Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi:

Evaluation of Anti-Inflammatory, Anti-diabetic activityof Indian *Bauhinia vahlii (stembark)*

Das Surya Narayan[1*], Patro Varanashi Jagannath[2], Dinda Subas Chandra[3]

[1]*Gayatri College of Pharmacy, Jamadarpali, Sambalpur, Odisha, India*
[2]*Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, India*
[3]*School of Pharmaceutical Education & Research, Berhampur University, Berhampur-760007, Odisha, India*

ARTICLE INFO

Article history:
Received 10 August 2012
Received in revised from 11 September 2012
Accepted 8 December 2012
Available online 28 December 2012

Keywords:
Phytochemicals
Anti-inflammatory
Antidiabetic
Bauhinia vahlii stem bark

ABSTRACT

Objective: To evaluate the anti-inflammatory and antidiabetic property of *Bauhinia vahlii* (stem bark) with preliminary phytochemical profile of the extracts. **Methods:** The dried whole plant material (1 400 g) was packed in soxhlet apparatus and extracted successively with Pet. Ether (PE) to defat the drug, petroleum ether was removed from the powdered defatted drug which was then extracted with benzene (BE), chloroform(CE) and 95% of Ethanol (EE) as increasing polarity and all extracts screened for anti-inflammatory and antidiabetic activity using carrageenan induced paw edema and streptozotacin induced diabetic respectively. The toxicity and phytochemical screening were done using standard procedure. **Result:** The preliminary phytochemical tests revealed the presence of alkaloids, flavonoids, phytosterol, phenolic compounds, and glycoside. While carbohydrates, protein, gums and amino acids were absent. The acute toxicity study of various extracts of *Bauhinia vahlii* was conducted and dose of 353 mg/kg is fixed for anti-inflammatory and antidiabetic perperty. The pet ether, chloroform and ethanolic extract of *Bauhinia vahlii* significantly decreased the paw edema induced by carrageenin in rats at a dose of 353 mg/kg comparable to standard ibuprofen (100 mg/kg). Similarly in case of antidiabetic property, the ethanolic and chloroform extract of *Bauhinia vahlii* at a dose level 353 mg/kg, showed significant reduction in blood sugar level from 2 to 24 h in progressive manner comparable to standard glibenclamide (5mg/kg).

1. Introduction

Plants are indispensible sources of medicine since time immemorial. Studies on natural product are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses and discovery of potential chemotherapeutic agents. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs [1].

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses [2]. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can easily be induced [2]. The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses [3]. Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary.

Diabetes mellitus is a group of disorders with different aetiologies. It is characterized by derangements in carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action [4]. Approximately 140 million people worldwide suffer from diabetes [5].The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable[6]. Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed [7], because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries.

Diabetes mellitus is ranked seventh among the leading

*Corresponding author: Das Surya Narayan, Assoc. Prof, (Pharm Analysis & Quality Assurance) Gayatri College of Pharmacy, Jamadarpali, Sambalpur, Odisha.
Tel: -09437158382
E-mail: suryadas2007@rediffmail.com , suryadas2007@gmail.com

causes of death and third when it's fatal complications are taken into account [8]. Traditional preparations of plant sources are widely used almost everywhere in the world to treat this disease. Therefore, plant materials are considered to be the alternative sources for finding out new leads for hypo- /antihyperglycemic agents.

Bauhinia vahlii[9] is a gigantic climbing ever green tree and largest creeper in India and can grow up to 10-30 m long. The woody stem can get as thick as 20 cm. The spreading stout branches are covered with rusty fine hair. Branchlets[10] densely pubescent and terminating in a pair of revolute tendrils; young branches, tendrils, petioles, underside of leaves especially along the nerves and inflorescence clothed with dense ferruginous tomentum. Leaves [11] very variable in size, often up to 18 in. diam., as broad as long or broader, deeply cordate, 11-15 nerved, cleft through about 1/3 of the length, sub-coriaceous, dark green and glabrescent above more or less downy beneath; lobes obtuse, rounded; petiole 3-6 in. long, stout. Flowers[11] white, on long slender pedicles, in terminal corymbose or corymbose racemes.

