Scientific Evaluation of anti-inflammatory potential of *Macrottyloma uniflorum* seeds extracts

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**ABSTRACT**

Phytochemicals possessing the antioxidant properties naturally present in food attract a greater interest to healthcare researchers due to their desirable health effects on human health as they can be explored for protection against oxidative deterioration. Macrottyloma uniflorum is a leguminous plant belonging to the family Fabaceae and commonly known as Horse gram. Aqueous and ethanol extracts of seeds of Macrottyloma uniflorum were evaluated for their anti-inflammatory effects using the scientific protocol on experimental rats. Extraction was carried out using the cold maceration method, and the anti-inflammatory activity was evaluated using a digital plethysmometer in the experimental rats injected with carrageenan to produce paw edema. Preliminary phytochemical studies confirmed the presence of various bioactive compounds such as alkaloids, glycosides, carbohydrates, proteins and amino acids, terpenoids, tannins, and phenolic compounds in both extracts while flavonoids were found only in ethanol extract. Both extracts of M. uniflorum seeds (200 mg/ml) significantly (p<0.01) reduced the paw edema volume induced by carrageenan. The ethanol extract of M. uniflorum seeds exhibited more potent anti-inflammatory activity than water extract, that might be due to the presence of flavonoids in ethanol extract. The activity of the extracts was compared with diclofenac sodium (10mg/kg b.wt.) as a reference drug. From the results, it may be suggested that the antioxidative potential of phenolic constituents and flavonoids is the primary factors for the anti-inflammatory activity of M. uniflorum seeds extracts.

**INTRODUCTION**

Natural products are concoction compounds or substances which possess their beneficial effects on the health of the human being. For many years regular items have assumed an imperative part in human services and counteractive action of maladies (Phillipson, 2001). Plant based medications keep on occupying a vital space in the treatment of various diseases around the world. Nowadays, the practice of traditional medicine contributes a unique opportunity to address public health challenges. Traditional medicine such as traditional...
Chinese medicine, Ayurveda, traditional Korean medicine and Unani have been practiced worldwide and proved of their potential for curing different illnesses. Inflammation is chiefly the outcome of the clinical infections characterized as pathophysiological symptoms described by edema and pain. Inflammation is a primary response to tissue damage, lipid peroxidation, or an underlying disease. The signs and manifestations of inflammation are erythema, swelling, warmth, pain, and loss of capacity of the influenced zone (Hemamalini et al., 2010). Recent research suggests that inflammation is the main gateway for many diseases like diabetes, rheumatoid arthritis, cardiovascular diseases, and cancer, etc.

According to recent research, inflammation is the primary gateway for some chronic diseases such as diabetes, cardiovascular disease, cancer, and rheumatoid arthritis. Numerous synthetic anti-inflammatory agents are available, but the side effects of these drugs are unavoidable. NSAIDs are the best choice for the treatment of wide range of pain and inflammatory conditions due to their efficacy. However, NSAIDs cause several undesirable side effects such as gastrointestinal ulcers, liver toxicity, haemorrhage, renal diseases, and suppression of immunity (Lopes et al., 2019). It is essential to search the anti-inflammatory drugs from a natural source with fewer or no side effects. Flavonoids are broadly distributed in the medicinal plants and have been evaluated and are reported to possess anti-inflammatory effects (Middleton et al., 2000) that might be due to their antioxidant property. Macrotyloma uniflorum is a leguminous plant belonging to the family Fabaceae and commonly known as Horse gram. It is a crop with great nutritional value and extensively used in ethnomedicinal practices in India (Pati and Bhattacharjee, 2013).

In traditional practices it has been used for the treatment of a variety of diseases and disorders including asthma, bronchial infections, kidney stones, vitiligo, urinary discharge, hemorrhoids, cardiovascular diseases, etc. It also is reported to possesses anti-ulcer and anti-diabetic activity. Owing to the presence of important bio active compounds, it helps in the dietary management of obesity (Bhartiya et al., 2015). Phytochemicals possessing the antioxidant properties that are naturally present in food attracts a greater interest to the healthcare researchers due to their desirable effects on human health as they can be explored for protection against oxidative deterioration (Segev et al., 2010). It is inappropriate to use the traditional medicines or practices that have not proven with any scientific study as that may result in a negative impact as well as giving undesirable side effects. Hence, extensive research is needed to reveal the efficacy and safety of many traditional medical practices. Flavonoids and other phenolic constituents present in M. uniflorum are effective antioxidants. They are thought to act by removal of free radicals and reactive oxygen species (Vandana et al., 2015). Therefore, the present study was undertaken to scientifically evaluate the anti-inflammatory activity of M. uniflorum seeds using a standard experimental protocol.

MATERIALS AND METHODS

Plant material

Macrotyloma uniflorum seeds were procured from a local plant nursery in Selangor, Malaysia in May 2016 and was identified and authenticated by expert from School of Pharmacy, KPJ Healthcare University College-71800, N.S.Malaysia. (Ref. No.: KPJUC/CRI/PA/2016(07).

