Original Article

In vitro anti-inflammatory and antioxidant potential of leaves of *Canavalia gladiata*

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ABSTRACT

Objective: The objective of this study was to evaluate the biological potential of different extracts of leaves of *Canavalia gladiata* by *in vitro* anti-inflammatory and antioxidant methods. **Materials and Methods:** An *in vitro* anti-inflammatory study was carried out by human red blood cell membrane stabilization method and antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) methods. **Results:** The *in vitro* membrane-stabilizing test performed on petroleum ether, chloroform, ethyl acetate, methanol, and aqueous extracts, at dose level 200 and 400 mg/kg and results showed dose-dependent activities and maximum activities founded at a higher dose as 51.89%, 58.99%, 68.12%, 67.34%, and 52.34% protection, respectively, as compared to control groups. The standard drug diclofenac (10 mg/kg), which showed 78.22% protection in membrane stabilization test and antioxidant potential at different doses levels 100, 200, and 400 mg/kg of extracts, found as 44.01%, 50.45%, 72.99%, 70.25%, and 78.10%, respectively, in DPPH free radical scavenging activity methods. The data's statistically found significant as ($^aP < 0.01$ and $^bP < 0.05$) as compared to control. **Conclusions:** The present investigations have been confirmed the anti-inflammatory and antioxidant activities of leaves of *C. gladiata* that supports the traditional claims of *C. gladiata* as a medicinally active plant.

Keywords: *Canavalia gladiata,* anti-inflammatory, antioxidant, 2,2-diphenyl-1-picrylhydrazyl hydrate methods, human red blood cell membrane stabilization

INTRODUCTION

Canavalia gladiata (Leguminosae family) is widely cultivated in tropical regions such as Southeast Asia and other regions. It is called sword bean since its fruits have a shape similar to a straw cutter. It contains components such as urease, canavanine, hemagglutinin, and C. gibberellin I and II.[1] In traditional medicine, its seeds, pods, stems, and roots are known to have efficacy in treating dysentery, nausea, hemorrhoids, sinusitis, back pain, obesity, diarrhea, and hiccups.[2,3] In addition, several studies have reported the effects of *C. gladiata* on allergy, inflammation, cancer, gastritis, antioxidant, and skin whitening. However, there has not been sufficient research to verify the differences between ripe and unripe green sword beans.[4,5] Inflammatory diseases are the most important cause of morbidity and mortality in worldwide. It is a body self-protective mechanism against any tissue injury and it involved a multifaceted array of enzyme activation, inflammatory mediator release, fluids extravasations, tissue breakdown, cell migration, and cell repair which are host defense mechanism activated in ill condition.[6,7] The role of inappropriate critical inflammation is becoming accepted in many diseases that affect patient life, including cardiovascular diseases, inflammatory and autoimmune disorders, neurodegenerative conditions, infection, and cancer. Edema formation, leukocyte leakage, and granuloma formation are the result of synergism between various inflammatory mediators that increase vascular permeability or the mediators that increase blood flow.[8] Carrageenan-induced paw edema method is widely used for determining the biphasic phase of inflammation. Histamine, 5-hydroxytryptamine, and bradykinin are the first detectable mediators in the early phase of carrageenan-induced

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inflammation,[9,10] whereas prostaglandins are detectable in the late phase of inflammation.[11] The present investigation has confirmed the anti-inflammatory and antioxidant activity of leaves of *C. gladiata* that supports the traditional claim of *C. gladiata* as a medicinally active plant.

MATERIALS AND METHODS

Air-dried plant material *C. gladiata* leaves were successively extracts with different solvents such as petroleum ether (PE), chloroform (CE), ethyl acetate (EE), methanol (ME) and aqueous extract (AE), vacuumed dried the extracts and further used for biological activities.

In vitro anti-inflammatory activity

This activity was tested with the human red blood cell (HRBC) membrane stabilization method.[10] The blood was collected from a healthy human volunteer who had not taken any NSAIDS at least 2 weeks before the start of experiment and mixed with equal volume of Alsever solution contains 2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Extracts such as PE, CE, EE, ME, and AE of *C. gladiata* leaves were prepared (200 and 400 mg/mL), respectively, using distilled water and to each concentration 1 M of phosphate buffer, 2 M hypo saline, and 0.5 M of HRBC suspension, which were added under controlled conditions. It was incubated at 37°C for half an hour and centrifuged at 3000 rpm for 20 min. The hemoglobin content of the supernatant solution of each extracts was estimated UV spectrophotometrically at 560 nm. Diclofenac 10 mg/ml was used as a reference standard and a control was prepared by omitting the test extracts. The calculation of the percentage of HRBC membrane stabilization was performed in triplicate. The percentage of HRBC membrane stabilization or protection was calculated using the formula mentioned below:

$$\text{Percent inhibition} = \frac{\text{Abs of control} - \text{Abs of treated}}{\text{Abs. of Control}} \times 100$$

