International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Original Article **CODEN: IJPNL6**

Anti-inflammatory activity of *Cuscuta Campestris* hydro alcoholic extract on anti-inflammatory animal model

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Received on: 20-03-2016; Revised on: 21-05-2016; Accepted on: 18-06-2016

ABSTRACT

Cuscuta campestris Yunck having common names like Golden Dodder, field Dodder and Bay Parri which is widely distributed in America, Africa, Asia, the Pacific Ocean, South and East America. Cuscuta campestris grows on other plant as a host having antibacterial, parasitic properties and anti-inflammatory due to presence of certain chemicals. The main purpose of research work was to find out the anti-inflammatory activity of plant extract. For evaluation of anti-inflammatory activity of Cuscuta campestris, adult Wistar rats of both sexes weighing between 150-200 g were used. Edema was induced in sub planter region of right hind paw of Wister rats by injecting 0.1 ml of 1% suspension of Carrageenan in normal saline. After establishing animal model, treatment was started by giving the plant extract 100 mg/kg and 200 mg/kg. Control was treated by normal saline. To compare the activity of extract, standard drug (Acetyl Salicylic Acid) was used. The results were documented on 0, 1, 2 and 3 hours after treatment and percentage inhibition was recorded. From the results, it was concluded that; the hydro alcoholic extract (200mg/kg) of plant Cuscuta campestris produced better inhibition of paw edema as compared to standard anti-inflammatory drug available.

Key Words: *Cuscuta campestris,* Anti-inflammatory Animal model, Extraction and comparison of extract and Acetyl Salicylic Acid activity

1. INTRODUCTION

Inflammation is body's vascular and cellular response (immune response) against foreign agents or tissue injury (physical, chemical and microbiologic, surgery or cancer) [1]. When this process is aggravated excessive tissue damage occurs which may lead to loss of organ function. Depending upon the nature and / or magnitude of stimuli, the inflammatory responses may be acute or chronic. At first instant mast cells release vasoactive amines i.e. histamine and serotonin which causes vasodilatation and increase the cell permeability, consequently intracellular fluid and proteins (macrophage inflammatory protein) escapes and join with damaged tissue fluid together they cause localized redness and edema. Moreover, due to rapture of cell membrane the phospholipids degraded by phospholipase A2 enzyme into arachidonic acid, where upon by the action of cyclooxygenase (COX1 and COX2) and lipoxygenase (5-LOX) enzymes eicosanoids i.e. prostaglandins (PGE2, PGF2α), thromboxane (TXA2) and leukotrienes (LTA4, LTB4, LTC4, and LTD4) are formed which play an important role in the process of inflammation[2]. If acute inflammation is not treated it may acquire complex chronic condition[3].

The persistent presence of micro-organisms or

antigen stimulates the innate immunity that leads to recruitment of various cells (macrophages, lymphocytes and platelets) to the site of damage tissue. The T helper lymphocytes (Th1 and Th2) produce pro-inflammatory cytokines (The-1; INF γ, IL-6, TNF-α and Th-2; IL-4, IL-5, IL-10, IL-13) and chemokine (Gro-α and IL-8). All these polypeptides facilitate the movement of inflammatory cells towards the affected area and increase the formation of reactive oxygen species (ROS), which may cause mutations that may lead to cancer or apoptosis[4]. Chronic inflammatory diseases include arthritis[5], asthma[6] atherosclerosis[7], and cancer[8]. A number of synthetic and semis synthetic anti-inflammatory and analgesic drugs are used for treatment.

The use of traditional plants in the treatment and prevention of diseases is attracting attention by scientists worldwide particularly in developing countries[9, 10]. The metabolic extract shows potent analgesic activity[11]. In current study anti-inflammatory and analgesic activity of hydro-alcoholic extract was performed.

According to Lee, Meng-Shiou (2011); in the seed of *Cuscuta campestris,* Quercetin concentration increases 23 fold by heating. Quercetin showed the anti-inflammatory and anti-proliferative activities.

