ISSN 0975-6299 Vol 2/Issue 1/ Jan-Mar 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

PHARMACOGNOSY

PHARMACOLOGICAL EVALUATION OF *CASSIA AURICULATA* BARK EXTRACT

Corresponding Author

MAHENDRA SHIRADKAR

Novel Drug Discovery & Developement, Lupin Research Park, Hinjewadi, Pune-411057

Co Authors

G. PAWANKUMAR[2] AND KAPIL SHAH[3]

[2]Nethaji Institute of Pharmaceutical Sciences, Toopranpet, Choutuppal, Nalgonda-508252
[3]Gangamai College of Pharmacy, Nagao, Dhule-424005

ABSTRACT

This project was designed with the objective of investigating glucose lowering potential, anti-mutagenic activity and anti-fertility activity of *Cassia auriculata* bark extract in the diabetic animals. The albino rats were treated with methanolic extract. The extracts were found to posses promising anti-diabetic, antimutagenic and anti-fertility activities..

KEYWORDS

Anti-diabetic, antimutagenic, antifertility, Cassia Auriculata

INTRODUCTION

Diabetes is a disorder of β-cells of langerhans that can be diagnosed by its symptoms like weight loss, excessive hunger, thirst and urination. It is a fifth leading cause of deaths as well the leading cause of adult blindness, responsible for 50% of heart attacks, 70% of strokes, and 85% of gangrenous leg amputations[1]. It is therefore reckoned with high level of research interest continuing on diabetes and the side effects of the currently available antidiabetic drugs[2]. Therefore, development of newer and more powerful herbal antidiabetic drugs with minimum side effects is essential.

Untoward mutations are associated with a no. of serious diseases like cancer, aging, arthritis, cardiovascular diseases and infectious diseases[3]. Many of the pollutants, residue from the chemical, fertilizer and pharma industries and the toxins from the fertilizers present in the food, UV rays due to decrease in the ozone layer are common agents of mutagenic damages in human population. Prevention of genetic mutation through the use of food and food additives having anti-mutagenic properties is an ideal mean for preventing mutagenic damages[4]. Various plants have been tested for their antimutagenic property however; still there is a need for more effective and useful antimutagenic phytochemicals.

'Population Explosion' was the term used as a synonym for the rapid population growth in developing countries like India. It has affected our economic growth and overall development severely. The control of human fertility is the most important and urgent of all biosocial and medicinal problems confronting mankind today[5]. One approach is being pursued to identify new antifertility agents in the search for their presence in natural sources. Many plant preparations are reported for their anti-fertility activity in the ancient Indian literature.

Cassia auriculata Linn (Family: Caesalpiniaceae) commonly known as *Tanners Senna*, is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine. The plant is used in the traditional system of medicine for urinary disorders, female antifertility, leprosy, worm infestation, diarrhoea, disease of pittam; bark is used in skin conditions; bark as astringent; leaves, flowers and fruits as anthelmintic; seeds for eye troubles, diabetes[6,7,8].

In continuation with our ongoing research with said plant, it was thought worthwhile to evaluate the said plant for its pharmacological activities.

MATERIALS AND METHODS

Plant material

Cassia auriculata bark (authenticated at Natural Remedies, Bangalore, India by Dr. H. N. Shivaprasad) was collected from Madikeri, Coorg District, Karnataka, India. The bark was dried, powdered and passed through 40-mesh sieve and stored in an airtight container for the further use.

Preparation of the extract

The powdered bark material was extracted using 90% methanol as a solvent in a soxhlet extraction apparatus. The solvent was completely removed by using rotary flash evaporator to get semisolid mass [10.2 % w/w][9]. This methanol extract was stored in a desiccator and weighed quantity was dissolved in distilled water and given to the animals. The

methanolic extract of the bark of *Cassia auriculata* was named as MCA.

Experimental animals

Albino rats (Rattus norvegicus) of Sprague Dawley strain were used for the experiment. They were housed in polypropylene cages under controlled temperature conditions (25 ± 2 °C) with 12:12 hrs light and dark cycle. They were fed with standard pelleted diet and water ad libitum. The study was duly approved by IAEC.

Acute toxicity study

Acute toxicity study was performed in rats divided into different groups of 6 each. After an overnight fast, the test drug was administered orally in graded doses [100-500 mg/kg]. They were observed continuously for the first 2 h for toxic symptoms and up to 24 h for mortality[10].

