EFFECT OF CASSIA AURICULATA EXTRACT ON NOCICEPTION , EXPERIMENTAL DIABETES AND HYPERLIPIDEMIA IN MICE AND RATS

Mathew George*, Lincy Joseph and Ramaswamy.
Nandha College of Pharmacy, Erode, India.

Correspondence: **Mathew George**
Nandha College of Pharmacy, Erode, India.
E-mail- mathewlincg@yahoo.com

Objective: Find out the effect of *Cassia auriculata* extract on nociception , experimental diabetes, and hyperlipidemia in mice and rats.

Methods: Aqueous extract of *Cassia auriculata* (CAEt) were Administered orally and different doses of the extract on "nociception in mice, blood glucose, glucose-6-
phosphatase , serum and tissue lipids in rats with alloxan induced diabetes" were studied. Diclofenac and Chlopropamide were used as standard reference drugs respectively. Aqueous extract of *Cassia auriculata* (CAEt) at doses of 150,300,500, mg/kg body weight.

Results:
Anti-nociceptive:Results indicate (doses of 150, 300,500 , mg/kg)that aqueous extract of *Cassia auriculata* possessed anti-nociceptive activity in mice.

Anti-diabetic& Anti-hyperlipidemic: Results indicated (doses of 150, 300,500 mg/kg for 30 days)suppressed the elevated glucose and,lipid levels in diabetic rats . *Cassiaauriculata.* 500mg/kg was found comparable to standard reference drugs.

Conclusion: Our findings shows that extract possesed anti-nociceptive, anti-diabetic and anti-hyperlipidemic activities.

Key words: Anti-nociceptive, Anti-diabetic, Anti-hyperlipidemic *Cassia auriculata* extract, Alloxan.

INTRODUCTION:
Renewed intrest on biological activities of medicinal plants emerged in early 1980's as the Council of Scientific and Industrial research have published the information on the screening of biological activities of many medicinal plants using experimental models[1]. Recently the use of herbal preparations in remedies for various medical conditions have been rapidly increasing especially in India. It is believed that herbal preparations are safe although the ingredients have never been vigorously substantiated. *Cassia auriculata* (family-Caselpenaceae) is used as anti-bacterial, anti-microbial, wound healing ,anti-oxidant and analgesic drug .
There is a growing intrest in co-relating phytochemical constituents of plants with its pharmacological activities. Scientists have started co-relating the botanical properties of plants with their pharmacological activities[2].

Diabetes mellitus is characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism[3]. The liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and severely affected during diabetes[4]. Liver participates in the uptake, oxidation , and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. During diabetes a profound alteration in the concentration and composition of lipids occurs[5]. Many traditional plant treatments for diabetes mellitus are used through out the world. A few of traditional plants for

treatment for nocicepton, and diabetes have received scientific scrutiny, and the World Health Organization has recommended that this area warrents attention[6].

Acute pain is generally welll accounted for in terms of nociception and excessive noxious stimulus giving rise to an intense and unpleasant sensation . Drugs which are used against nociception are called anti-nociceptive drugs., like opioid analgesic[7].

MATERIALS AND METHODS:

Animals(For anti-diabetic and anti-hyperlipidemic activites): All the experiments were carried out using male Wistar rats aged 8-9 weeks(180-200g body weight). The animals were housed in polypropylene cages and provided with water and standard pellets diet *ad libitum* . The animals used in the present study were approved by the Ethical Committee.

Animals (For antinociceptive activity):
Adult male ICR Balb/c mice (20-25 g) were used for the antinociceptive experiment. The animals were maintained under standard laboratory conditions (light period of 12h/day and temperature 27 °C±2°C),with access to food and water *ad* libitum. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations The experiment was performed in the morning according to the guidelines for the care of laboratory animals.