Fruit [12] is a flat woody pod with fine rusty hairs, 20-30 cm long. The plant has been reported to have agathisf lavone,flavonoid,betulinic acid,triterpene,campesterol and steroid in leaves[13], catechin,gallic acid methyl ester,benzenoid,mopanol and 4-O-methyl ester[13]. The plant is also reported to contain kaemferol, quercetin, rutin , betulinic acid[14] and Quercitrin, Stigmasterol, Sitosterol[16] in leaves.

The purpose of the present study was to evaluate the anti-inflammatory, anti-diabetic activity of Bauhinia vahlii stem bark extracts using carrageenan induced rat paw edema and Streptozotocin induced diabetic. The extracts were also studied for its acute toxicity effects and preliminary phytochemical screening.

2. Materials and methods

2.1 Materials

Streptozotocin (STZ) was purchased from Sigma Chemicals Co. (St. Louis, MO, USA), Carrageenan was procured from Himedia laboratories, Mumbai, India. All other chemicals and solvents(Pet.ether,Benzene,Chloroform and Ethanol) used were of analytical grade and obtained commercially from Merck- Limited, India, Mumbai.

2.2. Collection and identification of plant material

The Plant *Bauhinia vahlii* were collected in the month of October from Padiabahal, Sambalpur, Odisha , India. The plant material was taxonomically identified by Dr. (Mrs.) Uma Devi, Head, Department of Botany, Govt. Women's College, Sambalpur, Odisha. A voucher specimen (GWC/B-315/09) has been deposited in the Herbarium of the Department of School of Pharmaceutical Education & Research, Berhampur University, Berhampur-760007, India for future reference.

2.3. Experimental animals

Swiss albino mice (20-25 g) and Male Wistar rats (150-200 g) were purchased from the animal house of Gosh enterprises, Kolkata and housed in polypropylene cages at room temperature with proper ventilation. Prior to the experiments, mice and rats were fed with standard diet for 1 week in order to adapt to laboratory conditions. They were fasted over night but allowed free access to water before the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee (Reg. No.1339/ac/10/CPCSEA).

2.4. Preparation of plant material

The whole plant was first sun dried for several weeks, crushed by hands and dried again. Then the crushed parts of the plant were ground into coarse powder with the help of a mechanical grinder. By using the concept of the nature of solubility and distribution of the active ingredients, powdered material (1 400 g) was packed in soxhlet apparatus [17] and extracted successively with Pet. Ether (60-80,) to defat the drug, petroleum ether was removed from the powdered defatted drug which was then extracted with benzene, chloroform and 95% of Ethanol as increasing polarity. The whole each mixture then underwent filtration through what man filter paper. The filtrates (Pet. Ether, Benzene, Chloroform and Ethanol filtrate) obtained were evaporated by rotary evaporator at 5 to 6 rpm and at 40 °C temperature. It rendered a gummy concentrates. The gummy concentrate was designated as crude extract of respective solvent which was then freeze dried and preserved at 4 °C.

2.5. Phytochemical screening

Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, glycosides, saponins, tannins and terpenoids were carried out for all the extracts by standard procedure [18-21] (Table 1).

2.6 Acute toxicity study (LD_{50})

Acute toxicity study [22-23] was performed according to Organisation for Economic Co-operation and Development was performed as per Karber s method. Swiss albino mice of either sex weighing between 20-25 g were divided into six groups with six animals each.and of 90 d were used to determine LD_{50} of various extracts of *Bauhinia vahlii*. The gum acacia solution (2%) was used as a vehicle to suspend various extracts. The extracts were administered orally at different dose levels to group of six mice each, which have

been fasted overnight. The LD_{50} values of various extracts are calculate and dose is fixed as 353 mg/kg.

