Hypoglycemic, Analgesic and Anti-Inflammatory Activities of Methanol Extract of Sida Rhombifolia L. Leaves on Experimental Mice

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Abstract

The aim of this study was to investigate the analgesic, anti-inflammatory and hypoglycemic activities of methanol extract of Sida rhombifolia L. leaves along with the presence of major phytochemicals. The crude leaf extract of Sida rhombifolia L. was investigated for analgesic, anti-inflammatory and hypoglycemic activities using various experimental models. Anti-diabetic activity was determined by Oral Glucose Tolerance Test (OGTT). To determine the analgesic activity, acetic acid induced writhing model was used on mice. For anti-inflammatory activity test Inflammation (paw edema) was induced by injecting 0.1ml of 1% Carrageenan. The extract caused a significant (p<0.001) dose-dependent reduction of blood sugar, inflammation and pains induced by different agents used. The phytochemical screening showed the presence of Glycoside, alkaloid, flavonoid, saponin, Tannin and steroid type of compounds. Leaf extract possesses anti-inflammatory and analgesic effects which may be mediated through the phytochemical constituents of the plant.

Keywords: Sida Rhombifolia L, Hypoglycemic Activity, Analgesic Activity, Anti-Inflammatory Activity, Carrageenan

Introduction:

Celsius invented inflammation on 2000 years ago with four latin words: Rubor, calor, tumor and dolor. Inflammation has different phases. First phase is caused with permeability increase and exudation of fluid into interstitial space, secondly inflammation of leukocytes from blood to tissue, thirdly granuloma formation. Anti-inflammatory tests have three phases: acute, sub acute and chronic repair process [Patel et al. 2012]. Inflammation is a complex biological response of vascular tissue by pathogens, irritants with redness, warm, swelling and pain. [Palladino et al. 2003; Ferrero et al. 2007]. Inflammation is of two types: acute and chronic. Acute inflammation is an initial response of harmful stimuli and chronic is the damage to the body prostaglandins, prostacyclins and thromboxanes that are involved in inflammation, pain are synthesized by cyclo-oxynenes (Cox). [Pilloto et al.2010].

Inflammation is a series of factors with infection, trauma and injury to the tissues. [Calixto et al. 2004]. Inflammation is initiated by enzyme activation, mediator release, cell migration, fluid extravasations [Risso et al. 2010]. Inflammation releases leukocytes against injury that synthesize some biomolecules and releases during swelling and redness [Opdenakker et al. 1998]. During inflammation prostaglandin is synthesized and induces inflammation [Ricciotti and FitzGerald 2011]. Sustain inflammation can lead undesired health effect. Inflammation has been indicated in several diseases including cancer. [Grivennikov et al. 2010; Moore et al. 2010].

Inflammation blocking agents play an important role in treating pathologies associated with inflammatory reaction [Sosa et al. 2002]. Though inflammation has many cause but mechanisms are same to all. The inflammatory agent activates phospholipase A2 in the site, release arachidonic acid and metabolites. Inflammatory mediators like cytokine, histamine, serotonin, prostaglandin increase cell permeability to the site of action [Dassoler et al. 2004]. Any interruption in this sequences result in the reduction of mediator release and return to normal hemodynamic condition [Lope et al. 1987]. People seek medical help due to pain that is undesirable physical and emotional experience. For undesirable side effect of pain killer drugs, treatment for chronic pain is public health problem [Ballantyne 2005]. Analgesic that reduces pain can be classified into three classes: opioid analgesic (morphine), non-opioid analgesic (NSAIDs) and adjuvant analgesic (which is taken...
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for other purpose but reduces pain in certain situations). Because of high efficacy, opioid analgesics gives maximal analgesia [Fields 2011].

