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Anti-inflammatory Effect of an Extract of *Agave americana* on Experimental Animals

Arup Kumar Misra¹, Sushil Kumar Varma², Ranjeet Kumar³

¹Department of Pharmacology, All India Institute of Medical Sciences, Jodhpur, Rajasthan, ²Department of Pharmacology, Mahatma Gandhi Institute of Medical Sciences, Wardha, Maharashtra, ³Department of Pharmacology, Raipur Institute of Medical Sciences, Raipur, Chhattisgarh, India

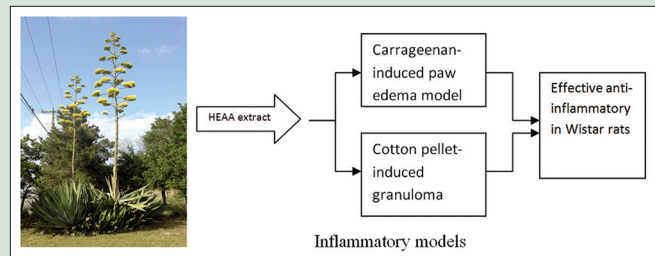
ABSTRACT

Background: Medicinal plants have the potential treasure to treat medical conditions for a long time based on error and trial method. *Agave americana* has been known as century plant or *American aloe*. The leaves contain genins, saponins, steroidal flavonoids, isoflavones, and coumarins. It has long been used for anti-inflammatory effects. **Objectives:** This study was designed to evaluate the anti-inflammatory activity of the extract of *A. americana*. **Materials and Methods:** *A. americana* leaves extract is collected and the extract is done by percolation method. Inflammation is induced in experimental animals' carrageenan-induced paw edema model and cotton pellet-induced granuloma model. The extract of *A. americana* and standard, aspirin and indomethacin are given orally for the anti-inflammatory effects. The anti-inflammatory effects were measured at regular intervals of time to see the percentage inhibition of paw edema and reduction of weight of granuloma with percentage protection to compare the effect of the extract with the control and standard. **Results:** The extract of *A. americana* showed improved in percentage inhibition of paw edema in graded doses of hydroalcoholic extract of *A. americana* (HEAA) as compared with control in carrageenan-induced paw edema model. The percentage inhibition of 400 mg/kg of HEAA at the 3rd h is almost comparable with the standard, aspirin. On the other hand, graded doses (200 and 400 mg/kg) showed a highly significant reduction ($P < 0.001$) in the weights of granuloma in comparison with the control in cotton pellet-induced granuloma model. The percentage inhibition of weight of granuloma by 400 mg/kg of HEAA is quite comparable to the standard, Indomethacin. **Conclusion:** Anti-inflammatory activity is seen with the graded dose of HEAA. The plant contains flavonoids and genins which have been shown to have anti-edematous effects in the acute phase of inflammation. Thus, the plant contributes to its anti-inflammatory activity. Thus, higher doses of *A. americana* leaves need to be used to have the better understanding of the mechanism of anti-inflammation.

Key words: *Agave americana*, anti-inflammatory, aspirin, flavonoids, genins, indomethacin

SUMMARY

- The hydroalcoholic extract from *Agave americana* (100, 200, and 400 mg/kg, HEAA) were studied in carrageenan-induced paw edema model and cotton pellet-induced granuloma model in Wistar rats. The plant contains flavonoids and genins which have been shown to have anti-edematous effects in the acute phase of inflammation. The results were good with the higher doses of 200 and 400 mg/kg of HEAA. Its *in vivo* anti-inflammatory activity is in good agreement with the traditional use of *A. americana*.