A. ANTI-DIABETIC ACTIVITY:

Diabetes Induction

Diabetes was induced in rats fasted for 24 hours by interperitoneal injection of Streptozotocin (Sigma) freshly dissolved in citrate buffer (pH 4.5) immediately before use. Streptozotocin was given at a dose of 65 mg/Kg body Weight[11]. The Streptozotocin treated animals were given 5% glucose solution for 24 hours to prevented Streptozotocin induced hypoglycemic mortality[12]. The diabetic state of the animals was assessed by measuring blood glucose concentrations 72 hrs after Streptozotocin treatment in fasting condition. The rats with a blood sugar level above 300 mg/dl as well as polydipsia, polyuria and polyphagia were selected for the experiment.

Determination of effective dose of plant extract

A preliminary experiment was undertaken to determine the effective dose of *Cassia auriculata* bark extract. The LD50 test was used for this purpose. Then the diabetic rats were selected and fed with different doses of plant extract (100, 250, 500 and 1000 mg/kg body weight) through oral intubations. Blood samples, collected at 0 hrs, 3 hrs, 6 hrs and 9 hrs, were assessed for blood glucose levels. The results at 3 hrs, 6 hrs, and 9 hrs were compared with that at 0 hrs and with control groups (without drug treatment) and the effective dose of the extract were determined.

Determination of antidiabetic potential

Eighteen Streptozotocin induced diabetic animals were divided into 3 groups containing 6 animals in each group.

Group I: Healthy control (received saline water),

Group II: Diabetic control (received saline water), and

Group III: *Cassia auriculata* bark extract treated animals .

The bark extract was prepared for oral intubations by dissolving it in normal saline. The extract was fed at an effective dose of 500 mg/kg body weight. The daily treatment to each group was carried out in the morning hrs in fasting condition for 30 days.

Collection of blood and liver tissue

Blood samples were collected from the tail vein at 0 hrs (1st day), 7th day, 15th day and 30th day and the glucose levels were estimated. Blood samples were collected 3 hrs after the administration of morning dose. The blood samples were allowed to clot and serum was separated by centrifugation. The animals were sacrificed after 30 days using light ether anaesthesia and liver was removed. The serum and tissue sample were kept at –20 °C until assayed for biochemical parameters. Changes in body weight of animals were monitored daily.

Biochemical analysis

The serum of all the animals was subjected to biochemical analysis. Glucose determinations were made with a One Touch profile (Lifescan Inc. Milipitas, California,

U.S.A.). The results were validated by o-toluidine method (Glucose Kit, Siddham Diagnostic, India). The determination of serum cholesterol[13] and serum triglycerides[14] and liver glycogen[15] was carried out by routine techniques.

Statistical analysis

The data obtained were statistically analysized using the student's t-test.

B. ANTIMUTAGENIC ACTIVITY:

Microorganism

Euglena gracilis (strain Z) was obtained from S.H. Hutner, Haskins Laboratory, Pace University, New York, NY, USA and maintained on Cramer-Myers (CM) medium[16] under static conditions at 27 °C and with permanent lighting (16.4 W/m^2).

Chemicals

Acridine orange [CAS 65-61-2] was purchased from Merck, Darmstadt, Germany. Stock solutions of AO, was prepared by dissolving them in distilled water.

Mutagenicity assay[17,18,19]

E. gracilis cells diluted to concentration 8 × 10^5 cells/ml CM medium were used in the experiments. Aliquots (0.2 ml) of the cell suspension were dispensed into test tubes, along with 2.3, 11.4 or 22.8 µM AO, indicated concentrations of methanolic extract of *Cassia auriculata* barks (5.5, 11.0, 110, 220, 330 µg/ml), at last the content of the incubation mixture was completed to a final volume (5 ml) by the addition of CM medium. Following a 24-h co-treatment, the cells were centrifuged at 3000 rpm for 20 min, the resultant pellet suspended in fresh CM medium and again centrifuged. After the pellet was re-suspended in fresh CM medium, the cells were finally cultivated 14 days at 27 °C under permanent illumination (16.4 W/m^2). The experiments were repeated in three independent series. Just before the counting, the movement of *E. gracilis* was stopped by adding a drop of EtOH and the counting of green and white (mutant) colonies was carried out in a Bruker chamber under a microscope. The viability of the Euglena cells was estimated by counting the total white mutants in the presence of mutagen (positive control) compared to the number of the spontaneous white mutants in the absence of mutagen (negative control). We defined the relative decrease of the bleaching as the anti-mutagenic potency (AP) which was calculated by the formula,

$$AP\ (\%) = \frac{Bo - Br}{Bo} \times 100$$

Where Bo is the AO-induced E. gracilis bleaching (%) and Br is the AO-induced and antimutagen-reduced E. gracilis bleaching (%). The statistical significance of all the calculated values were determined by paired Student's t-test. The values represent the means ± standard deviation (SD).

