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Anti-Inflammatory, Antioxidant and Anti-Diarrheal Effects of Ethanol Extract of *Stephania Japonica*

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Abstract

The present study was carried out to investigate anti-inflammatory, antioxidant and anti-diarrheal effect of ethanol extract of *Stephania japonica.* This study showed that the plant extract has significant ($p<0.05$) anti-inflammatory effect at all phases of carrageenan induced inflammation at a dose level 2g/kg. The DPPH free radical scavenging effect of the extract was compared with standard antioxidant ascorbic acid. IC_{50} values were found 33.57 µg/ml for the extract and 15.57 µg/ml for ascorbic acid. *S. japonica* extract at dosage level 2g/kg and 1g/kg decreased the gastrointestinal motility 36.56 and 21.53 %, respectively, in rats. The ethanol extract of the plant also reduced the total number of feces as well as wet feces of rats in castor oil-induced diarrheal model. The results revealed that the extract possesses promising anti-inflammatory, antioxidant and antidiarrheal activity.

Keywords: Anti-inflammatory, Antioxidant, Anti-diarrheal, *Stephania japonica*

Introduction

Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago (Solecki, 1975) and forest is referred to as GOD's own pharmacy (Treben, 1993). The era of natural product isolation from plant started just over 200 years ago, when Friedrich Sertürner isolated the first pharmacologically active pure compound from a plant: morphine from opium produced by cut seed pods of the poppy, *Papaver somniferum* (Hamilton and Baskett, 2000). Today drug discovery from natural products is thought as end of an era or an endless frontier (Jesse and Vederas, 2009).

The plant Stephania *japonica* belongs to the family Menisperaceae consisting 70 genera and approximately 400 species with a great deal of interest on account of presence of pharmaceutically important compounds from this family: picrotoxin, tubocurain, trilobine isotrilobine etc. Traditionally this plant has been used for facial paralysis (Yusuf *et al.*, 2007), abortifacient (Murty and Venkaiah, 2010; Mitra and Mukherjee, 2009), cuts and wounds (Gupta *et al.,* 2010) and stomachic (Tomas SC Li, 2002). Various alkaloids, such as tertiary phenolic biscoclaurine type alkaloid stepholine (Tomita and Ibuka, 1963), hasubanonine (Watanabe and Matsumura, 1963) were isolated from roots of *S. japonica*. The root extract has shown multidrug resistance modulator effect (Hall and Chang, 1997). The objective of the present study was to find out anti-inflammatory, antioxidant and anti-diarrheal effect of the ethanol extract (whole plant) of *S. japonica*.

Materials and Methods

Collection of plant materials

Plant materials (whole plant) were collected from plantation area of BCSIR laboratories Chittagong in August 2008. The plant was taxonomically identified by Industrial Botany Research Division of BCSIR laboratories Chittagong. A voucher specimen (Y-117) was deposited in the herbarium of BCSIR Laboratory, Chittagong.

Preparation of plant extract

The fresh plant *S. japonica* was washed with distilled water, chopped into small pieces, air dried under shade and ground into coarse powder and stored in an airtight container. Plant powder was macerated in pure ethanol (95%) for 7 days at room temperature (28±1)°C with occasional shaking. Every seven days later, ethanol extract was filtered off through a cotton plug and then with a Whatman No. 1 filter paper. The extract was concentrated under reduced pressure below 50°C

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through rotary vacuum evaporator (EYELA Rikakikai co., Ltd. Tokyo). The concentrated extracts were collected in a petri dish and air dried at room temperature. The whole process was repeated three times. The concentrated extract (60g blackish-green) was stored at 4°C until use.

Animals and diet

Wistar Albino rats of either sex obtained from the animal house of BCSIR laboratories, Chittagong, weighing between 180-200 g were used for the present study. The animals were acclimatized to room temperature (28±5)°C with a relative humidity of (55±5)% in a standard wire meshed plastic cages for 4 to 5 days prior to commencement of the experiment. During the entire period of study the animals were supplied with standard pellet diet and water ad libitum. In this study, all the animal experimentation was carried out according to the guidelines of Institutional Animal Ethics Committee (IAEC).

