Research Articles

Antioxidant, anti-inflammatory, analgesic and anti-proliferating activities of Grewia heterotricha Mast.

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ABSTRACT

Introduction and Aim: Plants are considered to be novel source of active compounds having pharmacological properties and help in the development of therapeutic agents. Hence, this study was undertaken to evaluate the antioxidant, anti-inflammatory, analgesic and anti-proliferating activity of aqueous and methanolic leaf extracts of Grewia heterotricha Mast.

Materials and Methods: The aqueous and methanolic leaf extracts of the plant were assessed for their in-vitro antioxidant activity by DPPH radical scavenging activity, in-vivo anti-inflammatory activity by carrageenan induced rat paw edema method, in-vivo analgesic activity by acetic acid-induced writhing test and in-vitro anti-proliferating activity by MTT assay.

Results: The methanolic extract had shown very significant DPPH radical scavenging activity with IC50 value 98.95μg/ml than aqueous extract and showed a significant reduction in the paw volume of rats at the concentration of 100 mg/kg body weight indicating potent anti-inflammatory activity compared with the reference standard Diclofenac sodium. Both the extracts showed significant analgesic effect (p<0.001) in acetic acid-induced writhing test and in-vitro anti-proliferating activity by MTT assay.

Conclusion: The results suggest that both aqueous and methanolic extracts of Grewia heterotricha Mast. leaves possess potent antioxidant, analgesic, anti-inflammatory and anti-proliferating properties, which supports the use of the plant in traditional medicine. Further investigation is required to illuminate on its active compounds.

Keywords: Analgesic; anti-inflammatory; DPPH; cytotoxic.

INTRODUCTION

In recent years people are suffering from many oxidative-stress related diseases such as inflammatory diseases, neurodegenerative disorders, heart diseases and cancer (1). Free radicals and reactive oxygen species produced in living systems can mutate DNA and result in tissue damage. Tissue damage then provokes an inflammatory response by the production of mediators and chemotactic factors (2). Inflammation in tissues is characterized by redness, pain, heat and swelling and loss of function in the injured area. An uncontrolled and persistent inflammation may lead to chronic diseases like asthma, rheumatoid arthritis or cancer (3). Generally, synthetic drugs like non-steroidal anti-inflammatory drugs (NSAIDS) and corticosteroids are used in the treatment of inflammations. Even though these drugs are effective, they produce severe side effects such as peptic ulceration, osteoporosis and GIT bleeding (4, 5). Cancer is one of the major causes of mortality in developed and developing countries and its severity is next only to cardiac diseases. Therefore, there is a need for new therapies and drugs to treat and prevent life-threatening diseases (6).

Herbal medicines have been taken an important place in curing many diseases for ages. According to World Health Organization, 80% of the population in the world use herbs to treat diseases because of their low cost and less adverse effects (7). Plant-derived bioactive components such as flavonoids, polyphenols and steroids known to possess antioxidant, anti-inflammatory, cancer cell growth inhibition and induction of apoptosis (8, 9). Hence plant-derived drugs are drawing the attention of biomedical scientists.

Grewia heterotricha Mast., belongs to the family Malvaceae, widely used as folk medicine in wound healing, fever, bronchitis and to cure some skin and intestinal infections (10). Previously it was reported that leaves of this plant were rich in flavonoids, phenolics and terpenoids. In the present study, antioxidant, analgesic, anti-inflammatory and anti-proliferating activity were assessed for aqueous and methanolic extracts of Grewia heterotricha Mast.
MATERIALS AND METHODS

Collection of plant material

G. heterotricha Mast. plants were collected from in and around Udupi, Karnataka, India during monsoon and prior to monsoon season. The authentication of the plant material was done by Dr. K. Gopalakrishna Bhat, Taxonomist, Taxonomy Research Centre, Poornaprajna College, Udupi, India. The voucher specimens of plant were deposited at Pilikula Herbarium, Mangaluru and provided with accession number 2395. After collection, the leaves were washed and shade dried. Finally leaves were powdered and stored in air-tight container.