2.7. Experimental design

Thirty-Six experimental animals were randomly selected and divided into six groups denoted as Group I, Group II, Group III, Group IV, Group V and Group VI, consisting of 6 Wistar rats in each group. Each group received a particular treatment i.e. control, standard and the four doses of the extract. Prior to any treatment, each rat was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Group III to Group VI received the crude extract orally at the doses of 353 mg/kg of body weight. Group II received intraperitoneal administration of Ibuprofen at a dose of 100 mg/kg as standard for anti-inflammatory study, while Group I was kept as control giving 2% gum acacia solution in normal saline water.

Similarly forty-two animals were selected and divided into seven groups denoted as Group I, Group II, Group III, Group IV, Group V, Group VI and Group VII consisting of 6 Wistar rats in each group. Each group received a particular treatment *i.e.* normal control, diabetic control, standard and the four doses of the extract. Prior to any treatment, each rat was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Group IV to Group VII received the crude extract orally at the doses of 353 mg/kg of body weight. Group III received intraperitoneal administration of Glibenclamide at a dose of 5 mg/kg-body weight as standard drug for anti-diabetic study, while Group II was kept as diabetic control giving 2% gum acacia solution in normal saline water.

2.8. Anti-inflammatory Activity

The anti-inflammatory activity of *Bauhinia vahlii* was studied using acute (carrageenan induced paw edema) models of inflammation. The experiment protocols were approved by the Institutional Animal Ethics Committee prior to the conduct of the animal experiments (Reg. No.1339/ac/10/CPCSEA).

This model is based on the principle of release of various inflammatory mediators by carrageenan. Edema formation due to carrageenan in the rat paw is biphasic event. The initial phase is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome[24,25]. Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasations, increased tissue water and plasma protein exudation along with neutrophil extravasations, all due to the metabolism of arachidonic acid[26]. The first phase begins immediately after injection of carrageenan and diminishes in two hours. The second phase begins at the end of first phase and remains through third hour up to five hours.

2.9. Method: Carrageenan-Induced Paw Edema in Rats

Anti-inflammatory activity was evaluated using the Carrageenan induced rat paw oedema according to the technique of Winter *et al* [26-31]. The animals were housed in cages under standard laboratory condition. They had free access to standard diet and water. The animal were divided into 6 groups of six animals each and fasted for 12 h before the experiment. The initial right hind paw volume of the rats were measured using a plethysmometer and then 0.1 mL of 1% w/v carrageenan solution in normal saline was subcutaneously injected into the sub plantar region of the right hind paw. The volume of right hind paw was measured at 1, 2, 3, and 4 h after carrageenan injection, and the paw volume was determined. The data were expressed as paw volume (ml), compared with the initial hind paw volume of each rat. Cosolvent (2% gum acacia solution, p.o), various extracts of *Bauhinia vahlii* as suspension in 2% gum acacia solution (p.o) and ibuprofen (10 mg/kg, p.o) [25] was administered 30 min before carrageenan injection. The group received Co solvent was treated as control.The hind paw volume was measured plethysmometrically before and after the carrageenan injection, at hourly intervals for 4 h (Table 2).

$$\% \text{ inhibition of edema} = \left(\frac{Vc - Vt}{Vc}\right) \times 100$$

Where, V_T = mean paw volume of test group

V_C = mean paw volume of control group

2.10. Anti diabetic Activities

2.11. Induction of diabetes in rats [32-33]

Rats were made diabetic with an intraperitoneal injection of Streptozotocin (STZ 60 mg/kg body weight) dissolved in citrate buffer (0.1 M, pH 4.5). Diabetes was confirmed in STZ rats by measuring the fasting blood glucose level 48 h after the injection of STZ. Rats with blood glucose level above 250 mg/dL were considered to be diabetic and were used in this experiment.