Preparationof extracts

Thoroughly washed and shade dried seeds of M. uniflorum were ground to coarse powder and the equal portions were separately macerated using ethanol and water for 6 days by cold maceration technique. The extracts obtained after filtration were concentrated at 40°C - 50°C using a rotary vacuum evaporator under reduced pressure (Udegbunam et al., 2011). The color and consistency and the percentage yield of both ethanol and water extracts were noted and the extracts were stored suitable until future use.

Preliminary phytochemical analysis

The preliminary phytochemical screening was conducted for both the extracts using the prescribed chemical tests to identify the presence of secondary metabolites such glycosides carbohydrates, alkaloids, amino acids and proteins, fats and oils, saponins, terpenoids, flavonoids, sterols, phenolic constituents, and tannins as well as gums and mucilages (Priya and Ravindhran, 2015). The results were recorded in Table 1.

Evaluation of anti-inflammatory activity

Animals

Wistar albino rats (150-200g) were procured from KPJUC Vivarium, Nilai, Malaysia and placed in standard housing conditions for experimental animals. The animals were maintained under room temperature (25±40C) with a 12h light/12h dark cycle and 35-60% relative humidity with free access to food, a commercial pellet, and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of KPJUC, Nilai, Malaysia.
Table 1: Preliminary phytochemical analysis extracts of *M. uniflorum* seeds

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Ethanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates and Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins and Amino acids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oils and Fats</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sterols</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic compounds and Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gums and Mucilage</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volatile oils</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = present; - = absent.

Experiments were performed in compliance with the Code of Practice for the Care and Use of Animal for Scientific Purpose (Ref.No:KPJUC/CRI/BPS/EC/2016/25).

Screening of anti-inflammatory activity

The animals were divided into four groups (n=6) and fasted for 24 h before the experiment. The anti-inflammatory activity of the ethanol and water extract of *M. uniflorum* seeds was assessed by carrageenan-induced hind paw edema method (Shankhajit *et al.*, 2010) using a digital plethysmometer. Initially, the left paw of each rat was marked just beyond the tibiotarsal junction and the volume of the paw up to the mark was measured. Group 1 received 0.5% Sodium CMC (10ml/kg) and served as normal control group. Group 2 and 3 received the respective extract (200 mg/kg b.wt.) and group 4 received the standard drug, diclofenac sodium (10mg/kg b.wt.) 30 min before the administration of carrageenan (0.1 ml of 1% w/v). Carrageenan was administered in the sub plantar region of the left hind paw each rat in every group. The paw volume was measured at 1<sup>st</sup> h, 2<sup>nd</sup> h, 3<sup>rd</sup> h, 4<sup>th</sup> h, 5<sup>th</sup> h and 6<sup>th</sup> h after the administration of the respective extract and the standard drug. The results are presented in Table 2.

Statistical analysis

The results were expressed as mean ± SEM. Difference between the control and the treatment groups were analyzed for significance by ANOVA followed by Dunnet “T” test using SPSS software (v:20, SPSS, USA), p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Extracts Characteristics and yield

Organoleptic characteristics and the percentage yield of ethanol and water extract of *M. uniflorum* seeds was observed and recorded. Ethanol and water extract were found to be brown and reddish-brown in colour. Both extracts were semisolid in consistency with ethanol extract being sticky in nature. *M. uniflorum* seeds yielded 6.2% and 4.2 % extract for ethanol and water respectively.

Preliminary phytochemical analysis

Based on the preliminary phytochemical analysis both ethanol and water extracts of seeds of *M. uniflorum* showed the presence of alkaloids, carbohydrates and glycosides, proteins and amino acids, terpenoids, as well as tannins and phenolic constituents. However, the flavonoids were only found in the ethanol extract. On the other hand, proteins and amino acids, oils and fats, sterols, gums, and mucilage, as well as volatile oils were absent in both the extracts.

Anti-inflammatory activity

Anti-inflammatory action of both ethanol and water extracts of *M. uniflorum* seeds (200 mg/ml) was evaluated using Wistar albino rats induced with paw edema by injecting the Carrageenan. A significant (p<0.01) anti inflammatory activity was found in the experimental rats treated with both ethanol and water extracts at 3rd and 4th h. Whereas, a highly significant (p<0.001) anti-inflammatory activity was noted at 5th h and 6th h. The ethanol extract exhibited more potent anti-inflammatory activity than water extract. Presence of flavonoids may be the reason in more potent activity of ethanol extract. The activity of the test extracts were equiv-
salent to the standard drug, diclofenac sodium (10 mg/kg b.wt.). The significance of the anti-inflammatory agents can’t be ignored due to their frequent use in numerous diseases, for example, joint inflammation and rheumatic fever. Rheumatic disorders are portrayed by chronic irritation. It is a regular practice to test natural compounds for the anti-inflammatory activity anticipating that active ones will be helpful against rheumatic diseases. Clinically, anti-inflammatory drugs are chosen based on their impact on the pain, stiffness or swelling of the influenced part. The activity on swelling being the most important in the selection of an anti-inflammatory agent. Among the in vivo techniques used to assess the anti-inflammatory medications, the carrageenan-induced rat paw edema method is the most reliable (Subhha et al., 2011). This method is often used to assess the anti-inflammatory activity of traditional remedies. The carrageenan initiated edema consists of two-stages. The primary stage completes within an hour of carrageenan aggravation and is attributed to the arrival of cytoplasmic catalysts serotonin from the pole cells. The second stage is mediated by an expanded arrival of prostaglandins in the inflammatory (Phelan et al., 2003).