1.1 Main objective of study

The main objectives of study are;

1. Extraction of plant by using a suitable extraction technique like maceration
2. To find out the anti-inflammatory activity of hydro alcoholic extract of plant Cuscuta Campestris on anti-inflammatory animal model.
3. To compare the anti-inflammatory activity of plant extract with the standard anti-inflammatory drug.

2. MATERIAL AND METHOD

2.1 Chemicals and Instruments:

B-Carrageenan, Acetic Acid, Ethanol, Acetyl Salicylic, Acid Distilled Water, Normal Saline, distilled Water and Plant extract. The instruments were as; Soxhlet apparatus, Grinder, Cutter, Baker, Flask, Aluminum Foil, Disposable syringe, vernier caliper and Stop watch.

2.2 Plant material

In the month of August, the whole parasitic plant *Cuscuta campestris grown on Dilbersia sisso* was collected from the area of Khanpur, District Rahim Yar Khan Punjab, Pakistan. This herbarium specimen was deposited to Department of Pharmacognosy, University of Karachi for identification of plant.

2.3 Preparation of extracts

Plant Cuscuta campestris was collected from Khanpur, District Rahim Yar Khan Punjab, Pakistan. Fresh Plant was brought to Pharmacology Laboratory, Faculty of Pharmacy, Hamdard University, Karachi. Plant was weighed which was two (2) kilogram at calibrated electric weighing machine. Then, it was cut into small pieces by the help of knife and it was grinded in a grinding machine. Then, this plant material was put in the conical flask of capacity 5 Liter (L) containing 3 L of hydro alcoholic solution (50:50) which was prepared by mixing 1.5 L of Ethanol 98% (analytical grade by Merck) and 1.5 L of doubled distilled water. The flask containing the mixture was put in the laminar wood and remains there for 03 days. After three days, this was passing through Whatmann No. 01, filter paper and the plant extract was collected. The extract was concentrated by Rota evaporator (Rota R-200, Buchi, Switzerland). When the concentrated extract was collected, it was further dried in vacuum oven (Vacucel, Einrishtungen, and GmbH). Then a crude extract was achieved weighing 4 % w/v. This extract was stored in refrigerator at 4^{O}c.

2.4 Animal selection and induction of inflammation

Adult Wistar rats of both sexes weighing between 150-200 g were used for experiment. They were housed in standard environmental condition like, ambient temperature (25^{0} ± 1^{0}C), relative humidity (55±5 %) and 12/12h light dark cycle. Animals had free access to standard pellet diet and water. All animal experiments were carried out in accordance with the guidelines of CPCSEA. The institute animal ethical committee gave the approval for conducting experiments on selecting male Wistar rats.

After 1 hour, edema was induced in sub planter region of right hind paw of Wister rats by injecting 0.1 ml of 1% suspension of Carrageenan in normal saline Winter et al methodology was adopted to determine the Anti-inflammatory activity of Plant Cuscuta campestris. After the placing the mark of identification on Wister rats, they were weighed ranging from 150 to 200 grams. The Wister rat was divided in to 4 groups each containing 6 rats (N=6). Group I was of Wister rats; named as reference drug group, received Acetyl salicylic acid 1mg/kg P.O. Group II and III was treated with plant Cuscuta campestris extract in 100 mg/kg P.O and 200 mg/kg respectively. Group IV was control and received Distilled water. At 0, 1, 2, and 3rd hour, the paw volume was measured by venire caliper. Actual volume was determined by comparing the initial and

subsequent values, and then compared with control. Via % inhibition formula, the inhibition of inflammation was determined by using the following equation.

% inhibition = 100 (1-Vt/Vc)

Where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

2.5 Statistical analysis

Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests and IBM SPSS-19 version.