Abbreviation Used: HEAA: Hydroalcoholic extract of *Agave americana* leaves; SEM: Standard error mean; ANOVA: Analysis of variance; n: Number; g: gram; w/w: weight by weight; h: hour; kg: kilogram; mg: milligram

Correspondence:

Dr. Arup Kumar Misra,
Department of Pharmacology, All India Institute of Medical Sciences, Jodhpur - 342 005, Rajasthan, India.
E-mail: arup2003m@gmail.com
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INTRODUCTION

“Herb” is a plant valued for its various natural substances that are capable of producing physiological and pharmacological effects on the human body.^[1] It is one of the integral parts of modern society. It played a major role in maintaining and improving the quality of life and health of human health for ages. The chemical constituents of these plants alter the pathophysiological aspects of disease in the human body. The immense potential of its medicinal properties was documented for a long time by the application of reverse pharmacology based on error and trial method. There has been a surge in plant research due to its immense potential for various diseases all over the world.^[2]

Many species of plants are still left unexplored, despite intensive research on various herbal plants. Thus, *A. americana* is chosen for the present study for its important medicinal properties. It has been known as century plant or American aloe.^[3] It has been known from antiquity that different parts of *A. americana* have been used traditionally to treat a variety of symptoms and disorders. The leaves contain constituents such as steroidal

saponins, isoflavones, and coumarins which were found to be useful for wound healing, in the treatment of diarrhea, dysentery, etc.^[4-8] The leaves have antimicrobial properties and hence used in mucosal inflammation, digestive disorders, and base for the plaque.^[8] In Ayurveda, the plant is also used for the treatment of rheumatoid arthritis, sciatica.^[9]

It appears from the perusal of literature that the plant *A. americana* has different important pharmacological parameters. Thus, the extract of leaves of *A. americana* was evaluated in experimental models to prove the medicinal properties by reverse pharmacology.

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MATERIALS AND METHODS

Animals

The present study was conducted in experimental animals, i.e., Albino Wistar rats. The animals were kept in polyvinyl wire mesh cages in the animal room of Department of Pharmacology. Wistar rats of either sex weighing between 150 and 250 g (8–12 weeks old) were chosen for the carrageenan-induced paw edema model and cotton pellet-induced granuloma models. They were maintained under standard laboratory condition (12 h light and dark cycle), temperature (22°C + 3°C), humidity (60% ± 10%) with access to food and water *ad libitum* as per the Organization for Economic Cooperation and Development guidelines, revised draft guidelines 425 and by the Committee for the Purpose of Control and Supervision of Experiments on Animals.^[10] The animals were given rest for 1 week to adapt to the new surrounding before subjecting them to experimentation.

Plant extract and preparation

The *A. americana* leaves were collected from the college nursery and authenticated by a botanist. The leaves collected were shade-dried, powdered, and stored in an airtight container. The powders were macerated for 24 h in 70% w/w ethanol. The hydroalcoholic extracts were prepared by percolation method using 70% w/w ethanol (solvent). The hydroalcoholic extracts were then dried and scraped to obtain the power form of extract. The yield of leaf extract by percolation is about 15%–20%. Before each experiment, the fresh solution of *A. americana* leaves extract was prepared by dissolving in distilled water and then given orally. Aspirin or acetylsalicylic acid and indomethacin solution were prepared by 2% suspension in gum acacia in distilled water. About 1% carrageenan was prepared in normal saline on the day of the experiment.

Drugs and chemicals

In the anti-inflammatory model, aspirin or acetylsalicylic acid (Alkem Ltd.), indomethacin (Jagsonpal Pharmaceutical Pvt. Ltd.), carrageenan (Analar, BDH) mercury plethysmometer, and electronic weighing balance were used.

Ethical clearance

Ethical clearance was taken from Institutional Research Ethics Committee and Institutional Animal Ethics Committee (IAEC) before the commencement of the study with approval letter number MGIMS/IAEC/Aug/5/2012.