Chemicals:Alloxanmonohydrate,Chlorpropamide,Diclofenacsodium Naloxone were obtained from Himedia Laboratories , S.G Pharmaceuticals Ltd., Pfizer India Ltd.Mumbai, respectively. All other reagents used were analytical grade.

Plant material: *Cassia auriclata* plant was collected freshly from in and around Erode district of Tamilnadu, India.Leaves and flowers of plant
dried under shade, made into coarse powder by grinding.
Plant was identified and authenticated at the herbarium Tamilnadu Agricultural University, Coimbatore.

Preparation of plant extract:

Aqueous Extract:
To 20 g of each dried plant powder form , 500 ml water were added and contents of flask were mixed thoroughly by gentle shaking . Flasks were kept for four days with frequent shaking . After the completion of maceration process the filtrates were obtained and water evaporated to get the dried extract. (evaporation by keeping flasks in electric mantle at 80 ^{0} C).The residual extract was dissolved in water and used in the studies[8].

Induction of experimental diabetes:
To induce diabetes in rats alloxan monohydrate(150 mg/Kg)was administered intraperitoneally to a group of rats . The alloxan was freshly prepared as 5 % weight/ volume solution in distilled water. After two days , blood samples were taken from these animals after two hours of oral dosing of CA extract ,chlorpropamide(400mg/kg) and equivalent amount of 2% weight/volume aqueous accacia solution as (control).Blood sugar levels of the animals are determined by the O-toluidine method of Fings et al (1970).
Rats with moderate diabetes having glycosuria and hyperglycemia(that is with a blood glucose of 200-300mg /dl) were used for the experiments[9].

Induction of experimental pain for anti-nociceptive study:
Hot plate Method: Hot plate (model 7280, Ugo Basile, Italy) was maintained at 55.0 °C ±0.2° C and the animals were placed into the perspax cylinder on the heated surface and the time (sec) to discomfort reaction (licking paws or jumping) was recorded as response latency.

Prior to and after aministration of extract (aqueous) at doses of 150,300 and 500 mg/ kg orally. A latency period of 20 seconds was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury.

GROUPS AND TREATMENT:

Hot plate Method: The number of animals(mice) six in each group(n=6 in each

group). Hot plate (model 7280, Ugo Basile, Italy) was maintained at 55.0±0.2° C and the animals were placed into the perspax cylinder on the heated surface and the time (sec) to discomfort reaction (licking paws or jumping) was recorded as response latency.

Prior to and after aministration of extract (aqueous) at doses of 150,300 and 500 mg/ kg orally. A latency period of 20 seconds was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury.Diclofenac sodium was used as standard reference drug(dose 0.075 mg/kg body weight).

Experimentalprocedurefor Anti-diabetic&Anti-hyperlipidemic activites:

In the experiment , a total of 36 rats (30 diabetic surviving rats and six normal rats) were used . The rats were divided into six groups of six animals in each group.

Group 1: Normal untreated rats

Group 2: Diabetic control rats given 1 ml of aqueous solution daily using an intra gastric tube for 30 days.

Group 3: Diabetic rats given CAEt(150 mg/ Kg body weight) in 1 ml of aqueous solution daily using an intra gastric tube for 30 days

Group 4: Diabetic rats given CAEt(300 mg/ Kg body weight) in 1 ml of aqueous solution daily using an intra gastric tube for 30 days

Group 5: Diabetic rats given CAEt(500 mg/ Kg body weight) in 1 ml of aqueous solution daily using an intra gastric tube for 30 days.

Group 6: Diabetic rats given Chlorpropamide(400 mg/ kg body weight) in 1 ml of aqueous solution daily using an intra gastric tube for 30 days.

At the end of 30 days , the animals were deprived of food overnight and sacrificed by the decapitation. Blood samples were collected in two different tubes (ie.,) one with anti-coagulant - potassium oxalate and sodium fluoride for plasma and another without anti-coagulant for serum preparation. Plasma and serum were separated by centrifugation. Liver was immediately dissected out, washed in ice cold saline and patted ,dry and weighed.