Carrageenan is obtained from seaweed called Rhodophyceae. It is a sulphated polysaccharide used to induce inflammation and in experimental animals particularly in anti-inflammatory studies (Abdul et al., 2013). Histamine and serotonin are released in the first phase followed by second phase caused by the release of bradykinin, protease, prostaglandin, and lysosome. Based on this, it would be claimed that suppression of the first phase involves the inhibition of release of early mediators including the histamine and serotonin. The second phase is probably due to the inhibition of cyclo oxygenase. Diclofenac sodium inhibits the cyclooxygenase enzyme and it is a lipooxygenase inhibitor. It is a well-known drug effective against the Carrageenan induced paw edema; hence it was used as a reference drug in this study. The data obtained from the present study suggests that seeds of *M. uniflorum* indeed possess anti-inflammatory activity against the edema resulted from the Carrageenan sub plantar injection. The reduction in the Carrageenan induced paw edema by the extracts of *M. uniflorum* seeds may be attributed to their inhibitory action on the lipooxygenase enzyme. The extracts of *Macrotyloma uniflorum* seeds showed anti-inflammatory activity on carrageenan induced inflammation in Wistar albino rats that might be due to the inhibition of the mediators responsible for inflammation. The chemical substances and antioxidants derived from plants are of potential interest in the therapeutic intervention for chronic inflammatory disorders (Giresha et al., 2017).

The few secondary metabolites from *M. uniflorum* have shown the ability to interfere with the complex network of biochemical pathways connected to inflammatory processes (Rochfort and Panozzo, 2007). A study by (Salman et al., 2016) on *M. uniflorum* seeds reveals the presence of anthocyanins, flavonoids, phenolic compounds and tannins, the results of the present study also reveals the presence of these compounds in the *M. uniflorum* seeds extracts. The presence of flavonoids, terpenoids, tannins, and phenolic compounds noticed in the extracts of *M. uniflorum* seeds might be responsible for their anti-inflammatory property. Besides that, the ethanol extract of *M. uniflorum* seeds exhibited more potent anti-inflammatory activity than water extract of *M. uniflorum* seeds that might be due to the presence of flavonoids in ethanol extract. Phenolic compounds have the most desirable effects on human health owing to their antioxidant properties, such as protection of oxidative damage (Uma et al., 2013). The presence of flavonoids has been suggested to inhibit prostaglandin synthetase and shown similar relationships between flavonoids and anti-inflammatory effects (Asif and Kumar, 2011). These findings suggest that the antioxidative potential of phenolic compounds and flavonoids might be reason for the anti-inflammatory activity of *M. uniflorum* seeds.

CONCLUSIONS

From the findings of this study it is concluded that *Macrotyloma Uniflorum* seeds have the potential to treat inflammation. Elaborated scientific research on *Macrotyloma uniflorum* is needed to identify and isolate the actual bio active constituents, responsible for anti-inflammatory activity and describe the exact mechanism of action. The persistent efforts for the study on *Macrotyloma uniflorum* may contribute to the development of a novel anti-inflammatory agent from *Macrotyloma uniflorum* seeds.

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Author contributions

KA, AK and BM conceived the project. KA, SS, AK and SP performed the experiments and wrote the
Table 2: Anti-inflammatory activity of extract of M. uniϕlorum seeds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before Induce Edema</th>
<th>Paw volume (ml) (mean ± SEM)</th>
<th>After Induce Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
<td>2h</td>
<td>3h</td>
</tr>
<tr>
<td>Control</td>
<td>0.44±</td>
<td>0.61±</td>
<td>1.41±</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td>0.12</td>
</tr>
<tr>
<td>Standard</td>
<td>0.45±</td>
<td>0.78±</td>
<td>1.62±</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.04</td>
<td>0.1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.41±</td>
<td>1.12±</td>
<td>1.01±</td>
</tr>
<tr>
<td>Extract</td>
<td>0.02</td>
<td>0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>Water</td>
<td>0.43±</td>
<td>1.04±</td>
<td>1.05±</td>
</tr>
<tr>
<td>Extract</td>
<td>0.03</td>
<td>0.08</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM; (n=6); ***p<0.001- highly significant, **p<0.01-significant, and *p<0.05 – less significant.

The authors declare that they have no funding support for this study.

The authors declare that they have no conflict of interest for this study.

REFERENCES