Assessment of anti-inflammatory activity

Anti-inflammatory activity of hydroalcoholic extract of *A. americana* (HEAA) leaves was studied in the following two models were carrageenan-induced paw edema model and cotton pellet-induced granuloma models. Group 1 (control) was treated with distilled water 10 ml/kg and Group 5 (standard) was given aspirin 300 mg/kg in carrageenan-induced paw edema model and indomethacin 10 mg/kg in the cotton pellet-induced granuloma, respectively. Group 2, 3, and 4 were given test drug, HEAA in dose 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively. A pilot study was done and the above doses were found effective and hence selected for the study.^[7]

Carrageenan-induced paw edema model

The albino Wistar rats of either sex were divided into 5 groups, 6 animals in each group (total 30). Group I served as control and was given only 2% gum acacia (10 ml/kg, p.o.).^[11,12]

Group 1 – Control-received distilled water (10 ml/kg, p.o)

Group 2 – HEAA 100 mg/kg orally

Group 3 – HEAA 200 mg/kg orally

Group 4 – HEAA 400 mg/kg orally

Group 5 – Standard drug aspirin 300 mg/kg orally.^[13]

Wistar rats of either sex weighing 150–250 g were used for the test. Both hind paws were marked at the tibiotalar junction. Right hind paw was kept as the control in each animal. Acute inflammation was induced by injecting 0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline on the plantar aspect of left hind paw after 60 min of oral administration of standard and test drugs. Each paw volume was measured by dipping in Mercury plethysmometer up to the mark as described by Chattopadhyay *et al.*^[14] Readings were taken at 1, 2, and 3 h.^[13] The difference between the two readings was taken as the volume of edema, and the inhibitory percentage of inflammatory reaction was determined for each animal by comparing with control and calculated by the formula described by Sudjarwo Agus.^[15]

Percentage of inhibition = $(1 - Et/Ec) \times 100$ ^[16]

Where Ec = Edema of the control (Group I)

Et = Edema of the treated (Group II–V)

Cotton pellet-induced granuloma

The albino Wistar rats of either sex were divided into 5 groups, 6 animals in each group (total 30 animals). Group I served as control and was given only 2% gum acacia (10 ml/kg, p.o.).^[17]

Group 1 – Control-received distilled water (10 ml/kg, p.o)

Group 2 – HEAA 100 mg/kg orally

Group 3 – HEAA 200 mg/kg orally

Group 4 – HEAA 400 mg/kg orally

Group 5 – Standard drug indomethacin 10 mg/kg orally.^[18]

Sterile cotton pellet (2 ± 1 mg) soaked in 0.2 ml of distilled water containing penicillin (0.1 mg) was implanted subcutaneously on the 1st day on the inner side of right thigh of each rat with aseptic precautions under ketamine (80 mg/kg i.m) anesthesia.^[19] Animals were given the test and standard drug orally once a daily for 7 days. The pellets remained in bodies of rats for 7 days. On the 8th day, the rats were sacrificed and wet cotton pellets with granuloma were dissected out. Weights of these cotton pellets were measured. Then, they were dried in hot air oven at 60°C for 24 h and again the dry weight has determined. Change in weight of granuloma was calculated by subtracting the weight of cotton.^[7]

Statistical analysis

All the results were expressed as mean ± standard deviation. The differences between experimental groups were compared by one-way analysis of variance followed by Students *t*-unpaired test. The results were considered statistically significant when **P* < 0.05, ***P* < 0.01, ****P* < 0.001 as compare to control.

RESULTS

Anti-inflammatory activity of hydroalcoholic extract of *Agave americana* assessed by carrageenan-induced paw edema method in rats

In the carrageenan-induced rat paw edema test, the *A. americana* extract of leaves at 200 and 400 mg/kg showed gradual inhibition (*P* < 0.01 and *P* < 0.001, respectively) of paw edema at the end of 1st h after giving the carrageenan in the rat paw in comparison of control. At the 1st h, the doses of 100, 200, and 400 mg/kg showed percentage inhibition 15.15%, 22.72%, and 27.27% in comparison to control.

On the other hand, the *A. americana* extract of leaves at 100, 200, and 400 mg/kg, respectively, showed significant inhibition ($P < 0.001$) of paw edema at the end of 2nd and 3rd h after giving the carrageenan in the rat paw in comparison of control. At the 2nd h, the doses of 100, 200, and 400 mg/kg showed percentage inhibition 43.42%, 52.63%, and 59.21% in comparison to control, and at the end of 3rd h, the graded doses of *A. americana* showed percentage inhibition of 55.12%, 66.66%, and 76.92% in comparison with control. Whereas, the standard drug aspirin showed highly significant inhibition of paw edema of rat ($P < 0.001$) at 1st, 2nd, and 3rd h with percentage inhibition of 46.96%, 65.78%, and 80.76%, respectively, in comparison to control [Table 1 and Graphs 1, 2]. The percentage inhibition of 400 mg/kg of HEAA at the 3rd h is almost comparable with the standard, aspirin.