Analytical procedure:

Fasting blood glucose was estimated by O-toludine method.(Sasaki et al)[10].Plasma insulin level was assayed by enzyme linked immunosorbent assay(ELISA) kit, using human insulin as standard. Haemoglobin was estimated by the method of Drabkin and Austin[11] and glycosylated haemoglobin by the method of Sudhakar Naik and Pattabiraman[12]. Lipids were extracted from serum and tissue by the method of Folch et al[13]. Total cholesterol and triglycerides were estimated by the method of Zlatkis et al[14] and Foster and Dunn[15] respectively. Free fatty acids and phospholipids were analysed by the method of Falholt et al[15] and Zilversmit et al[16]. Hexokinase and glucose-6 phosphatase were assayed by the method of Brandstrup et al[17] and Koida and Oda[18].

Statistical analysis for anti-nociceptive activity:

Numerical result are expresses as mean ± SD, unless otherwise stated . one way analysis of variance(ANOVA) was used for statistical comparison; $P<0.05$ being the criterion for statistical significance. The significant treatment means were further subjected to Duncan multiple post test[20].

Statistical analysis for anti-diabetic activity:

All values were expressed as the mean obtained from number of experiments (n). Data from all the tables of normal animals, diabetic control animals , reference drug treated and CAEt treated animals were compared by ANOVA followed by Duncans multiples range test(DMRT)[19].

Results:

1.Anti-nociceptive activity

The table 5 shows the time course of the anti-nociception producedby the CA Et (150,300,500,mg/kg,positive control like

diclofenac sodium). CA Et 500mg/kg and Naloxone(Opioid antagonist) were decreased the antinociceptive activity of CA Et. Intraperitoneal administration of the extract resulted in significant and dose -dependent prolongation of the response latency in the hot plate test. The effect reached a peak at approximately at 60 minutes after administration and then gradually decreased.

2.Blood glucose and plasma insulin

Table 1 shows levels of blood glucose, plasma insulin, total haemoglobin, glycosylated haemoglobin, changes in body weight and urine sugar of normal and experimental rats. There was a significant elevation in blood glucose and glycosylated haemoglobin levels, while the plasma insulin and total haemoglobin levels decreased significantly in alloxan induced diabetic rats when compared with normal rats.

Administration of CA Et and Chlorpropamide tends to bring parameters significantly towards the normal. The effect of CAEt at a dose of 500mg/kg bodyweight was more highly significant than 150 mg, & 300 mg mg/kg body weight and therefore the dose was used for further biochemical studies.

In diabetic rats, the urine sugar was (+++) but in the case of CAEt treated rats at a dose of 150 mg and 300 mg/kg body weight showed decreased urine sugar(++) and (+) respectively. CAEt at a dose of 500mg /kg body weight , showed urine sugar as seen in normal rats. These effects were compared with Chlorpropamide.

3.Serum & Tissue Lipids

The effect of CAEt on serum and tissue lipids of normal and experimental rats are summerised in tables 2 and 3 respectively. A marked increase in the frequency of cholesterol ,free fatty acids , triglycerides, and phospholipids were observed in diabetic control rats. Treatment with CAEt significantly reduced the lipid levels.

4.HepaticHexokinase & Glucose -6-phosphatase

The activities of carbohydrate enzymes are represented in table 4. Activity of hexokinase in liver decreased markedly while the glucose-6-phosphatase activity increased significantly in diabetic conrol rats. Treatment with CAEt in diabetic rats increased the hexokinase activity and decreased the glucose-6-phosphatase ativity.

Table1

Blood glucose , plasma insulin, total haemoglobin glycosylated haemoglobin, changes in body weight and urine sugar of normal and experimental animals.