Due to side effects (hypertension, highperglycemia, osteoporosis, cardiovascular disease) clinical use of these drugs suffer from disadvantages [Gautam and Jachak 2009]. Factor of discovering new compounds for pain treatment has understood a complex mechanism of pain transmission to nervous system because of having many receptors, enzyme and signaling path ways [Besson 1999]. Pharmacological mechanism is considered the potential of medicinal plants for discover of new compounds in the treatment of pain disorder (fewer side effect) [McCurdy and Scully 2005]. One of the most common endocrine disorders is diabetes mellitus and more than 100 million people are suffering from it due to population growth, aging, increase obesity and physical inactivity [Nair et al. 2006; Sarah et al. 2004]. In India more people are suffering from type 2 diabetes than other country [Jared 2011; Safdar et al. 2004] by recent statistic, approximately 7.8% (438 million) people is expected to have diabetes by the year 2030 [Ramachandran et al. 2010]. A higher number of people are affecting by this disease due to stress, rapid development of cities, lifestyle ease and metro life. The expenditure of treatment of diabetes exceeds $100 billion per year [Umarra et al. 2010]. Because of having side effect and high cost, the treatment of diabetes with synthetic drugs are not preferable but traditional drugs are preferable for less side effect.

At least 400 plants have anti-diabetic activity according to literature [Arulrayan et al. 2007]. Diabetes mellitus is common endocrine disease have microvascular (retinopathy, neuropathy, and nephropyh) and macrovascular (heart attack, stroke and peripheral vascular disease) complications [Patel et al. 2011]. Due to deficiency of insulin people suffer from diabetes with high sugar level [Ponnumsy et al. 2011]. Type 2 is the most common form in which body does not properly use insulin [Li et al. 2004]. Now treatment of this disease with synthetic drug is available but because of having more side effects the search of effective and less side effect hypoglycemic agent is compulsory [Saxena and Vikram 2004]. The hypoglycemic effect of several plants used as anti-diabetic remedies and the mechanism is being studied. Traditional medicines from medicinal plants offer great potential for discovery of new anti-diabetic drugs [Jung et al. 2006].

**Materials and method:**

**Drugs and chemicals**

Carrageenan was purchased from Otto chemicals, India. The standard drug Diclofenac-Na was received as a gift from Square Pharmaceuticals Limited of Bangladesh. Acetic acid, methanol and other chemicals supplied from laboratory of Bangladesh University were analytical grade.

**Experimental animals**

Eight weeks-old Swiss albino mice (27-30g) purchased from Jahangirnagar University, Dhaka, Bangladesh and were housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr light: 12 hr dark cycle). The mice were fed with standard pellet diet taken from, Jahangirnagar University, Dhaka. The animals used in this study were cared in accordance with the guidelines of animal experimentation of our institute.

**Plant Materials**

The flowering plant of Sida rhombifolia L. leaves were collected from near Jahangirnagar University fields, Dhaka, Bangladesh.

**Drying and Grinding**

The collected plants were separated from undesirable materials or plants or plant parts. They were dried in the sun for one week after cutting into small pieces. The plant parts were ground into coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

**Preparation of Plant Extract**

About 300 gm of powdered sample was taken in a clean, flat-bottomed glass container and soaked in 1500 ml of 90% methanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by apiece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate was kept in an open space to evaporate the solvent thus crude extract was obtained. Fine powders of the flowering plant of Sida rhombifolia L. leaves are dissolved in 90% methanol and then evaporation the solvent.

**Phytochemical Screening**

Phytochemical studied of methanolic extract of plant material extract was carried out for preliminary chemical investigation for the direction of practical pharmacognosy text book [Trease and Evans 1983; Ali 2012; Ghani 2011]

**Anti-inflammatory activity**

Inflammation (paw edema) was induced by injecting 0.1ml of 1% Carrageenan in physiological saline into the sub plantar tissues of the left hind paw of each mouse. [Winter et al. 1962] The methanol extract of Sida rhombifolia L. leaves rind 300 mg/kg were administered orally 30 min prior to Carrageenan administration. The paw edema size was measured at 0, 1, 2, 3 & 4 hours by using dial caliper [Al-Haboubi and Zeitlin 1983]. The percentage reduction of paw edema in drug treated group was compared with the control group. Diclofenac sodium (5 mg/kg p.o.) was used as reference standard. 0 hour reading was considered as an initial normal paw size. Data was collected from the paw thickness and percentage reduction of paw edema of the treated animals. Percentage reduction of paw edema was calculated by using the formula.