Preparation of extract

The powdered leaves (75g) of the plant were extracted with 350ml of methanol using Soxhlet extractor for 24hr. The extract was concentrated by evaporation using rotary vacuum evaporator to obtain dark viscous semi-solid (% yield-2.84%) and labelled as methanol extract of G. heterotricha (MEGH). The leaf powder was mixed with water, stirred continuously for 48 h on a magnetic stirrer to accomplish aqueous extract. The mixture was filtered, and the filtrate was then concentrated (% yield-3.09%) and labelled as aqueous extract of G. heterotricha (AEGH). The extracts were stored in refrigerator and were used for further study.

Antioxidant activity

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Sasidharan et al., Different aliquots of plant extracts (100, 200, 300, 400 and 500 μg/mL in methanol) were mixed with 2 mL of DPPH solution (0.004%), incubated for 30 min at room temperature and the absorbance was read at 517nm in a spectrophotometer (Systronics, model 166) against a DPPH control. Ascorbic acid was used as a standard. Percent inhibition was calculated using the following formula:

\[
\% \text{ Inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

Where, \(A_0\) and \(A_1\) stand for absorbance of the blank and absorbance of tested extract solution respectively.

Animals

Adult Wistar albino rats (150 g -200 g) of either sex was used for the in vivo evaluation. Animals were housed under standard laboratory conditions and were fed with standard animal feed and water ad libitum. The animal experiments were performed after the approval of the protocol by the institutional animal ethical committee.

Acute toxicity study

OECD guidelines 423 were followed while studying the acute toxicity [2]. (Acute toxicity class method).

In vivo Anti-inflammatory activity

Carrageenan induced rat paw edema

The carrageenan induced rat paw edema method was carried out to evaluate acute anti-inflammatory activity. Albino Wistar rats of either sex weighing between 150-200 g were divided into four groups with six animals in each group. After one hour of oral administration of either control vehicle, Diclofenac sodium or plant extracts; 0.1 mL of (1% w/v) in saline was injected into the plantar tissue of the left hind paw of all animals. The degree of inflammation left paw was measured by comparing with the right paw. Increase in paw volume was measured in the interval of 0 min, 30 min, 60 min 120 min, 240 min and 24hour following carrageenan injection, using a plethysmograph (12). The percentage inhibition of inflammation was calculated as inhibition of edema volume in extract treated groups and was compared with control.

In vivo analgesic activity

Acetic acid induced writhing test

The analgesic activity was determined by acetic acid induced writhing (abdominal constriction) method(13) using Albino Wistar rats of either sex weighing between 150-200 g of either sex selected by random sampling technique. Six groups (six animals each) were injected with 1% acetic acid at the dose of 25 ml/kg intraperitoneally (14). Animals in group I were given distilled water which served as control, group II with Standard drug Diclofenac (10mg/kg) as standard and Group III, IV, V and VI were given the methanol and aqueous extracts at the dose of 100 mg/kg orally 30 minutes prior to the administration of the writhing agent. The number of writhes produced in the animals were recorded for 30 minutes and compared with the control.

The percentage protection was calculated using the formula,

\[
\% \text{ Inhibition} = \left[ \frac{(Vc - Vt)}{Vc} \right] \times 100
\]

Where, \(Vt\) = Mean number of writhing in test animals; \(Vc\) = Mean number of writhing in control.

Statistical analysis

Statistical analysis was done using one-way ANOVA, followed by Tukey’s post hoc multiple comparison tests. p<0.001 were considered as significant. Data are represented as mean ± S.E.M.

Cytotoxicity assay

The in vitro cytotoxicity activity was carried out by MTT assay using MCF7 cell lines (15). MCF-7 (Human Breast Cancer cell line) cells were procured from National Centre for Cell Sciences (NCCS), Pune. 200μl cell suspension was seeded in a 96-well plate at required cell density (20,000 cells per well), without the test sample. The cells were allowed to
grow for about 24 hours. Appropriate concentrations of the test sample (150-550 μg/ml) were added. The plates were incubated for 24 hrs at 37°C in a 5% CO₂ atmosphere. After the incubation period, the plates were taken out from incubator, spent media was removed and MTT reagent was added to a final concentration of 0.5mg/ml. The plates were incubated for 3 hours. The MTT reagent was removed and then 100μl of DMSO solution was added. The absorbance was read in an Elisa reader at 570 nm and 630 nm was used as reference wavelength. The IC₅₀ value was determined by using linear regression equation. Standard drug Camptothecin was used as a positive control.