Groups	Body wt.g Initial	Final	Fasting blood glucose mg/dl	Plasma insulin (iu/ml)	Haemoglobi ng/dl	Glycosylated Hb.(mg/g Hb	Urine sugar[A]
Normal	197±11.40	207± 8.80	98.50±9.04[a]	15.04±0.1[a]	13.85±1.72[a]	0.22±0.01[a]	Nil
Diabetic control	202±16.70	152± 14.66*	233±16.40[b]	5.35±1.95[b]	5.60±0.45[b]	1.81±1.07[b]	+++
Diabetic+ CA Et (150mg/kg)	193±17.70	199± 16.33*	216±20.80[b]	5.90±1.41[b]	7.91±1.61[c]	0.68±0.03[c]	++
Diabetic+ CA Et (300mg/kg)	199±18.30	209± 11.32*	159±15.20[c]	8.05±1.64[c]	9.54±0.93[d]	0.48±0.04[d]	+

Diabetic+ CA Et (500mg/kg)	203±20.68	215± 13.72*	114.3±11.30 [d]	14.16±0.67[d]	12.5±1.91[e]	0.37±.0 .04[e]	Nil
Diabetic+ Chlorpro pamide 400 mg//kg)	194±11.80	207± 13.43*	125.6±11.32 [d]	12.70±0.65[e]	11.36±2.01[d]	0.47±0.04[d]	Trace

Values are given as mean ±SD for six rats in each group.
Duncan procedure, Range for the level 2.89, 3.03,3.13,3.20,3. 25.
Diabetic control was compared with normal , *p<0.001
Experimental groups were compared with diabetic control *p<0.001.
Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).
A- indicates 0.25 % sugar and (+++) indicates >1% sugar

Table2
Changes in level of cholesterol, free fatty acids, triglycerides and phospholipids in serum of normal and experimental animals.

Groups	Cholesterol (mg/100ml)	Free fatty acids (mg/100ml)	Triglycerides (mg/100ml)	Phospholipids (mg/100ml)
Normal	74.00 ±1.49[a]	70.43±5.06[a]	44.53±3.36[a]	80.25±1.57[a]
Diabetic control	99.66±5.03[b]	83.86±6.67[b]	63.83±2.50[b]	98.75±4.28[b]
Diabetic+CAEt 500mg/kg	84.46±3.18[c]	75.06±1.55[c]	54.93±3.70[c]	84.50±1.86[c]
Diabetic+Chlorpropamide. 400 mg/kg	91.26±2.37[d]	78.51±0.87[d]	59.46±2.70[d]	89.00±1.12[d]

Values are given as mean ±S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).
Duncan procedure , Range for the level 2.95, 3.09,3.20.

Table3
Changes in levels of cholesterol, free fatty acids, triglycerides and phospholipids in liver of normal and experimental animals.

Groups	Cholesterol (mg/100g wet tissue)	Free fatty acids (mg/100g wet tissue)	Triglycerides (mg/100g wet tissue)	Phospholipids (mg/100g wet tissue)
Normal	329.04 ±2.88[a]	608.70± 31.68[a]	348.88±14. 07[a]	2.66± 0.11[a]
Diabetic control	513.70± 6.88[b]	914.22± 49.27[b]	622.35±9.4 0[b]	2.54± 0.08[b]
Diabetic+CAEt (500mg/kg)	421.14± 5.40[c]	775.09± 47.86[c]	441.98±12. 05[c]	2.02± 0.05[c]

Diabetic+Chlorpropamide. (400 mg/kg)	442.98± 6.36 [d]	807.67± 26.30 [c]	530.19±11.7 [d]	2.29± 0.10 [c]

Values are given as mean ±S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).
Duncan procedure , range for the level 2.95, 3.09,3.20.

Table4

Changes in activities of hexokinase and glucose-6-phosphatase in liver of normal and experimental animals.