C. ANTIFERTILITY ACTIVITY

It was evaluated by determining the anti-implantation and early abortifacient activity of methanolic extracts[20]. The experimental protocols have been approved by the Institutional Animal Ethics Committee. Rats were divided into 3 groups of 6 animals each and they were paired with males in a ratio of 2:1. First groups received vehicle (1% Tween 80) and was considered as control, methanol extract was administered orally (by an oral catheter) at two different doses of 100 and 200 mg/kg to second and third groups, respectively. The treatment of the drug was such that the animals were allowed for mating, after continuous administration of the drug for seven days and during which period the animals were all in estrous stage. Mating was allowed by

placing the treated female rats with untreated male rats in its cages. In the following day the mating was confirmed by the presence of sperm in the vaginal smear or a sperm plug in the vaginal opening.

Female rats that gave a result of sperm positive were further treated with the same dose levels of the extract for another five days. Pregnancy starts from the day of mating and the next day was taken as Day-1. Pregnancy is also confirmed by continuous diestrous stage of the female rats. The pregnant rats were segregated and housed in separate cages during gestation. A weekly examination was conducted for observing the weight variations. On day ten of pregnancy the rats were dissected under light anesthesia to observe the number of implantations, number of corpora lutea in the overy and number of resorptions if any. Again the rats were examined on day 20 to record the number of implantation sites, a normal and degenerated fetus including gross abnormalities.

Statistical analysis

The early abortifacient and antiimplantation activities were calculated by using the following formulae[20],

$$\% \text{ Abortifacient activities} = \frac{\text{Mean number of resorptions}}{\text{Mean number of corpora lutea}} \times 100$$

$$\% \text{ Antiimplantation activity} = \frac{\text{Mean number of implantations}}{\text{Mean number of corpora lutea}} \times 100$$

RESULTS

Anti-Diabetic Activity:

A significant reduction in the body weight of the diabetic animals was observed after 72 hours of streptozotocin injection. The body weight of the animals treated with *Cassia auriculata* bark extract remained almost constant without any significant changes. (Table –1)

Table 1

Effect of Cassia auriculata bark extract treatment of diabetic rats for 30 days on body weight and blood glucose levels (Mean of 5 Values ± SEM)

Groups	Body Weight		Initial	7th Day	% Deviation	15th Day	% Deviation	30th Day	% Deviation
	Initial	Final							
Healthy Control (Group-I)	207.14 ± 6.4	198.15 ± 5.1	81 ± 2.1	87 ± 3.6	5.4*	79 ± 4.2	2.2	85.12 ± 5.2	5.8*
Diabetic Control (Group-II)	165.62 ± 11.1	161.12 ± 20.1	456 ^{c} ± 26.1	476^{c} ± 22.1	3.9*	510^{c} ± 271	11.5*	532.2^{c} ± 3.7	16.5*
Diabetic + Drug treatment (Group-III)	201 ± 5.4	190 ± 8.3	497 $^{d, h}$ ± 21.1	256$^{d, h}$ ± 16.6	43.6	70$^{d, g}$ ± 7.9	82.4	78.5^{d,g} ± 7.8	80.9

Group –II and III were compared with Group-I (P # 0.05 = a, P # 0.01= b, P # 0.001= c, Non-significant = d). Group-III was compared with Group-II (P#0.05 = e, P# 0.01= f, P # 0.001= g, Non-significant = h).

A significant increase in the blood glucose levels was observed in diabetic animals after 72 hours of streptozotocin (0 hrs or Ist day). Animals treated with the methanolic bark

extract of *Cassia auriculata* showed a significant reduction in the blood glucose levels on the 7th day with a percentage deviation of 43.6% from the blood glucose on 1st day. On 15th day the deviation was 82.4%. The 30 days administration of *Cassia auriculata* bark extract caused a lowering of blood glucose levels with a deviation of 80.9% when compared with the blood glucose level on 1st day (Table-1).