2.12. Estimation of blood glucose

Blood samples were collected from the tail tips at 12, 24, 36 and 48 h after streptozotacin administration and found that stable hyperglycemia produced after 48 h. The blood sugar level was measured by digital display glucometer (One touch -Johnson & Johnson Ltd.). Initial blood sample were taken before the oral administration of the standard drug (Glibenclamide) and extracts. The blood glucose levels were measured at 2, 4, 8, 12 and 24 h after oral administration of Glibenclamide and various extracts of *Bauhinia vahlii* (Table 3).

2.13. Statistical Analysis

For Anti-inflammatory screening all experimental results were expressed as mean±SEM and data were assessed by

ANOVA Method followed by student's *t*-test. $P<0.05$ was considered as statistically significant. While for Anti-diabetic activity, all results are expressed as mean± standard error. The data was analyzed statistically using ANOVA followed by Dunnett's Multiple Comparison Test using SPSS 10.0 statistical software. The level of significance was fixed at 5%.

3. Result

3.1. Phytochemical constituents of the plant

The preliminary phytochemical tests revealed the presence of alkaloids, flavonoids, phytosterol, phenolic compounds, and glycoside. While carbohydrates, protein, gums and amino acids were absent (Table 1).

3.2. Acute toxicity study

The acute toxicity study of various extracts of *Bauhinia vahlii* was conducted and the LD_{50} of all extracts found to lay between 3 000-4 000 mg/kg body weight. The LD_{50} is in a higher dose range so the extracts are safe and 1/10 average of all extracts *i.e.* 353 mg/kg was taken as dose for pharmacological screening procedure.

3.3. Anti-inflammatory activity

The pet ether, chloroform and ethanolic extract of *Bauhinia vahlii* significantly decreased the paw edema induced by carrageenin in rats at a dose of 353 mg/kg comparable to standard ibuprofen (100 mg/kg) shown in Table 2.

3.4. Antidiabetic activity

The result of antidiabetic effect depicted in Table 3. From the table it is revealed that the ethanolic and chloroform extract of *Bauhinia vahlii* at a dose level 353 mg/kg, showed significant reduction in blood sugar level from 2 to 24 h in progressive manner comparable to standard glibenclamide.

4. Discussion

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. *B. vahlii* belongs to family Caesalpiniaceae is a common plant of Western Odisha. Phytochemical evaluation of the various extracts of *Bauhinia vahlii* reveals the presence of alkaloids, flavonoids, phytosterol, phenolic

Table 1
Preliminary Phytochemical Investigation of various extracts and powder of *Bauhinia vahlii*.

Test	Inference				
	PD	PEE	BE	CE	EE
Test for Carbohydrates	−	−	−	−	−
Test for Gums and Mucilages	+	−	−	−	−
Test for Proteins and Amino Acid	+	−	−	−	−
Test for Fixed Oils and Fats	+	+	+	+	+
Test for Phytosterols	+	+	+	+	+
Test for Glycosides	−	−	−	+	−
Tests for Saponins	+	−	−	−	+
Tests for Flavonoids	+	+	−	+	+
Tests for Alkaloids	+	−	−	−	+
Tests for Tannins and Phenolic Compounds	−	+	+	−	+

Table 2
Anti-inflammatory activity of various extracts of *Bauhinia vahlii* (Stem bark).

Treatment	Dose (mg/kg)	Mean paw volume (mL) ± SEM			
		Time in minutes			
		60	120	180	240
Control(2% gum acacia)	−	0.48±0.03	0.78±0.09	0.85±0.12	0.89±0.14
Ibuprofen	100	0.29±0.07* (39.6)	0.28±0.07* (64.1)	0.24±0.06* (71.80)	0.23±0.13* (74.2)
Pet.Ether. Ext	325	0.33±0.13(31.25)	0.41±0.09* (47.4)	0.49±0.01* (42.4)	0.42±0.07* (52.8)
Benzene. Ext	325	0.45±0.09(6.3)	0.73±0.12 (6.4)	0.78±0.15 (8.2)	0.78±0.11(12.3)
Chloroform. Ext	325	0.36±0.07(25)	0.34±0.09* (56.4)	0.35±0.07* (58.8)	0.32±0.11* (64)
Ethanol. Ext	325	0.36±0.12* (25)	0.42±0.13* (46.2)	0.57±0.17* (33)	0.50±0.09* (43.8)

Each value is mean±SEM (n=6), * Denotes significant difference compared to control value at $P<0.05$.