Groups	Hexokinase(units[A]/g protein)	Glucose-6- phosphatase (units[B]/gm protein)
Normal	146.66±6.09 [a]	0.169±0.014 [a]
Diabetic control	106.48±4.74 [b]	0.242±0.023 [b]
Diabetic+CA Et500mg/kg	129.70±10.44 [c]	0.187±0.012 [ac]
Diabetic+Chlorpropamide 400mg/kg	124.20±6.40 [c]	0.200±0.008 [c]

Values are given as mean ±S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05(DMRT).
Duncan procedure ,Range for the level 2.95, 3.09,3.20.
A-μ moles of glucose phosphosylated/ min.
B-μ moles of pi liberated /min.

Table5

Effect of *CA Et* on hot plate reaction time in mice

Group (mg/kg)	Time(minutes) Pre-	30	60	120	150	One-wayANOVA df	F	P
Control -saline	5.61±0.39[ax]	5.46±0.54 [ax]	5.46±.53[ax]	6.20±0.61[a x]	5.32±0.68 [ax]	4,25	2.37	ns
CA Et 150	5.61±0.37 [ax]	6.04±0.37 [ax]	5.94±0.12 [ax]	6.06±0.42 [ax]	6.27±0.42 [ax]	4,25	2.08	ns
CAEt 300	6.16±0.66 [ax]	6.84±0.34 [bax]	7.53±0.58 [by]	7.6±0.26 [bx]	7.32±0.69 [by]	4,25	5.606	<0.05
CAEt 500	5.74±0.19 [ax]	8.82±0.26[cy]	9.40±0.72 [by]	8.6±0.30 [cy]	8.86±0.62[by]	4,25	19.82	<0.05
CAEt 500+Naloxone	5.22±0.16 [ax]	8.22±0.22[dy]	8.8±0.68 [cy]	8.4±0.28[dy]	8.64±0.58[cy]	4,25	3.62	<0.05
Diclofenac	12.4±0.69 [ax]	16.4±0.48 [cy]	18.2±0.80[by]	15.2±0.24 [cy]	13.4±0.48[by]	4,25	57.20	<0.05
ANOVA F	1.535	51.510	35.458	45.728	21.86			
df	5,28	5,28	5,28	5,28	5.28			
p	Ns	<0.05	<0.05	<0.05	<0.05			

CAEt= *Cassia auriculata extract*
Values are expressed in = Mean ±SD(sec).,n=6 in each group
Ad Sign ificantly different at p<0.05, in the same column.
Xy Significantly different at p<0.05, in the same column

DISCUSSION:

Our findings show that CA Et possesed antinociceptive, anti-diabetic, and anti-hyperlipedimic activities. The phyto compounds may be present in the crude extract of CA Et ,that may account for the anti-nociceptive activity. Administration of CA Et showed significant anti nociceptive activity in the hot plate. This results indicate that the plant extract possesses centrally and peripherally mediated antinociceptive properties[21].

The hot plate method is one of the most common test for evaluating the analgesic efficacy of drugs/compounds in rodents[22]. However,care must be taken for drugs or compounds that produce false positive results by modifying the behavior of the rodents[23] . The inhibition of antinociception by Naloxone (an opioid antagonist). So the hot plate results were positive and mechanism of CA Et antinociception might involve opioid receptors. The potency of antinociception was less than Diclofenac sodium at similar doses. The results from the present study strongly indicate that the aqueous extract of CA possesses antinociceptive activity.

Alloxan is well known for its selective pancreatic islets ßcells cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interfere with cellular metabolic oxidative mechanisms[24]. Intraperitoneal administration of alloxan 150 mg/kg effectively induced diabetes in normal rats as reflected by glycosuria, hyperglycemia, polyphagia. polydipsia and body weight loss when compared with normal rats[25]. In our present study we have observed that an aqueous extract of CA can reverse these effects . The possible mechanism by which CA Et brings about its antihyperglycemic action may be by potentiation of pancreatic secretion of insulin from B-cells of islets or due to enhanced transport of blood glucose to peripheral tissue. This was clearly evidenced by the increased level of insulin in diabetic rats treated with CA Et.