The liver glycogen level was significantly lowered (p#0.001) in the diabetic control animals (Group-II) in comparison to healthy control animals (Group-I). In animals treated with *Cassia auriculata* bark extract (Group-III), showed a significantly increased level of glycigen (p#0.001) as compared to diabetic control animals. The glycogen level was also raised significantly (p#0.001) liver of healthy control animals (Table-2).

Table-2

Effect of Cassia auriculata bark extract treatment of diabetic rats for 30 days on liver glycogen, serum cholesterol and serum triglycerides. (Mean of 5 Values ± SEM)

Groups	Liver Glycogen (mg/gm)	Serum cholesterol (mg/gm)	Serum triglyceraldehyde (mg/gm)
Healthy Control (Group-I)	6.97 ± 0.22	120.33 ± 3.63	86.22 ± 4.13
Diabetic Control (Group-II)	3.75 ^{c} ± 0.15	231.37 ^{c} ± 4.01	127.45 ^{c} ± .01
Diabetic + Drug treatment (Group-III)	7.99 $^{b, g}$ ± 0.22	117.34 $^{d, g}$ ± 4.06	73.53 $^{d, g}$ ± .96

Group –II and III were compared with Group-I (P # 0.05 = a, P # 0.01= b, P # 0.001= c, Non-significant = d). Group-III was compared with Group-II (P # 0.05 = e, P # 0.01= f, P # 0.001= g, Non-significant = h).

Anti-mutagenic activity:

The mutagenic effect of AO on E. gracilis was tested in three concentrations, 2.3, 11.4 and 22.8 µM, which induced, respectively, 43 ± 2 %, 58 ± 2 % and 65 ± 3 % of white mutant cells. The AO concentrations were chosen so as to ensure no significant change in the viability of cells compared to the negative controls (Euglena cells in the absence of the mutagen). Similarly, no spontaneous white mutants were found in any sets of positive controls.

Anti-fertility:

The methanol extract of *Cassia auriculata* barks showed significant reduction in the number of corpora lutea and increase in the number of resorptions in comparison to the control. The extract showed 69% antifertility activity on oral administration of 100 mg/kg whereas a remarkable 100% antifertility activity resulted on the administration of 200 mg/kg as compared to the untreated control group (Table 3). All the data were expressed as mean ± SD and subjected to students' "t" test for statistical significance of satisfied probability level[21].

Table 3

Data showing anti-fertility activity of methanolic bark extract of cassia auriculata

Sample	Dose	Number of Corpora Lutea	Number of implantation sites	Number of resorbed implantation	% Antiimplantation activity	% Early abortifacient activity	% Total antifertility activity
Vehicle Group I	2 ml/kg	11.33± 1.82	10.5± 2.06	NA	NA	NA	NA

Extract Group II	100 mg/ml	8.83 ± 1.47	4.66± 1.63	2.16 ± 0.08	47.1	24.5	71.6
Extract Group III	200 mg/kg	3.33 ± 1.03	0.33± 0.77	0.33± 0.77	90	10	100

DISCUSSION

Anti-diabetic Activity:

In the present investigation the body weight of diabetic rats was reduced after 72 hrs of streptozotocin injection, which is in accordance with earlier findings[22,23,24]. Thereafter, there was no significant change in the body weight of the animals suggesting that the drug has no effect on degradation of depot fat[25]. A lowering of cholesterol level was observed in diabetic rats fed with bark extract. The exact mechanism of action of herbal product is not known; therefore, further studies are needed. More studies are also required to find out whether this could probably be due to the alterations in insulin level or by increasing the insulin sensitivity by the plant extract. Work in this direction is in progress.

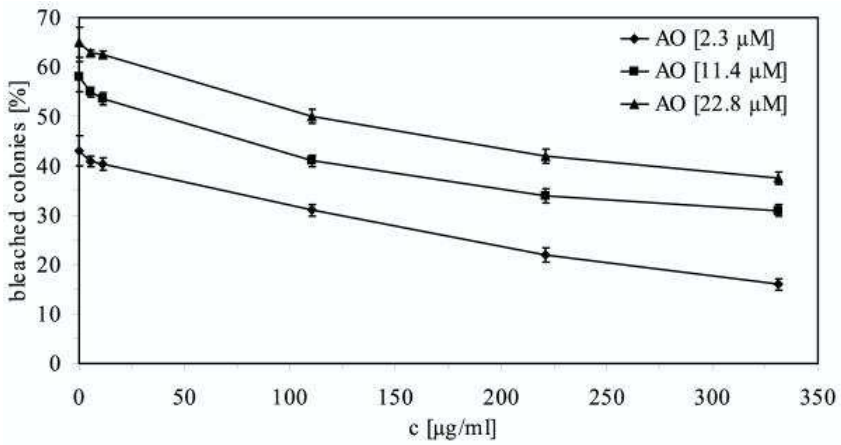
Anti-mutagenic Activity:

The dose-dependent inhibitory effect of the methanolic extract of *Cassia auriculata* barks on the mutagenicity of AO applied at the above 3 concentrations is displayed in Figs.1. As shown in Figs.1 the plot of the percentage of bleached mutants vs. concentration for the methanolic extract of *Cassia auriculata* barks display typical curving even though over different concentration ranges. The lowest concentration that causes statistically significant ($p < 0.05$) reduction in the percentage of white colonies (as compared to the positive control) is 0.7 and 11.0 µg/ml and the decrease of the percent proportion of mutant colonies gradually continued with further increasing the concentration.

Possible mechanism of their antimutagenic activity of methanolic extract of *Cassia auriculata* barks, could be based on the reports that the barks are excellent scavengers of reactive oxygen species (ROS) such as singlet oxygen and/or superoxide anion radical (as another Ayurvedic preparation "Triphala" is reported to have same mechanism of action[26] which is well documented) as ROS play a central role in multistage mutagenesis and carcinogenesis[27].

Figure 1

A plot of percentage of bleached colonies of E. gracilis vs. concentration of C. auriculata bark extract at 3 concentrations of acridine orange (AO). Symbols and brackets denote means ± standard deviation of 3 independent determinations.



CONCLUSION

The blood glucose levels in animals fed with methanolic extract of *Cassia auriculata* bark decreased significantly on 7th, 15th and 30th day, when compared with the glucose levels on 1st day. Glucose level in diabetic control animals remained almost constant without any significant changes. A decrease in blood glucose levels with a concomitant increase in liver glycogen levels of diabetic treated rats are suggestive of the fact that the antihyperglycemic activity of the extract is probably due to increased uptake of glucose for the formation of glycogen by enhanced glycogenesis. Further studies are in progress to find out the exact mechanism of action. While In summary, the result presented here showed that the methanolic extract of *Cassia auriculata* barks inhibited the AO-induced mutagenicity in the E. gracilis assay. methanolic extract of *Cassia auriculata* barks demonstrated significant antimutagenic activity against the chloroplast damaging effects of AO with a high potency. Formulation of this methanolic extract of *Cassia auriculata* barks formulation into proper delivery system is essential so as to incorporate it in one's diet, which may results in providing a measure of protection against oxidative damage from free radicals.

Based on the results of the present study it can be concluded that methanolic extract of *Cassia auriculata* has potential antifertility activity at a doses of 100 and 200 mg/kg. The activity is in dose dependent manner. Estrogen secretion by corpus lutea at early stages of pregnancy provides the nutrition for early embryo and prevents early abortion by decreasing the contractility of the uterus[28]. *Cassia auriculata* may prevent pregnancy by antiestrogenic activity

REFERENCES

1. David William and Thomas Lemke, Foye's Principles of Medicinal Chemistry, 5th Edn, Vol 2, Lippincott Williams and Wilkins publications: 629-643, (2005).
2. Purohit A and Sharma A, The effect of liver damage on the activity of g-strophanthin in the rat. J cell Tissue Culture, 6 (1): 537-540, (2006).
3. Amen B N, Identifying environmental chemicals causing mutations and cancer. Science, 204: 587-593 (1979).
4. Ferguson L R, Detection of grlA and gyrA mutations in 344 Staphylococcus aureus strains. Mut Res, 161: 193-198 (1994).
5. Parekar A S, The role of the Committee on Safety of Drugs. Brit Med Bull, 26: 01-09 (1970).
6. Bates S H, Jones R B and Bailey C J, Brit J Pharm, 130: 1944-1948 (2000).
7. Balasaraswati R, Sadasivam S, Ward M and Walker J M, An antiviral protein from Bougainvillea spectabilis roots; purification and characterisation. Phytochemistry, 47 (8): 1561-1565 (1998).
8. Bolognesi A, Polito L, Olivieri F, Valbonesi P, Barbieri L, Battell M G, Carusi M V, Benvenuto E, Del Vecchio Blanc F, Di Maro A, Parente A, Di Loreto M and Stripe F, New ribosome-inactivating proteins with polynucleotide:adenosine glycosidase and antiviral activities from Basella rubra L. and bougainvillea spectabilis Willd. Planta, 203 (4) : 422-429 (1997).
9. Theodorou N A, Vrbova H, Tyhurst M and Howell S L, Problems in the use of polycarbonate diffusion chambers for syngeneic pancreatic islet transplantation in rats. Diabetologia, 18: 313-318 (1980).
10. Litchfield J T, Wilcoxon F, A simplified method of evaluating dose-effect