Assay for anti-inflammatory activity

Anti-inflammatory activity of *S. japonica* extract was assessed by following the model described by Winter et al. (1962). Twenty five Wistar Albino rats were randomly divided into five equal groups (n=5): control group, positive control group and three individual treated groups. Control group received only distilled water 2ml/rat, positive control group received 40mg/kg standard drug diclofenac sodium and treated groups received *S. japonica* extract at the dose 2g/kg, 1g/kg and 0.5g/kg body weight, respectively. The initial right hind paw volume of each rat was measured using plethysmometer (UGO Basile, Italy). According to the model, acute inflammation (paw edema) was induced in all albino rats by subplantar injection of 0.1ml of 1 % (w/v) carrageenan one hour after dosing. The volumes of right hind paws were measured at 1st, 2nd, 3rd and 4th hour after carrageenan injection by using the plethysmometer. The paw edema was determined using the following formula (Olajide *et al.,* 2000):

$$\% \text{ inhibition} = \frac{(C_t\text{-}C_0)\text{ control}-(C_t\text{-}C_0)\text{ treated}}{(C_t\text{-}C_0)\text{ control}} \times 100$$

DPPH antioxidant assay

The antioxidant activity of *S. japonica* extract was assessed in comparison to standard antioxidant ascorbic acid (BDH, England) depending on the scavenging effect of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The whole procedure was performed according to established procedure (Braca *et al.* (2001). Different concentrations (20, 40, 60, 80, 100 and 200 µg/ml in methanol) of ascorbic acid solution (1ml) as well as *S. japonica* extract solution (1ml) were mixed with 3 ml of 0.4 mM DPPH solution. The mixtures were kept in dark for 30 minutes to measure the absorbance at 517 nm using UV-Visible Spectrophotometer (Cintra, Australia) and ascorbic acid was used as a positive control. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The degree of decolorization of DPPH from purple to yellow indicated the scavenging efficiency of the extract. The scavenging activity against DPPH was calculated using the equation: Scavenging activity (%) = [(A - B) / A] x 100.

Where A is absorbance of control (DPPH solution without the sample), B is the absorbance of DPPH solution in the presence of the sample (extract/ ascorbic acid). The scavenging activity (%) was then plotted against log concentration and from the graph IC_{50} (Concentration for 50% inhibition) value was calculated by linear regression analysis.

Gastrointestinal motility test with barium sulfate milk

Gastrointestinal motility test with $BaSO_4$ (BDH, England) milk was performed according to method described by Chatterjee (1993). Rats fasted for over night were randomly divided into five groups (n=5). Control group received only distilled water, positive control group received commercially available anti-diarrheal drug loperamide 1mg/kg, and three individual treated groups received (p.o.) *S. japonica* extract 2g/kg, 1g/kg and 0.5g/kg body weight, respectively. Thirty minutes later 2ml of 10% barium sulfate (BDH, England) suspension were administered (p.o.) in all groups of rats. After 30 minutes rats were sacrificed. The total length of small intestine and the distance traveled by $BaSO_4$ milk was measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileo-cecal junction).

Castor oil-induced diarrhea

The method described by Shoba and Thomas (2001) was followed for this study. Twenty five Wistar Albino rats were randomly divided into five equal groups (n=5): control group, positive control group and three individual treated groups. Control group received only distilled water 2ml/rat, positive control group received loperamide 1mg/kg as standard and treated groups received *S. japonica* extract at the

dose 2g/kg, 1g/kg and 0.5g/kg body weight, respectively. Rats were housed in separate cages having blotting paper placed below for collection of fecal matters. Diarrhea was induced by oral administration of castor oil (2ml/rat). Extract and drugs were given orally 1 hour before the administration of standard dose of 2 ml of castor oil. The number of both hard and soft pellets was counted at every hour over 5 hour period for each rat. A numerical score based on stool consistency was assigned as follows: normal stool =1, semisolid stool =2 and watery stool =3.

Statistical analysis

All the values in the tests were expressed as mean ± SEM (standard error of the mean). Statistical differences between the mean of the various groups were analyzed by using Student's "t" test. Probability (p) values of 0.05 or less were considered as significant. All the graphical presentation and statistical calculations were prepared using "Microsoft Excel- 2003".