In the case of anti-diabetic activity, anti-hyperlipidemic activity, level of blood glucose, plasma insulin , total haemoglobin, glycosylated haemoglobin , changes in body weight and urine sugar of normal and experimental rats. There was a significant elevation in blood glucose and glycosylated haemoglobin level , while the plasma insulin and total haemoglobin level decrease significantly in alloxan induced diabetic rats when compared with normal rats. Administration of CA Et and Chlorpropamide tends to bring parameters significantly towards the normal. The effect of CAEt at dose of 500mg/kg bodyweight was more highly significant than 150 and 300 mg mg/kg body weight and therefore the dose was used for further biochemical studies . In diabetic rats , the urine sugar was (+++) but in the case of CAEt treated rat at a dose of 150 and 300 mgkg body weight showed decrease in urine sugar(++) and (+) respectively. CAEt at a dose of 500mg /kg body weight , showed urine sugar as seen in normal rats. These effects were comparable with Chlorpropamide.

Activity of hexokinase in liver decreased markedly while the glucose-6-phosphatase activity increased significantly in diabetese conrol rats[26]. Treatment with CAEt in diabetic rats increase the hexokinase activity and decreased the glucose-6-phosphatase activity.

We have observed a decrease in total haemoglobin during diabetes and this may be due to the formation of glycosylated haemoglobin. Increase in the level of haemoglobin in the animals given CA Et may be due to decreased level of blood glucose and glycosylated haemoglobin.

Anti hyperlipidemic effect of CA Et may be due to the down regulation of NADPH and NADH , a cofactor in the fat metabolism. Higher activity of glucose-6-phosphatase provides H+ which binds with NADP + in the form of NADPH and is helpful in the synthesis of fats from carbohydrates. When glycolysis slows down because of cellular activity, the pentose phosphate pathway still remain active in liver to break down glucose that continuously provides NADPH which converts acetyl radicals into long fatty

acid chains. Metabolic aberration in alloxan induced diabetic rats suggests a high turn over of triglycerides and phospholipids[27]. CA Et may antagonize metabolic aberration and there by restore normal metabolism by tilting the balance from high lipids to high carbohydrates turn over.

CAEt may be capable of oxidizing NADPH. Enhanced hexokinase activity in CA Et treated rats suggests greater uptake of glucose from blood by the liver cells .Activities of enzyme suggests that enhanced lipid metabolism during diabetes is shifted towards carbohydrate metabolism and it enhance the utilization of glucose at peripheral site. One of the possible actions of CA Et may be due to its inhibition of endogenous synthesis of lipids

It can be concluded from the data that CA Et significantly reduces the level of nociception , blood sugar, serum and tissue lipids .CA Et have beneficial effects on plasma insulin and hexokinase activity. Moreover its anti-hyperlipidemic effects could represent a protective mechanism against development of atherosclerosis.

Of course the information from the present study will be a golden coin in the wealth of pharmacological field

REFERENCE:

1. Somchic.M.R,.Sulaiman M.R,.Zuraine A, Antinociceptive and anti-inflammatory effect of *Centella asiatica*, Indian J Pharmacol/ Dec2004/vol36/issue6/377-80.

2. Cheng CL, KOO MWL Effects of *Cassia auriculata* on ethanol induced gastric mucosal lesions in rats, Life Sci2000,67:2647-53.

3. Sulaiman M.R,. Samsudin. L, Indian Journal of pharmacology/December 2004/Vol:37 Issue-7/277-80.

4. Baquer NZ.Glucose over utilization and under utilization in diabetes and effects of antidiabetic compounds . Ann Real Acad Farm 1998;64:147-80.

5. Swanston Flatt SK, Day C,Bailey CJ,Flatt RR. Traditional plant remedies for diabetes. Studies in the normal and streptozotocin diabetic mice.Diabetologia 1990;33:462-4.