3. RESULT AND DISCUSSION

We used different extracts, Normal saline and Acetyl Salicylic Acid in different doses. We measured the edema diameter in 0, 1, 2 and 3 hours respectively to compare the effects of normal saline, Hydroalcholic extract 100 mg/kg, Hydroalcholic extract 200mg/kg and Acetyl Salicylic Acid. Normal saline was given 10ml/kg dose and we measured the edema diameter as; 3.92±0.21 mm, 4.66±0.21 mm, 5.34±0.21 mm and 6.28±0.21 mm at 0, 1, 2, and 3 hours respectively, as shown in Table No. 3.1. Hydroalcholic extract was given as a dose of 100mg/kg and edema diameter was measured as 3.90±0.13 mm, 4.58±0.13 mm, 4.50±0.13 mm and 5.00±0.13 mm at 0, 1, 2 and 3 hours respectively as shown in Table No 3.1. Same Hydroalcholic extract was used with the double concentration as 200mg/kg and we get the edema diameter at 0, 1, 2 and 3 hours as, 4.06±0.26 mm, 4.32±0.26 mm, 4.5±0.26 mm, and 4.77±0.26 mm respectively as shown in Table No. 3.1. To compare the anti-inflammatory effect of Hydroalcohlic extract of Cuscuta campestris, we also compare the results with the effect of Acetyl Salicylic Acid by giving the dose of 100 mg/kg and obtained the following results; 3.82±0.06 mm, 4.16±0.06 mm, 4.45±0.06 mm and 4.70±0.06 mm at 0, 1, 2 and 3 hours respectively as shown in Table No.3.1.

On the basis of the results we obtained, it is concluded the Cuscuta campestris has comparable anti-inflammatory activity with Acetyl Salicylic Acid which is anti-inflammatory drug available. If we use the double strength concentration of extract, we got the good anti-inflammatory results as compared to Acetyl Salicylic Acid.

After giving the different extracts and acetyl Salicylic Acid to the Rats, we also calculated the percentage inhibition at different time intervals as 0, 1, 2, and 3 hours. When Hydroalcohlic extract 100 mg/kg was used the percentage inhibition was 0.551783 %, 1.680972818 %, 15.79275905 %, and 20.61554789 % respectively as shown in Table No. 3.2. After using Hydroalcholic extract 200mg/kg, the percentage inhibition was remarkable high as compare to extract with 100 mg/kg. The result was, 3.480475 %, 7.296137339 %, 15.73033708 % and 24.03820642 % at 0, 1, 2 and 3 hours respectively as shown in Table No. 3.2. On treated with Acetyl Salicylic Acid, we got the percentage inhibition as, 2.758913 %, 10.69384835 %, 16.60424469 % and 25.23215707 % at 0, 1, 21 and 3 hours respectively as shown in Table No. 3.2.

In Table 3.1 and Table 3.2, show the anti-inflammatory effect of Hydro alcoholic plant extract of Cuscuta campestris (100 and 200mg/kg) in Carrageenan induced paw edema in Wister rats. The hydro alcoholic extract of plant Cuscuta campestris (200mg/kg) prevented the formation of edema induced by Carrageenan and thus showed significant anti-inflammatory activity ($p<0.05$). The hydro alcoholic extract of plant Cuscuta campestris (200 mg/kg) reduced the edema induced by Carrageenan by 24.05 % after 3h injection of noxious agent as compared to the control vehicle treated group. Acetyl salicylic acid at 10mg/kg inhibited the edema volume by 25.23 %. On Carrageenan induced acute inflammation model the hydro alcoholic extract of plant Cuscuta campestris (200 mg/kg) produced better inhibition of paw edema.

4. CONCLUSION

From the above results and discussion; it is concluded that the plant extract of Cuscuta campestris have reportable anti-inflammatory activity. At the dose of 200 mg/kg, it has high anti-inflammatory activity as compare to 100 mg/kg. The percentage inhibition of Cuscuta Campestris plant's extract is similar to the standard drug like Acetyl Salicylic Acid which is used in all health care setup and as OTC drug. On large scale, this plant extract can be used to develop a dosage form having good anti-inflammatory activity.