Table 3
Antidiabetic activities of various extracts of *Bauhinia vahlii* stem bark.

Group		Blood Sugar level in mg/dL					
		Time (h) / Dose	2 h	4 h	8 h	12 h	24 h
I	Normal control	–	88.4±2.07	87.4±1.12	87.1±0.6	87.6±1.7	87.2±1.03
II	Diabetic control	2% Gum acacia solution	288.4±8.06	286.7±9.14	287.1±10.4	289.6±7.6	293.2±5.23
III	Glibenclamide	5mg / kg /Day	227.0±4.04*	171.0±4.57*	139.4±2.64*	122.2±2.3*	114.0±3.7*
IV	Pet.Ether Ext	320mg/kg	284.3±7.3	281.2±9.4	280.6±9.2	280.3±5.1	288.8±8.1
V	Benzene. Ext	320mg/kg	289.4±5.2	284.0±7.5	285.4±9.3	289.2±9.5	292.4±5.3
VI	Chloroform. Ext	320mg/kg	260.4±7.5*	246.0±3.5*	227.3±8.6*	212.7±6.3*	269.4±8.3*
VII	Ethanol. Ext	320mg/kg	268.4±5.8*	252.2±3.4*	233.4±8.1*	194.7±2.7*	194.7±2.7*

All values are expressed as mean± SEM, n=6, *P<0.05 significant compared to control.

compounds, and glycoside. Here anti-inflammatory activity was performed based on the folk lore information by using the method,

Carrageenan induced inflammation model for the estimation of anti-inflammatory effect. The development of oedima in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin, and prostaglandin [38-39]. Stem bark extracts of *B.vahlii* showed significant anti inflamatory activity. This significant anti-inflammatory effect may be due to the inhibition of any inflammatory mediators by the glycosides or steroids [39] present in the extract.

Similarly the treatment of diabetes with medicines of plant origin that proved much safer than synthetic drugs is an integral part of many cultures throughout the world and gained importance in recent years. India has a rich history of using various potent herbs and herbal components for treating various diseases diabetes [34] and several species of plants have been described as having a hypoglycemic activity [35-37].

The anti-inflammatory activity in carrageenan-induce rat paw edema and antidiabetic activity in streptozotocin-induced diabetic rats of Bauhinia vahlii were monitored at a dose level 353 mg/kg for a time interval of 1hr to 4 h and 2h to 24h respectively. From evaluation it was concluded that the Pet.ether, chloroform and Ethanol extract shows significant reduction of inflammation as compared to Ibuprofen (P<0.05) and Chloroform and Ethanol extracts cause a significant decrease in blood glucose level as compared to standard drug Glibenclamide (P<0.05).

The present result indicates the efficacy of Bauhinia vahlii as an effective therapeutic agent in the treatment of acute inflammations and diabetic and also the present study authentifies the folk lore information on the anti-inflammatory and antidiabetic property of the plant Bauhinia vahlii. Further and detailed studies are in process for the isolation of active constituent responsible for this property and to idendification of the possible mechanism for the said properties.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

This study was supported by the grants from AICTE (8024/RIFD/MOD-75/2010-11), New Delhi, India. The Authors are extremely grateful to Prof. Ashok Kumar Satpathy , Director and Prof (Dr).S.K.Mohapatra, Principal, Gayatri college of Pharmacy,Sambalpur, Odisha,India for providing necessary facilities to carry out this work.

References

[1] Balunas M J, Kinghorn A D. Drug discovery from medicinal plants. *Life Sci* 2005; **78**: 431-441

[2] Sosa S, Balicet MJ, Arvigo R, Esposito RG, Pizza C, Altinier GA. Screening of the topical anti-inflammatory activity of some Central American plants. *J Eth.pharmacol* 2002; **8**: 211-15.

