug (mg/kg)</th>
<th>Within 30 minutes</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>-ve control</td>
<td>-</td>
<td>134±1.00</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>46.66±14.63***</td>
<td>65.2%</td>
</tr>
<tr>
<td>Cassava extract</td>
<td>100</td>
<td>33.0±2.0***</td>
<td>75.4%</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>46.6±5.2±1.08***</td>
<td>65.2%</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>41.3±1.5.28***</td>
<td>69.2%</td>
</tr>
</tbody>
</table>

Value are express as mean ± SD for N=5,

A non-specific but widely used method for analgesic test is the acetic acid induced writhing test [12]. Acetic acid has been found to cause an increase in peritoneal fluid levels of prostaglandins (PGE2 and PGF2), hence causing inflammatory pain by inducing capillary permeability [13]. Writhing consists of a constriction of the abdominal muscle together with stretching of the hind limbs due to tissue damage and sensitization of the nociceptors nerves by inflammatory mediators [14, 15, 16]. ECLE was able to show a significantly (P<0.001) decrease in acetic acid induced writhing compared to negative control saline. 100 mg/kg showed the highest percentage inhibition 75%, which is higher than indomethacin (65.2%), while the lowest effect was showed by 250 mg/kg (table 2). The inhibition of acetic acid induced writhing in the rat by ECLE may therefore be due to inhibition of the activity of the COX and decrease in the synthesis of inflammatory mediators; prostaglandins and thromboxane’s from arachidonic acid [17]. It may also be as a result of blockage of calcium influx and or intracellular calcium dependent mechanisms [18].

Table 4: Antipyretic activity and inhibitory values (100,250,500 mg/kg) cassava ethanol leaves extract, Paracetamol against yeast induced pyrexia (fever)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>dose</th>
<th>Initial temp</th>
<th>Post yeast temp</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>-ve control</td>
<td>35.0</td>
<td>36±0.0±0.9</td>
<td>36±0.0</td>
<td>36±0.0</td>
<td>36±0.0</td>
<td>36±0.0</td>
<td>36±0.0</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>10</td>
<td>32.2</td>
<td>34±0.4</td>
<td>34±0.4</td>
<td>34±0.4</td>
<td>34±0.4</td>
<td>34±0.4</td>
</tr>
<tr>
<td>Cassava extract</td>
<td>100</td>
<td>34.1</td>
<td>35±0.0</td>
<td>35±0.0</td>
<td>35±0.0</td>
<td>35±0.0</td>
<td>35±0.0</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>35.3</td>
<td>36±0.3</td>
<td>36±0.3</td>
<td>36±0.3</td>
<td>36±0.3</td>
<td>36±0.3</td>
</tr>
</tbody>
</table>

Value are express as mean ± SD for N=5,

Fever is thought to be produced by several endogenous substances including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumour necrosis factor-α (TNF-α), macrophage protein-1 (MIP-1) and prostaglandins. Brewer’s yeast induces both TNF-α and prostaglandin synthesis. Non-steroidal anti-inflammatory drugs (NSAIDs) reduce fever by depressing inflammatory messages at both peripheral sites of tissue inflammation and within central nervous system thermoregulatory sites. These agents suppress peripheral production of pyrogentic cytokines such as TNF-α and interleukin-1β, while lowering the thermoregulatory set point by blocking central cyclooxygenase production of prostaglandin E2 [19]. Antipyretics on the other hand, such as aspirin and paracetamol have been widely used since the late 19th century. It is now clear that most antipyretics work by inhibiting the enzyme cyclooxygenase and reducing the levels of PGE2 within the hypothalamus. Recently, other mechanisms of action for antipyretic drugs have been suggested, including their ability to reduce pro-inflammatory mediators, enhance anti-inflammatory signals at sites of injury, or boost antipyretic messages within the brain. It is a well-established fact that free radicals play an important role in pain antipyretic agents are better understood, the indications for their clinical use are less clear. ECLE was able to show a significant (P<0.0001) reduction in fever induced in rats by subcutaneous injection of 15% suspension of brewers yeast.100 and 200 mg/kg showed percentage reduction of 34.83% and 34.66%, which is lower than paracetamol33.16% (table 4). The reduction of fever induced by brewer’s yeast in the rat by ECLE may be due to inhibition of the activity of the enzyme cyclooxygenase and reducing the levels of PGE2 within the hypothalamus or other mechanism.

Table 5: Phytochemicals Present or absent in the cassava leaf extract

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotenoids</td>
<td>(+)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>(+)</td>
</tr>
<tr>
<td>Tannins</td>
<td>(+)</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>(+)</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Saponsins</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
</tr>
</tbody>
</table>

(+)* present, (-) absent
inflammatory. Its biological activities are mediated through inhibition of lipid peroxidation and plasma activities. Several studies indicate that tannins are able to induce the analgesic effects and decrease the paw oedema induced by formalin and carrageenan [22, 23, 24] Carotenoid save more than mere pigments; they play an important role as antioxidants, carotenoids protect cells and tissues from harmful radical oxygen species (ROS), acting as scavengers of singlet molecular oxygen, peroxyl radicals. Researchers discovered that α- and β-carotenoids enhanced the anti-nociceptive activity of morphine [25]. Terpenoids significantly inhibit the development of chronic joint swelling. Terpenoids may affect different mechanism relevant to inflammations arising in response to etiological factors [26]. The presence of anti-inflammatory effects of the cassava leaves extract may be due to the presence of this terpenoids.

CONCLUSION

The ECLE has shown a significant anti-inflammatory, anti-pyretic and analgesic effects. These effects maybe because of the presence of phytocemicals; flavonoids, tannins, carotenoids and terpenoids present in the ECLE. However purification and mechanism of action of the bioactive component present in ECLE should be elucidated.

ACKNOWLEDGEMENT

Authors are thankful to Faculty of applied science, UCSI University for the funds to carry out the research project.

REFERENCES