Results and Discussion

Subplanter injection of carrageenan in rat showed a time dependent increase of paw thickness (Table I) and carrageenan induced inflammation was significantly ($p<0.05$) inhibited by *S. japonica* extract in all phases of the experiment at a dose level 2g/kg but highest reduction (42.21%) was observed at the 4th hr after carrageenan injection (Table II). The DPPH free radical scavenging activity of the *S. japonica* ethanol extract and ascorbic acid is shown in Table III. Among six different concentrations used in the study (20 to 200 µg/ml) ethanol extract showed highest scavenging activity 85.76% (Table III) at concentration 200 µg/ml and at the same concentration ascorbic acid showed 98.35% scavenging activity. Percentage scavenging activity was plotted against log concentration. From the graph IC_{50} (Concentration for 50% inhibition) values calculated by linear regression analysis (Figure 1) for *S. japonica* extract and vitamin C were found to be 33.57 and 15.57 µg/ml respectively. The IC_{50} values obtained for *S. japonica* extract and ascorbic acid indicate that *S. japonica* extract as well as ascorbic acid have ability to neutralize free radicals. Most of the plant extracts showing antioxidant activity are due to the presence of phenolic compounds (Ramarathnam *et al.*, 1997; Velioglu *et al.*, 1998). Phenolic natural compounds such as flavonoids possess antioxidant activity because of their redox properties which allow them to act as reducing agents and singlet oxygen quencher. The Inflammatory process may be defined as sequences of events that occur in response to noxious stimuli or infection. These responses are orchestrated by highly modulated interaction between mediators of inflammation and inflammatory cells (Sacca *et al.*, 1997). Activation of the inflammatory response depletes antioxidants and exposes the host to increased risk of oxidative stress (Sorci and Faivre, 2009). Recent evidence has indicated that cytokines (and chemokines), as well as their receptors, are involved in pathophysiology of many inflammatory diseases including sepsis, rheumatoid arthritis atherosclerosis and asthma. These pathological states seem to be linked with an imbalance of cytokine network and to excessive recruitment of leukocytes to the inflammatory sites (Haddad, 2002; Feldmann *et al.* 1998; Young, 1998). Because of this, the cytokine system constitutes a very interesting and promising

Table I: Effect of ethanol extract of *S. japonica* on carrageenan induced paw edema

Group	Paw edema (mm^3)			
	1st hr	2nd hr	3rd hr	4th hr
Control	0.26±0.02	0.46±0.02	0.69±0.03	1.01±0.05
DS	0.10±0.01*	0.17±0.01*	0.25±0.01*	0.39±0.02*
SJEx 2 g /kg	0.18±0.01*	0.30±0.01*	0.44±0.01*	0.58±0.02*
SJEx 1 g /kg	0.19±0.01^{NS}	0.35±0.02*	0.50±0.03*	0.62±0.03*
SJEx 0.5 g/kg	0.23±0.01^{NS}	0.44± 0.01^{NS}	0.61±0.02^{NS}	0.89± 0.02^{NS}

SJEx: *Stephania japonica* extract; DS, Diclofenac sodium 40mg/kg, Values are expressed as mean ± SEM (n=5) Degree of freedom is 8 in all cases; *$p<0.05$ significant compared to control NS: Not significant (Student's t test)

Table II: Effect of *S. japonica* extract in the inhibition of paw edema in rat

Group	% Inhibition of paw edema			
	1st hr	2nd hr	3rd hr	4th hr
Control	-	-	-	-
DS	60.6	62.8	63.1	61.5
SJEx 2 g /kg	31.01	34.07	36.58	42.21
SJEx 1 g /kg	25.8	24.4	27.64	38.36
SJEx 0.5 g/kg	11.2	4.2	11.7	12.4

Table III: DPPH free radical scavenging activity of *S. japonica* extract and ascorbic acid

Conc.	Log C	absorbance		% scavenging activity		IC_{50} µg/ml	
µg/ml		SJEx	AA	SJEx	AA	SJEx	AA
20	1.30	0.2717	0.2014	38.01	54.05		
40	1.60	0.215	0.1347	50.94	69.26		
60	1.78	0.1632	0.1164	62.76	73.44	33.75	15.57
80	1.90	0.1249	0.0483	71.50	88.98		
100	2.00	0.0887	0.0346	79.76	92.106		
200	2.30	0.0624	0.0072	85.76	98.35		