[3] E David E, Elumalai EK, Viviyan Therasa E, Thirumalai T. Evaluation of anti-inflammatory activity of the leaf extract of Wrightia tomentosa. Roem & Schult. *J Phar Resh* 2010; **3**:208-209

[4] Ansarullah, Bharucha B, Dwivedi M, Laddha NC, Begum R, A Hardikar AA,3 and Ramachandran AV. Antioxidant rich flavonoids from Oreocnide integrifolia enhance glucose uptake and insulin secretion and protects pancreatic β -cells from streptozotocin insult. *BMC Complement Altern Med*. 2011; **11**: 126.

[5] Sangameswaran, B and Jayakar, B. Anti-diabetic and spermatogenic activity of Cocculus hirsutus (L) Diels. *Afr J Biot* 2007; **6**(10):1212-1216.

[6] Paul D D, Tédong L, Asongalem EA, Dimo T, Sokeng S D, Kamtchouing P. Hypoglycaemic and antidiabetic effect of root extracts of Ceiba pentandra in normal and diabetic rats. *Afr. J. Trad. CAM* 2006; **3** (1): 129 -136

[7] Pareek A, Suthar M. Antidiabetic activity of extract of Berberis aristata root in streptozotocin induced diabetic rats. *Pharmacologyonline* 2010; **2**: 179-185.

[8] Trivedi NA, Majumder B, Bhatt JD, Hemavathi KG. Effect of Shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats. *Indian J Pharmacol*. 2004; **36**: 373-76.

[9] Baker in Hook.f. Bauhinia vahlii Wight & Arnott Fl.Brit Ind; 1876. 2:279.

[10]Prodr. Bauhinia vahlii Wight & Arnott.Fl.Pen.Ind; 1834. P. 297.

[11]Parker. Bauhinia vahlii Wight & Arnott, Fl.Punj; 1956; 3. P.180.

[12]Ali,P. Bauhinia vahlii Wight & Arnott.Acta Biol; 1967.8.P.241.

[13]Dugasani S, Balijepalli M K, Tandra S, Pichika M R. Antimicrobial activity of Bauhinia tomentosa and Bauhinia vahlii roots. *Pharmacog maga* 2010; **6**(23):204-207.

[14]Das S N, Dash P, Mistry S, Patro V J, Choudhary N S K. Antimicrobiological activity of Bauhinia vahlii (Stem Bark) against selected Human Pathogens. *IntRJPharmSci*.2010; **01**(01): 0036.

[15]Sultana, S, Ilyas M, Kamil M, ShaidaWA. Chemical Investigation of Bauhinia Vahlii. *J Indian Chem Soc* 1985; **62**(4): 337-338.

[16]Harborne J B. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.New Delhi: Springer, Rajkamal Electric Press, 3rd Edn.1998: p.1-32, 40-227.

[17]Shah B N, Nayak B S. Experimental Pharmacognosy. Jalandhar,India: Vikash & Co; 2008, p.190-200.

[18]Shah C S, Quadry J S. A Text Book of Pharmacognosy. Ahmedabad,India: B.S. Shah Prakashan; 2007-08: 13th Edn, p.83-86,97.

[19]Ali M. Text Book of Pharmacognosy, CBS Publishers and Distributers,New Delhi: , 2003, 2nd Edn. p. 64-65,96-97,139-40,272,283.

[20]TailangM, SharmaA. Phytochemistry:Theory & Practical.New Delhi,India:Birla Publications; 2008, Ist Edn.p.199.

[21]Ghosh MN. Evaluation of Analgesic activity. In:Fundamentals of experimental pharmacology, 2nd ed., Scientific book Agency,Calcutta; 2005,P.69-71.

[22]Agrahari AK, Khaliquzzama M and Panda SK. Evaluation of analgesic activity of methanolic extract of Trapa natans. var Bispinosa roxb Roots. *Journal of Current Pharmaceutical Research* 2010; **01**: 8-11.