Anti-inflammatory activity (%) = \((1-\frac{T}{C}) \times 100\)

Where T is the change of paw diameter in treated group and C is the change of paw diameter in control group.

**Analgesic activity**

For analgesic test all mice were divided into four groups. Each group comprises of 4 mice. Control group (received 0.5% methyl cellulose, per oral), Standard Group (received Diclofenac-Na10mg/kg intraperitoneally), and Sida rhombifolia L. leaves extract Group (received 300mg/kg Sida rhombifolia L. leaves extract per oral). The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 mins before intraperitoneal administration 10ml/kg of .7% acetic acid but Diclofenac-Na was administered intraperitoneally 15 minutes before the acetic acid injection, the mice were observed for specific contraction of body referred to as...
“writhing” for the next 10 minutes [Ahmed et al. 2004]. Percentage protection of acetic acid induced writhing was calculated by the formula.

\[
\text{Percentage protection} = \frac{(W_c - W_t)}{W_c} \times 100
\]

Where, \( W_c \) is the mean values of control group and \( W_t \) is the mean values of treated group.

Method for Evaluation of Hypoglycemic Activity

Oral Glucose Tolerance Test (OGTT) in diabetic mice. After fasting 16hr, diabetes was induced into mice by in intra-peritoneal injection (i. p.) of alloxan monohydrade (90 mg/kg) dissolved in saline. After 72hrs, plasma glucose levels were measured by glucometer (Tyson, Taiwan) using a blood sample from tail-vein of mice. Mice with blood sugar higher than 11.5 mmol/L were considered as diabetic.

All the mice were divided into 4 groups, each group containing 5 mice. The divided groups are NC (normal control), DC (diabetic control), STD (diabetic mice receiving 100mg/kg Metformin), ME (diabetic mice receiving methanolic extract). The mice were fasted overnight and next day blood samples were taken from all groups of animals to estimate fasting blood glucose level (0 min). All mice received 2gm/kg glucose. Without delay extract and were given per oral and four more blood samples were collected at 30, 60, 90 and 120 minutes intervals and blood glucose level was estimated in all the experiments by using glucometer.[Hossain et al. 2011]

Data Analysis

All values were expressed as mean ± Standard error of mean (SEM). Statistical comparison were performed by One-way analysis of variance (ANOVA), followed by Dunnett test using IBM-SPSS software version 20. Results were considered as significant of the differences between the test and control group data when \( p \) values less than 0.001 (\( p<0.001 \)).

Results:

| Tested groups | Methanolic Extract of Sida rhombifolia L. |
|---------------|------------------------------------------|
| Carbohydrate  | -                                        |
| Glycoside     | +                                        |
| Alkaloid      | +                                        |
| Saponin       | +                                        |
| Protein       | -                                        |
| Flavonoid     | +                                        |
| Tannin        | +                                        |
| Steroid       | +                                        |

Note: + = Indicates the presence of the tested group, - = Indicates the absence of the tested group.