Anti-inflammatory activity of hydroalcoholic extract of *Agave americana* assessed by cotton pellet-induced granuloma in rats

In cotton pellets-induced granuloma pouch method, the HEAA at 100 mg/kg, 200 mg/kg, and 400 mg/kg showed a highly significant reduction ($P < 0.001$) in the weights of granuloma with 20.24%, 43.26%,

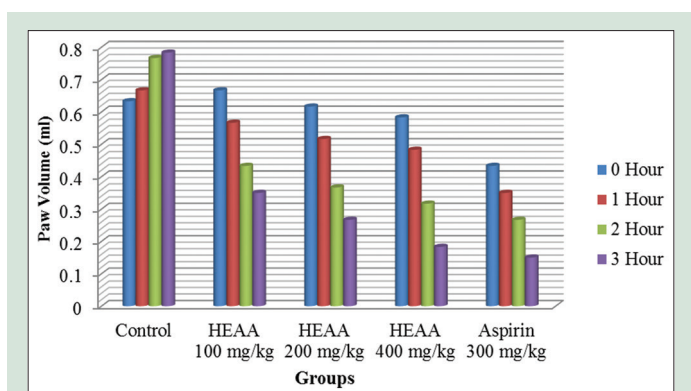
and 64.31% protection, respectively, as compared with control. On the other hand, graded doses (200 and 400 mg/kg) showed a highly significant reduction ($P < 0.001$) in the weights of granuloma with 43.26% and 64.31% protection, respectively, in comparison with control. The standard, indomethacin 20 mg/kg showed a protection of 74.28% with highly significant reduction ($P < 0.001$) in the weight of granuloma as compared with control. The percentage inhibition of weight of granuloma by 400 mg/kg of HEAA is quite comparable to the standard [Table 2 and Graphs 3, 4].

DISCUSSION

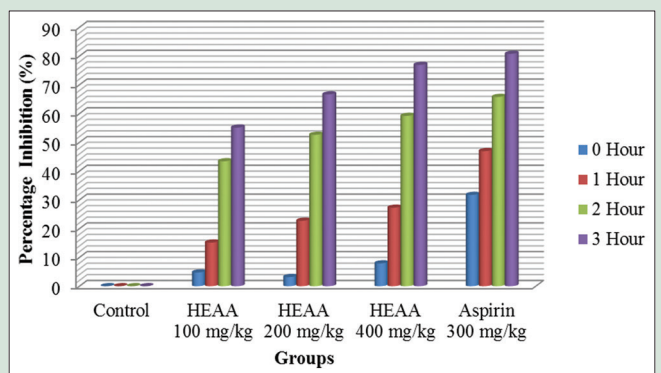
Carrageenan-induced paw edema

An anti-inflammatory drug of herbal origin is suitable for screening its anti-inflammatory properties in the acute as well as a subacute model of inflammation.^[20,21] Carrageenan model is common as it is highly reproducible with apparent no systemic side effects.

Carrageenan develops edema in the paw of the rat in a biphasic event.^[22] The initial phase of inflammation produced by carrageenan causes the release of histamine, kinin, and serotonin in the 1st h. While the second



Graph 1: Anti-inflammatory activity of ethanolic leaf extract of *Agave americana* by Carrageenan-induced paw edema method in rats



Graph 2: Anti-inflammatory activity of ethanolic leaf extract of *Agave americana* on rats by carrageenan-induced paw edema model showing percentage inhibition

Table 1: Anti-inflammatory activity of ethanolic leaf extract of *Agave americana* by carrageenan-induced paw edema method in rats