experiments. J Pharmacol Ex Ther, 96: 99-135 (1949).
11. Theodorou N A, Vrbova H, Tyhurst M and Howell S L, Problems in the use of polycarbonate diffusion chambers for syngeneic pancreatic islet transplantation in rats. Diabetologia, 18 : 313-318 (1980).
12. Andallu B and Varadacharyulu N C, Control of hyperglycemia and retardation of cataract by mulberry (Morus indica L.) leaves in streptozotocin diabetic rats. Ind J Exp Bio, 40: 791-795 (2002).
13. Zlatkis A, Zak B and Boyle A J, J Lab Clin Med, 41: 486-492 (1953).
14. Gottfried S P and Rosenberg B, Improved Manual Spectrophotometric Procedure for
15. Determination of Serum Triglycerides. Clin Chem, 19 : 1077-1078 (1973).
16. Montogomery R, Determination of glycogen. Arch Biochem Biophys, 67 : 378-381 (1957).
17. Cramer M Myers, Phthalate Esters as Peroxisome Proliferator Carcinogens. J Arch Microbiol, 17 : 384-388 (1952).
18. Ebringer L, Interaction of drugs with extranuclear genetic elements and its consequences. Teratogen Carcinogen Mutagen, 10 (6) : 477-481 (1990).
19. Foltínová P Grones, Identification of ‘genotoxic' and ‘non-genotoxic' alerts for cancer in mice: the carcinogenic potency database. J Mutation Res, 393 : 1-7 (1997).
20. Križková L, Lopes MH, Polónyi J, Belicová A, Dobias J, Ebringer L, Total gene deletions and mutant frequency of the HPRT gene as indicators of radiation exposure in Chernobyl liquidators. Mutation Res, 446 : 225-229 (1999).
21. Khanna U and Chaudhary R R, Strategies for initial management of hypertension. Indian J Med Res, 56 : 1575-1586 (1968).
22. Armillage P and Berry P, Statistical Methods in Medical Research, 2nd Edn, Blackwell Scientific Publication, Oxford: 186-192. (1985).
23. Oksanen A, Testicular Lesions of Streptozotocin Diabetic Rats. Hormone Res, 6: 138-144 (1975).
24. Dixit V P, Gupta R A and Kumar S, Effects of Alloxan diabetes on testicular function of male dog Canis familaris. Ind J Expt Biol, 18: 459-462 (1980).
25. Murray F E, Orth J, Gunsalus E, Weisz J, Li J B, Jefferson L S, Musto N A and Barlme C W, The pituitary-testicular axis in the streptozotocin diabetic male rat: evidence for gonadotroph, Sertoli cell and Leydig cell dysfunction. Int J Andrology, 4: 265-280 (1981).
26. Chakrabarti S, Biswas T K, Rokeya B, Ali L, Mosihuzzaman M, Nahar N, Khan, A K A and Mukherjee B, Advanced studies on the hypoglycemic effect of Caesalpinia bonducella F. in type 1 and 2 diabetes in Long Evans rats. J Ethnopharmacol, 84: 41-46 (2003).
27. Amen B N, Structure-activity investigation of the alteration of the physical state of the skeletal network of proteins in human erythrocyte membranes induced by 9-amino-1,2,3,4-tetrahydroacridine. Free Radical Res Commun, 7: 121-126 (1989).
28. Lunec, Effect of granulocyte colony-stimulating factor on neutropenia and associated morbidity due to chemotherapy for transitional-cell carcinoma of the urothelium. J Ann Clin Biochem, 27: 173-179 (1990).
29. Guyton C G and Hall E J, Textbook of Medical Physiology, 10th Edn, Harvard publication, Singapore: 948-970, (2000).