RESULTS

Table 1: DPPH radical scavenging activity of melatonin and aqueous leaf extracts of Grewia heterotricha Mast.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Concentration (μg/ml) / % Inhibition</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>MEGH</td>
<td>52.60±1.5</td>
<td>71.74±1.1</td>
</tr>
<tr>
<td>AEGH</td>
<td>19.26±2.0</td>
<td>31.11±1.9</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>82.97</td>
<td>85.95</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SD (n = 3)

In vivo Antinflammatory activity

Carrageenan induced rat paw edema

Anti-inflammatory activity was evaluated by carrageenan-induced paw edema model in Wistar rats. AEGH and MEGH extracts showed 50% inhibition of edema formation after 1 hr at the dose of 100mg/kg body weight. At 2nd and 4th hour, both extracts showed a statistically significant decrease in the paw volume as compared to control group (p<0.01). MEGH and AEGH showed potent activity of 84.84% and 74.84%, respectively compared with Diclofenac sodium (84.84%). MEGH is found to be more effective than AEGH. The results are shown in Table 2.

Table 2: Anti-inflammatory Activity of leaf extracts of G. heterotrichain Carrageenan Induced Rat Paw Edema Model

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Carrageenan induced edema (Volume in ml)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30min</td>
<td>60min</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.39±0.02</td>
<td>0.86±0.05</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10</td>
<td>0.11±0.16*</td>
<td>0.25±0.02*</td>
</tr>
<tr>
<td>MEGH</td>
<td>100</td>
<td>0.20±0.02</td>
<td>0.31±0.01*</td>
</tr>
<tr>
<td>AEGH</td>
<td>100</td>
<td>0.22±0.02</td>
<td>0.33±0.02*</td>
</tr>
</tbody>
</table>

Note: All the results are expressed in term of Mean ± SEM., n=6 animals in each group; *p<0.01 vs control, statistically significant.

Analgesic activity

Acetic acid induced writhing test

The leaf extracts of G. heterotricha reduced the number of abdominal writhes in 30 minutes after the administration of the plant extracts (100 mg/kg body weight) (Table 3). MEGH exhibited potential analgesic activity and reduced the number of abdominal writhes by 76.5%. The results are depicted in Table 3.

Table 3: Analgesic Activity of leaf extracts of G. heterotrichain Wistar rats by Acetic acid Induced Writhing Test

<table>
<thead>
<tr>
<th>Drug/Extract</th>
<th>Dose (mg/kg Body Weight)</th>
<th>Number of writhes per 30 min</th>
<th>% Inhibition of writhes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>41.83±3.27</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10</td>
<td>7.50±0.76*</td>
<td>82.07</td>
</tr>
<tr>
<td>MEGH</td>
<td>100</td>
<td>9.83±0.47*</td>
<td>76.5</td>
</tr>
<tr>
<td>AEGH</td>
<td>100</td>
<td>14.50±0.84*</td>
<td>65.33</td>
</tr>
</tbody>
</table>

Note: All the results are expressed in term of Mean ± SEM., n=6 animals in each group; *p<0.001 vs control, statistically significant.

Cytotoxicity assay

The cytotoxicity of MEGH and AEGH were determined by the MTT assay. The results were shown in Table 4. A dose-dependent decrease in the percentage cell viability was observed. It was observed that 10% cell viability was observed in...
MEGH whereas 40% MCF-7 cell viability in AEGH at the concentration of 550µg/ml. Therefore, anticancer activity was significant in MEGH (IC₅₀ value 386.48 µg/ml) compared to AEGH. There were no significant changes were observed in the morphology of control cells. However, in MCF-7 cells treated with leaf extracts exhibited the evident sign of cell shrinkage, decreased cell density, loss of cell adhesion, which are the characteristics of cell death (16) Fig 1a-d).

Table 4: Cytotoxic effect of leaf extracts of G. heterotricha

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration µg/ml</th>
<th>IC₅₀ value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 µM</td>
<td>150</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>45.4</td>
<td>45.4</td>
</tr>
<tr>
<td>AEGH</td>
<td>99.62±0.02</td>
<td>87.22±0.007</td>
</tr>
<tr>
<td>MEGH</td>
<td>94.42±0.005</td>
<td>74.4±0.001</td>
</tr>
</tbody>
</table>