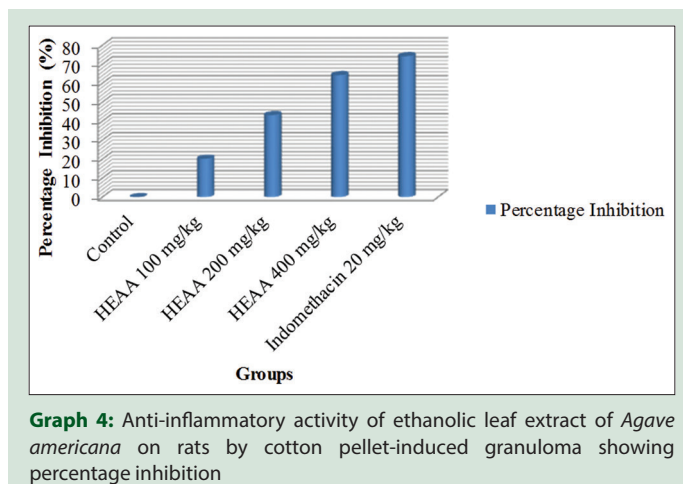
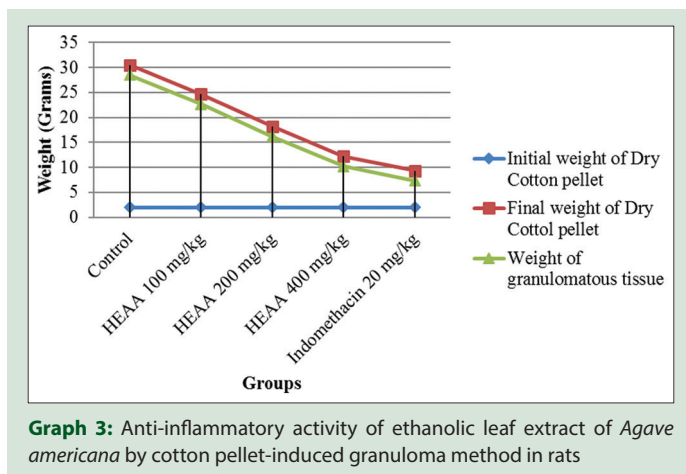
Group	Dose (mg/kg p.o.)	Paw volume (ml) (percentage inhibition)			
		0 h	1 h	2 h	3 h
Control (distilled water)	10 ml/kg	0.6333±0.02108 (0.0)	0.6667±0.03333 (0.0)	0.7667±0.03333 (0.0)	0.7833±0.03073 (0.0)
HEAA	100	0.6667±0.02108 (4.76)	0.5667±0.02108 (15.15)	0.4333±0.03333*** (43.42)	0.3500±0.0500*** (55.12)
HEAA	200	0.6167±0.03073 (3.17)	0.5167±0.03073** (22.72)	0.3667±0.02108*** (52.63)	0.2667±0.02108*** (66.66)
HEAA	400	0.5833±0.01667 (7.93)	0.4833±0.01667*** (27.27)	0.3167±0.03073*** (59.21)	0.1833±0.03073*** (76.92)
Aspirin	300	0.4333±0.03333*** (31.74)	0.3500±0.02236*** (46.96)	0.2667±0.02108*** (65.78)	0.1500±0.02236*** (80.76)

* $P < 0.05$.^[7] Number of animals ($n=6$). One-way ANOVA followed by student's unpaired t -test. Results are expressed in mean±SEM. * $P < 0.05$ significant, ** $P < 0.01$ very significant, and *** $P < 0.001$ highly significant as compared to control. HEAA: Hydroalcoholic extract of *Agave americana* leaves; SEM: Standard error mean; ANOVA: Analysis of variance

Table 2: Anti-inflammatory activity of ethanolic leaf extract of *Agave americana* by cotton pellet-induced granuloma method in rats

Group	Dose (mg/kg)	Initial dry weight of CP (mg)	Final dry of CP (mg)	Weight of granulomatous tissue	Percentage inhibition
Control (distilled water)	10 ml/kg	2	30.50±0.7638	28.50±0.7638	0.0
HEAA	100	2	24.67±1.116	22.67±1.116***	20.24
HEAA	200	2	18.17±0.7923	16.17±0.7923***	43.26
HEAA	400	2	12.17±0.6009	10.17±0.6009***	64.31
Indomethacin	20	2	9.333±0.4944	7.333±0.4944***	74.28