ACKNOWLEDGMENT

I am very thankful to Faculty of pharmacy, Hamdard University Karachi for providing us the instruments and research facility. I am also thankful to my teachers in general and supervisor in especial for such help and kindness. I am also very thankful to Mr. Muhammad Rafiq (Laboratory in-charge) for his help and support during my work.

Table: 3.1 Effect of Hydroalcholic extract of plant Cuscuta campestris on carrageenan induced paw edema in rat treatment groups

Treatment Groups	Doses	Edema Diameters (mm)			
		0 hr	**1 hr**	**2 hr**	**3 hr**
Normal Saline	10ml/kg	3.92±0.21	4.66±0.21	5.34±0.21	6.28±0.21
Hydroalcholic extract 100mg/kg	100mg/kg	3.90±0.13	4.58±0.13	4.50±0.13	5.00±0.13
Hydroalcholic extract 200mg/kg	200mg/kg	4.06±0.26	4.32±0.26	4.5±0.26	4.77±0.26
Acetyl salicylic acid	100mg/kg	3.82±0.06	4.16±0.06	4.45±0.06	4.70±0.06

Each value is mean ± SEM N=6 rats
a P < 0.01 b P < 0.05
One way ANOVA followed by Dunnet Multiple comparison test statistically significant when compared to control.

Table: 3.2 Percentage inhibition of paw edema exhibited by hydro alcoholic extract of plant Cuscuta campestris

Treatment	Percentage inhibition (%) at various time intervals			
	0 time	**1 hr**	**2 hr**	**3 hr**
Hydroalcholic extract 100mg/kg	0.551783	1.680972818	15.79275905	20.61554789
Hydroalcholic extract 200mg/kg	3.480475	7.296137339	15.73033708	24.03820642
Acetyl salicylic acid	2.758913	10.69384835	16.60424469	25.23215707

REFERENCES

1. Gabay, C., Kushner, I. Acute phase proteins and other systemic responses to inflammation. New England Journal of Medicine (1999) 340 (6):448-54.
2. Rocca, B., FitzGerald, G.A. Cycloxygenases and prostaglandins: shaping up the immune response. International Immunopharmacology (2002) 2: 603-30.
3. He M., Lau, H.Y., Ng, S.W., Bhatia, M. Chemokine in acute Inflammation, regulation, function and therapeutic strategies. Journal of Integrative Biology (2007) 1(1):18-27
4. Moss, S., Blaser, M.J. Mechanism of disease: Inflammation and origin of cancer. Nature Clinical Practice Oncology. (2005) 2 (2): 90-97.
5. Kaine, J.L. Abdacept for the treatment of rheumatoid arthritis. Current Therapeutic Research (2007) 68(6):379-99.
6. Yeretssian, G., Labbe K., Saleh, M. Molecular regulation of inflammation and cell death. Cytokine (2008) 43: 380-90.
7. Ross, R. Atherosclerosis is inflammatory disease. New England Journal of Medicine. (1999) 340 (2):115-126
8. Hofseth, L.J., Worgovich, M.J. Inflammation, Cancer and Targets of Ginseng. The Journal of Nutrition. (2007) 137:183-85.
9. Sofowora A, 1982. Medicinal Plants and Traditional medicine in Africa. Published by John Wiley and Sons Ltd. 1st edition 131: 168 –71.
10. UNESCO, 1996. Culture and Health Orientation Texts World Decade for cultural Development 1988-1997, Document CLT/DEC/PRO – 1996, Paris, France. p. 129.
11. Ghule R.S, Benkatnarayanan R., Thakare S.P. *et al*.. Analgesic activity of *Cuscuta campestris Yuncker* a parasitic plant grown *on Nerum indicum Mill.* Journal of Advanced Pharmacy Education and Research. 2011, 1: 45-51
12. Winter, Charles A., Edwin A. Risley, and George W. Nuss. "Carrageenin-induced edemas in hind paw of the rat as an assay for anti-inflammatory drugs." *Experimental Biology and Medicine* 111.3 (1962): 544-47