Table 1 Results of Phytochemical Screening

| Animal Group | 60 min   | 120 min  | 180 min  | 240 min  |
|--------------|----------|----------|----------|----------|
| Control      | 0.34±0.004 | 0.33±0.007 | 0.30±0.007 | 0.30±0.004 |
| Standard     | 0.16±0.008*** | 0.13±0.007*** | 0.12±0.008*** | 0.11±0.004*** |
| Extract(300mg) | 0.20±0.007*** | 0.18±0.010*** | 0.15±0.010*** | 0.13±0.007*** |

Table 2: Results of Anti-inflammatory effect of Sida rhombifolia L. leaf extract on carrageenan induced paw edema (mm) in mice. Experimental data were presented as mean ± SEM. By using the Dunnett test significant differences (***p<0.001) between the means were determined compare to control group where \( n=04 \). For statistical evaluation IBM-SPSS software version 20 was utilized.

| Animal Group       | Writhing Counting (Mean±SEM) |
|--------------------|------------------------------|
| Control Group      | 39.5±0.369                  |
| Standard Group     | 6.75±0.176***               |
| Extract Group (300 mg/kg) | 13.75±0.269***             |

Values were Mean ± SEM, \( (n=4) \); ***p<0.001 Dunnett test as compared to Control Group.

Table 3 Results of Analgesic effect of Sida rhombifolia L. leaf extract on acetic acid-induced writhing in mice
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Table 4 Oral Glucose Tolerance Test (OGTT) of Sida rhombifolia L. leaf extract in alloxan-induced diabetic (mM/L) in mice

| Animal Group | 0 min | 30 min | 60 min | 90 min | 120 min |
|--------------|-------|--------|--------|--------|---------|
| Control Group | 27.54±0.49 | 31.55±0.31 | 31.12±0.40 | 30.73±0.49 | 30.00±0.52 |
| Standard Group | 22.95±0.22 | 27.21±0.32*** | 23.72±0.34*** | 19.70±0.29*** | 16.83±0.25*** |
| Extract Group (300 mg/kg) | 26.93±0.20 | 28.43±0.25*** | 27.32±0.35*** | 26.05±0.19*** | 24.43±0.23*** |

Experimental data were presented as mean ± SEM. By using the Dunnett test significant differences (**p<0.001) between the means were determined control group where n=04. For statistical evaluation IBM-SPSS software version 20 was utilized.

Discussion:
In this study, hypoglycemic, analgesic and anti-inflammatory activities of methanol extract of Sida rhombifolia L. leaves were assessed in different well accepted animal models, including Oral Glucose Tolerance Test (OGTT) in alloxan induced diabetic mice, carrageenan induced mice paw edema, acetic acid-induced writhing test.

Diabetes mellitus is one of the most common chronic diseases which elevate the mortality basically by type-2 diabetes. Unrecognized diabetes mellitus and impaired glucose tolerance are mostly linked with acute stroke. [Cruickshank JK 1997] [Haddad PS 2005] [Gray CS 2004]. The present study, extract of Sida rhombifolia L had marked (p<0.001) in hypoglycemic effects in diabetic mice (Table-4).

Analgesic drugs acts on both CNS and PNS without significantly altering consciousness. [K. D. Tripathi 2004]. The acetic acid induced writhing is basically a revelation of peripheral pain [Sanchez-Mateo C C 2006] [Collier H O J 1968]. The acetic acid induces release of different endogenous chemical pain mediators like prostaglandin E2 (PG) [Zahid Hina 2012] [Deraedt Roger 1980]. The experiment showed (Table-3) that the extract exhibited statistically significant inhibition of writhing (p<0.001) at extract dose 300mg/kg body weight. The development of carrageenan-induced edema is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins [Kapewangolo L(Oxalidaceae).JornalBrasileiro de Fitomedicina 2: 4-8].

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The presence of flavonoid identified might be responsible for the analgesic and anti-inflammatory activities in methanolic extract.

Conclusion
This study showed that the methanol extract of Sida rhombifolia L. leaves contain Glycoside, alkaloid, flavonoid, saponin, Tannin and steroid type of compounds. Significant analgesic, anti-inflammatory activity and mild anti-diabetic were observed. However, to confirm this information further research should be conducted upon this topic.

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Ethical Statement

Conflict of Interests

The authors declare that there is no conflict of interest among them.

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