* $P < 0.05$.^[7] Number of animals ($n=6$). One-way ANOVA followed by Student's unpaired t -test. Results are expressed in mean±SEM. * $P < 0.05$ significant, ** $P < 0.01$ very significant, and *** $P < 0.001$ highly significant as compared to control. HEAA: Hydroalcoholic extract of *Agave americana* leaves; CP: Cotton pellet; ANOVA: Analysis of variance



phase is related to the release of prostaglandin-like substances, protease and lysosome in 2–3 h and these mediators produce edema in the rat paw.^[23,24] These mediators lead to a dilation of vessels with extravasations of fluid and plasma proteins with the formation of edema.^[9] These mediators as the metabolites of arachidonic acid and its cyclooxygenase pathway are capable of producing the characteristic signs of inflammation: vasodilatation, hyperemia, pain, edema, and cellular filtration.

Oral administration of HEAA extract at the dose 100 mg/kg, 200 mg/kg, and 400 mg/kg showed significant ($P < 0.001$) percent inhibition of edema at the end of 2 and 3 h as compared to control [Table 1 and Graphs 1, 2]. This action may be due to inhibition of histamine, serotonin, kinin, and prostaglandin by HEAA. *A. americana* extract contains flavonoids and genins as constituents which have been shown to have anti-edematous effects in the acute phase of inflammation.^[25] Thus, the plant contributes to its anti-inflammatory activity.

Cotton pellet-induced granuloma formation

It is widely used to assess the exudative and proliferative component of inflammation. Cotton pellets by absorbing fluid greatly influence the weight of granuloma and dry weight. It is well corrected with the amount of granulomatous tissue.^[26] The size of the granuloma is inhibited by NSAIDs by suppressing the generation of collagen fiber and mucopolysaccharides.^[27]

HEAA at the dose of 100, 200, and 400 mg/kg showed significant ($P < 0.01$, $P < 0.001$, and $P < 0.001$, respectively) inhibition of granuloma as compare to control [Table 2 and Graphs 3, 4]. The percentage inhibition of granuloma at a dose of 400 mg/kg of HEAA is almost comparable to the standard, aspirin. With increasing doses of HEAA, there is a gradual increase in the percentage inhibition of granuloma, indicating its anti-inflammatory activity.

A. americana extract contains flavonoids and genins^[27] as constituents which may be responsible for decreasing both final dry weight of cotton pellet and weight of granuloma which can attribute to their anti-inflammatory activity.

CONCLUSION

Herbal medicines have been used for various illnesses for many centuries. Due to better availability, affordable cost, and fewer side effects, they have immense potential. *A. americana* plant is one of the many species that are still left unexplored despite knowing the various medicinal values of this plants. Therefore, the present study was planned to evaluate the medicinal properties of *A. americana* plant.

The leaves of *A. americana* were found to have various pharmacological action due to its various constituents. The HEAA leaves mostly contain flavonoids, coumarins, flavanone, hecogenin, kammogenin, steroidal saponins, tetratriacontanol, and homoisoflavonoids.

Anti-inflammatory activity was noted with HEAA in a dose-dependent manner in acute (carrageenan-induced hind paw edema model) as well as subacute (cotton pellet-induced granuloma model) inflammatory experimental models. The anti-inflammatory activity of HEAA is due to its constituents, flavonoids, and genins. They decrease the chemical mediators of inflammation.

The extract of *A. americana* leaves in graded doses demonstrated significant anti-inflammatory on experimental animals in this study. The anti-inflammatory activities may be attributable to the flavonoids and genins of *A. americana*. It can be concluded that for a better understanding of the mechanism of anti-inflammatory effects, higher doses of *A. americana* leaves can be used for further studies. Thus, *A. americana* leaves need to be explored intensively so that it can be used to treat various disorders.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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