Note: All the results are expressed in term of Mean ± SEM

DISCUSSION

Free radicals or reactive oxygen species play an important role in many human diseases due to their toxic effect in biological systems. They can react with carbohydrates, proteins and lipids and cause damage to tissues and organs leading to inflammation and cancer (17). In recent years, herbal medicines have received large attention due to the presence of various phytoconstituents with multiple pharmacological properties. In the present work, the antioxidant activity of methanol and aqueous extract was evaluated by DPPH radical scavenging assay. The experimental data showed that both the extracts have good radical scavenging property and higher antioxidant capacity was found in methanolic extract. The secondary metabolites such as phenolic compounds, flavonoids, tannins act as antioxidants and scavenge free radicals and protect against oxidative degenerative diseases (18). In our previous report, it has been reported that leaf extract of G. heterotricha possess phenolic compounds, flavonoids, tannins. According to Varela-López, the natural products exert antioxidant and anti-inflammatory activity through up regulation of either NF-κB, MAPK or p38/JNK pathways (19). The potential DPPH radical scavenging activity observed in this study may be due to the presence of these secondary metabolites in the extracts that may mediate through any of the above signaling pathways. Our observation is in agreement with the studies of Izuegbuna et al. (20).

Inflammation produced in the body may result in diverse disease conditions. Currently, much interest is giving to medicinal plants with anti-inflammatory properties. Carrageenan-induced rat paw edema method is most extensively used method for anti-inflammatory studies. Edema was developed due to induction of carrageenan which is normally associated with the release of mediators such as prostaglandins, histamine, bradykinins and serotonin which triggers inflammation. These mediators act at three different phases of inflammation. Histamine and serotonin are involved in the initial phase that last up to 1.5 from 0 h after the injection of carrageenan. The second (1.5-2.5h) and third phase (2.5-6h) are attributed to bradykinin and prostaglandins respectively (21). In the present study, results showed that anti-inflammatory activity of both

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extracts is comparable to diclofenac sodium and a significant reduction in the paw volume was observed even 2 h post-induction. Similar results have been reported by Tamrat et al., in leaf extracts of Moringa stenopetala Bak and Carey et al., in methanol extract of Kigelia pinnata DC flower.

One of the major signs of inflammation is the pain that can be triggered by the action of inflammatory mediators or by direct stimulation of nociceptors (23). Pain and inflammation are associated with various disease conditions like arthritis, cancer, and vascular diseases. In acetic acid-induced writhing method pain is generated indirectly via endogenous mediators like prostaglandin, which stimulates peripheral nociceptive neurons. Prostaglandin inhibitory role of plant secondary metabolites have been reported in the recent past (24). In our result, methanol and aqueous extracts reduced writhing movement by inhibiting peripheral pain induced by the direct action of acetic acid. This suggests the potential analgesic activity of the extracts.

MTT assay was used to screen in vitro cytotoxic activity of methanol and aqueous plant extracts. This is based on the reduction of tetrazolium dye MTT to formazan crystals by mitochondrial lactate dehydrogenase produced by live cells. The results revealed an increase in the concentration of extracts up to 550 µg/ml, reduce the cell viability significantly (P < 0.001) in a dose-dependent manner in MCF-7 cell lines. Methanolic extract appeared to be more active compared to aqueous extract. Our observations are in agreement with the reports of Ala et al., (2018) about the dose dependent cytotoxic activity of plant extracts.

From the above results, it is observed that both extracts of G. heterotricha have good antioxidant, anti-inflammatory, analgesic and anti-proliferating properties. Moreover, results clearly illustrated that MEGH has higher pharmacological properties compared to AEGH. The bioactive compounds phenolics, flavonoids and tannins may be responsible for these biological properties.

**CONCLUSION**

Oxidative stress-related diseases like inflammatory diseases, cardiovascular diseases and cancer may result due to the formation of free radicals and these diseases are associated with pain. It is essentially needed to develop nontoxic anticancer, anti-inflammatory and analgesic drugs to improve healthcare. In our study, G. heterotricha plant leaves showed potent antioxidant, anti-inflammatory, analgesic and anticancer activities. Based on the results of this study, it is possible to conclude that methanolic leaf extract has these prominent biological activities and can be used as a source to develop non-toxic anti-inflammatory, anticancer and analgesic drugs. However, further studies are needed to identify the specific bioactive molecules involved in these pharmacological properties.

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**CONFLICT OF INTEREST**

The authors declared no conflict of interest in this work.

**REFERENCES**