[23]Rahman M, Bachar SC and Rahmatullah M. Analgesic and anti-inflammatory activity of methanolic extract of acalypha indica linn. Pak. *J. Pharm. Sci.* 2010; **23**(3): 256-258

[24]Chatpaliwar VA , Johrapurkar A A , Wanjari M.M , Chakraborty R R , *Kharkar V T Indian Drugs* 2002; **42**; 543- 45.

[25]Sundhararajan R and Ravichandiran V. Anti-inflammatory activity of Spermacoce ocymoides Burm.F. *Journal of Pharmacy Research* 2012; **5**(1):719-721.

[26]Asuzu I U, Sosa S, Loggia D R. The anti-inflammatory activity of Icacina trichantha tuber. *Phytomedicine*. 1999; **4**(2): 267-272.

[27]Areej M, Taweel AL, Kadriya S, El-Deeb, Maged S, Abdel K, Jaber S. GC/MS analysis of the fatty acids of three Clematis species growing in saudiarabia and their Anti-inflammatory activity. *Saudi Pharmaceutical Journal* 2007; **15**(3-4):224-227.

[28]Sati S C, Sati M D, Raturi R, Badoni P, Singh H. Anti-Inflammatory and Antioxidant Activities of Zanthoxylum Armatum Stem Bark. Global Journal of researches in engineering: *J General Engineering* 2011; **5**:86.

[29]Das BN, Saha A, Ahmed A. Anti-inflammatory activity of bark of Xeromphis spinosa. *Bangladesh J Pharmacol* 2009; **4**: 76-78.

[30]Pandurangan A, Khosa R L, Siva E. Evaluation of Anti-inflammatory and Analgesic Activity of Root Extract of Solanum Trilobatum Linn. *Iranian Journal of Pharmaceutical Researc*h 2008; **7** (3): 217-221.

[31]Vaiyapuri S, Dey A, Balasundaram, Balasubramanian R. Antihyperglycemic, Antihyperlipidemic and Antioxidant Effect of Phyllanthus rheedii on Streptozotocin Induced Diabetic Rats. *Iranian Journal of Pharmaceutical Research* 2008; **7** (1): 53-59.

[32]Mishra SB, Verma A, Mukerjee A, Vijayakumar M. Anti-hyperglycemic activity of leaves extract of Hyptis suaveolens L. Poit in streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Medicine*. 2011; 689-693.

[33]Yeh GY, Eisenberg DM, Kaptchuk T J, Phillips RS. Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes Care* 2003; **26**: 1277-1294.

[34]Verspohl EJ. Recommended testing in diabetes research. *J Planta Med* 2002; **68**: 581-590.

[35]De Sousa E, Zanatta L, Seifriz I, Creczynski-Pasa TB, Pizzolatti M.G, Sypoganicz B, Silva FRMB. Hypoglycemic effect and antioxidant potential of kaempferol-3,7-0-($\dot{\alpha}$)-dirhamnoside from Bauhinia forficate leaves. *J Nat Prod* 2004; **15**(3-4): 829-832.

[36]Colca J R. Insulin sensitizers may prevent metabolic inflammation. *J Biochem Pharmacol* 2006; **72**: 125-131.

[37]Georgewill OA, Georgewill UO, Nwankwoala RNP. Antiinflammatory effects of Morninga oleifera lam extract in rats. *Asian Pac J Trop Med* 2010; **3**(2): 133-135.

[38]Georgewill OA, Georgewill UO. Evaluation of the anti-inflammatory activity of extract of Vernonia Amygdalina. *Asian Pac J Trop Med* 2010; **3**(2): 150-151.

[39]Mohamed Saleem TK, Azeem A K, Dilip C, Sankar C, Prasanth NV, Duraisami R. Anti-inflammatory activity of the leaf extacts of Gendarussa vulgaris Nees.*Asian Pac J Trop Med* 2011; 147-149.