6. WHO Expert Committee on diabetes mellitus second report. Technical Reports Series 646. World Health Organisation. Geneva 1980;61.

7. Joshi SG. Cesalpinaceae-*Cassia auriculata*. Text book of medicinal plants. 2000;119.

8 .Pari L, Latha M. Antidiabetic effect of *Cassia auriculata* flowers: Effect on Lipid Peroxidation in Streptozocin diabetic rats. Pharmaceutical Biology 2002.

9 Siddiq O, Sun Y, Lin JC,Chum YW,Facilated transdermal transport of insulin ,J Pharma Sci 1987;76:341-5.

10. Sasaki T, Matzy S,Sonal A, Effect of acetic acid concentration on the colour reaction in the O-toludine boric acid method for blood glucose estimation, Rinsho Kagaku 1972:1;346-53.

11. Drabkin DL, Austin JM ,Spectrophotometric constants for common haemoglobin derivatives in human, dog and rabit blood. J Biol chem. 1992,98: 719-33.

12. Sudhakar Naik S, Pattabiraman TN. A new colorimetric method for the estimation of glycosylated haemoglobin, Clin Chem Acta 1981; 267-74.

13. Folch J,Less M,Solane SGH. A simple method for isolation and purification of total lipids from animal tissues,J Biol Chem 1957;26:497-509.

14. Zlatkis A ,Z ak B and Bogle GJ. A method for determination of serum cholesterol, J Clin Med 1953;41:486-92.

15. Falholt K, Falholt W. An essay

colorimetric method for routine determination of free fatty acids in plasma, Chem Acta 1973;46:105-11.

16. Zilversmit DB Davis AK,Microdetermination of phospholipids by TCA precipitation, J Lab Clin Med 1950;35:155-61.

17 .Brandstrup N, Kirk JE ,Determination of hexokinase in tissue,J Gerontol 1957;12:166-71.

18. Koida H Oda T,Pathological occurance of glucose-6-phosphatase in liver disease, C lin Chem Acta 1959; 4:554-61.

19. Benet P,Franklin NH, Statistical analysis in chemistry and chemical industry,Newyork:John wiley and son ,USA.208-27.

20. Hosseinzadeh H, Ramezani M, Salamani G. Antinociceptive, anti-inflammatory and acute toxicity effects of Zataria multiflora Boiss extracts in mice and rats. H Ethnopharmacol 2000;73:379-85.

21. Zakaria MNM ,Islam MW, Radhakrishhnan R, Chen HB, Kamil M, Al-gifri AN ,et al.Antinociceptive and anti-inflammatory properties of Caralluma Arabica. J Ethnophamacol 2001;76:155-8.

22. Carter RB, Differentiating analgesic and non-analgesic drug activities on rat hot plate: Effect of behavioral end point. Pain 1991;47:211-20.

23. Tjolsen A, Rosland JH, berge OG, Hole K. The increasing- temperature hotplate test: An improved test of nociception in mice and rats. J pharmacol Methods 1991; 25:241-50.

24 .Papaccio G, Pisanti fa, Latronico MV, Ammendola E, Galdieri M.Multiple low dose and single high dose treatments with streptozotocin do not generate nitric oxide. J Cell Biochem 2000;77(1):82- 91

25 .Calaberesi P, Chabner BA. Antineoplastic agent, In Goodman A, Rall JW (Eds). The pharmacological basis of therapeutics.pergmann press, New YORK. 1209-63.

26. Bopanna KN, Kannan J,Sushma G, Balaraman R ,Rathod SP.Antidiabetic and antihyperlipaemic effects of Neem seed kernel powder on alloxan diabetic rabbits, Indian J Pharmacol 1997;29:162-7.

27 Goodman LS, Gillman A, The pharmacological basis of therapeutics, 7th edition, MacMillion, NewYork,1985;1490-510.