In vitro antioxidant activity – 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical scavenging assay method

Free radical scavenging activity of *C. gladiata* successive leaves extracts used against stable DPPH was determined by the slightly modified method.[9,12] The solution of DPPH reacts with an antioxidant agent, which can donate hydrogen and reduce DPPH. The change in color (from deep violet to light yellow) was measured at 517 nm by UV spectrophotometer. The solution of DPPH in ME 6×10^{-5} M was prepared fresh every day before UV analysis. Three milliliter of this solution was mixed with 100 µg/ml, 200 µg/ml and 400 µg/ml concentration of individual plant extract. The samples were kept under dark place for 15 min at room temperature and individual sample absorbance was measured. The experiment was carried out in triplicate. The free radical scavenging activity was calculated by the following formula.

$$\text{Percentage Inhibition} = [(AB–AA)/AB] \times 100$$

Where AB = absorption of blank sample (t = 0 min), AA = absorption of test extract solution.

RESULTS AND DISCUSSION

In vitro anti-inflammatory activity

Successive extracts of *C. gladiata* leaves were evaluated by *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. The dose-dependent significant ($^aP < 0.01$ and $^bP < 0.5$) activities were observed in all extracts, maximum activities were found in higher dose as 67.34% in ME, 68.12% in EE, 58.99% in CE extract, 52.34% in AE, and 74.18% with standard drug diclofenac. The results are tabulated in Table 1.

Table 1: *In vitro* anti-inflammatory activity of *Canavalia gladiata* leaves extracts

Groups	Concentration (mg/kg)	Percent of stabilization activity
Control (vehicle only)	-	-
P.E.C.G.	200	39.45±0.45
	400	51.89±0.89[a]
C.E.C.G.	200	53.90±1.20
	400	58.99±0.67[b]
E.E.C.G.	200	57.23±0.47
	400	68.12±1.42[b]
M.E.C.G.	200	60.54±1.34[b]
	400	67.34±1.32[b]
A.E.C.G.	200	48.83±1.45[a]
	400	52.34±1.22[a]
Diclofenac	10	78.22±1.86[a]

*E.C.G.: Extract of *Canavalia gladiata*, P.E.: Pet. Ether, C.: Chloroform, E.: Ethyl acetate, M.: Methanol, A.: Aqueous, Diclofenac: Standard drug used. Data observed in triplicate and statistically found significant as $^aP<0.01$ and $^bP<0.05$

Table 2: *In vitro* antioxidant activity of *Canavalia gladiata* leaves extracts

Groups	Concentration (µg/ml)	Percent of scavenging activity (±SEM)
P.E.C.G.	100	17.37±0.015
	200	22.89±0.028
	400	44.01±0.035
C.E.C.G.	100	20.98±0.022
	200	37.96±0.054
	400	50.45±0.029[b]
E.E.C.G.	100	36.22±0.023
	200	50.23±0.034[a]
	400	72.99±0.045[a]
M.E.C.G.	100	30.02±0.032
	200	44.96±0.012[a]
	400	68.12±0.019
A.E.C.G.	100	35.23±0.023
	200	46.80±0.033
	400	70.25±0.039[a]
Rutin	100	40.98±0.042[b]
	200	52.05±0.034[a]
	400	78.10±0.025[a]

*E.C.G.: Extract of *Canavalia gladiata*, P.E.: Pet. Ether, C.: Chloroform, E.: Ethyl acetate, M.: Methanol, A: Aqueous, Rutin: Standard drug used. Data observed in triplicate and statistically found significant as $^aP<0.01$ and $^bP<0.05$

In vitro antioxidant activity

In vitro antioxidant activities of successive extracts of *C. gladiata* leaves were evaluated for different extracts such as pet. ether, CE, EE, ME, and AE at doses levels of 100 μg/ml, 200 μg/ml, and 400 μg/ml by DPPH free radical scavenging activity methods. The results of *C. gladiata* leaves extract were observed in all extracts, maximum activities were found in a higher dose as 68.12% in ME, 72.99% in EE, 50.45% in CE extract, 70.25% in AE, and 78.10% with standard drug Rutin. The results are tabulated in Table 2.

CONCLUSIONS

The present investigations have been confirmed the anti-inflammatory and antioxidant activities of leaves of *C. gladiata* that supports the traditional claims of *C. gladiata* as a medicinally active plant.

Statistical analysis

Results are expressed as mean ± SEM. Data are found significant $^{a}P < 0.01$ and $^{b}P < 0.05$ as compared to control group; statistical analysis by one-way ANOVA followed by Dunnet's t-test.

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