AA: Ascorbic acid

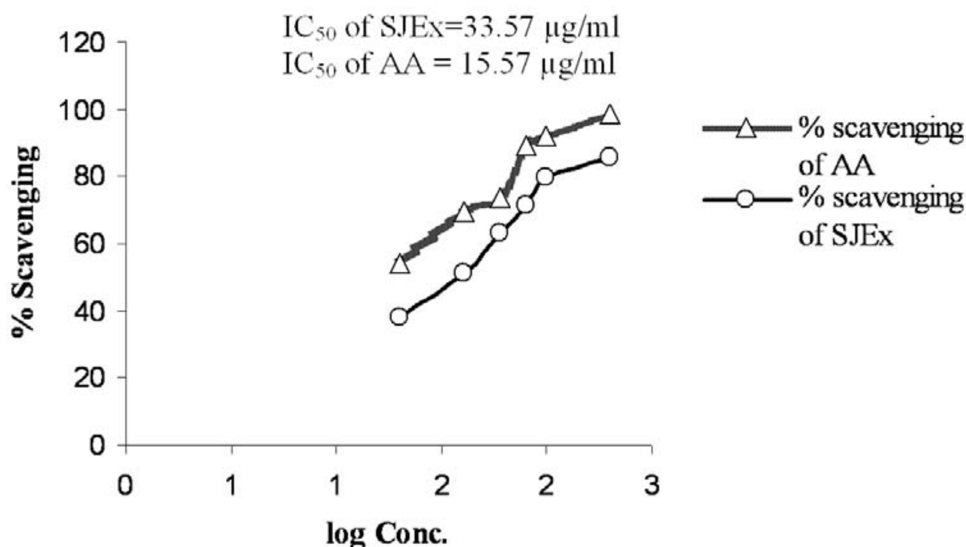

Fig. 1: Comparative % scavenging activities of *S. japonica* extract and ascorbic acid

target for the development of clinically relevant anti-inflammatory drugs. Carrageenan induced paw edema in rats is thought to be biphasic (Vinegar *et al.*, 1969). In the second phase bradykinin, protease, prostaglandin, and lysosome (Crunkhorn and Meacock, 1971) are released. A substantial body of evidence supports the concept that various plant-derived compounds with anti-inflammatory properties exert their effects through the modulation of cytokine system (Habtemariam, 2000). For instance, flavonoids, a class of compounds widely distributed throughout the plant kingdom posses interesting anti-inflammatory action (Gerritsen et al, 1995; Middleton, 1998; Di Carlo *et al*, 1999). So, resulting anti-inflammatory and antioxidant effect could be assumed either as the protective effect against oxidative stress or inhibition of enzymes of prostaglandin pathway or other enzymatic path ways of inflammatory process.

S. japonica extract at dosage level 2g/kg and 1g/kg decreased the gastrointestinal motility of rats 36.56 and 21.53 % respectively. However, loperamide (1mg/kg) exhibited more remarkable reduction (48.76%) with barium sulfate milk model at 30 min study (Table IV). In the rats with

Table IV: Effect of ethanol extract of *S. japonica* on gastrointestinal transit of rat

Group	% Gastrointestinal transit	% Inhibition of transit
Control	57.67±0.96	
Loperamide 1mg/kg	29.5±1.5**	48.76
SJEx 2 g /kg	36.5±0.56**	36.56
SJEx 1 g /kg	45.25±1.5**	21.53
SJEx 0.5 g/kg	55.04±0.91^{NS}	4.56

Values are expressed as mean ± SEM (n=5) **p<0.01 significant compared to control (Student's t-test)

castor oil-induced diarrhea the ethanol extract reduced the total number of feces as well as wet feces in dose dependent manner and the results were statistically significant (Table V). Castor oil-induced diarrhea is a secretory diarrhea due to the recinolic acid which induces diarrhea by a hypersecretory response (Almeida *et al*., 1995; Stewart *et al*., 1975). Ethanol extract of *S. japonica* successfully inhibited the castor oil-induced diarrhea and gastrointestinal motility. So, it can be assumed that anti-diarrheal action was mediated by anti-secretory as well as anti-peristaltic mechanisms.

Table V: Effect of ethanol extract of *S. japonica* on castor oil-induced diarrhea

Group	Total No. of feces in 5 h	Total No. of wet feces in 5 h
Control	22.2±1.06	16.8±0.58
Loperamide 1mg/kg	4.4±0.51**	0.8±0.49**
SJEx 2 g /kg	10.8±0.86**	2.4±0.24**
SJEx 1 g /kg	15.2±1.15**	4.4±0.81**
SJEx 0.5 g/kg	19.4±0.67*	14.6±0.74*

Values are expressed as mean ± SEM (n=5) *p< 0.05, **p< 0.01 significant compared to control (Student's t-test)

Conclusion

Present study justifies the traditional use of the plant for the remedy of different types of diseases related with inflammation and gastrointestinal problem. However further investigations are still necessary for isolation, purification and characterization of different biologically active compounds from the extract and elucidating their mode of actions.

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