www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Hypo glycemic and immuno-modulatory activity of *Tephrosia calophylla* root extract

Adinarayana K*, Mahesh kumar K, Dwaraka mai P, Charuseela P, Rekha J

Annamacharya College of Pharmacy, Rajampet, Y.S.R (Dist) Andhra Pradesh, India

ABSTRACT

The use of natural herbal products for the prevention and treatment of different pathologies is continuously expanding throughout the world. This is particularly true with regards to flavonoids, which represent a variety of class of secondary metabolites with potentially beneficial to human health effects. The genus *Tephrosia* is rich in certain flavonoids and contributing in greater extent for promising biological activities. In the current research potency of methanolic extract of *Tephrosia calophylla* roots was tested for hypoglycaemic and immunomodulatory activities. The prepared plant material was successively extracted with the solvents like petroleum ether, chloroform and methanol. According to the obtained yield percentage and the results of preliminary phytochemical screening, the methanol extract was selected for our current research. The results of acute toxicity study on *TCR* extract has shown that the methanol extract was safer to be use against animal models at appropriate doses as per OECD guide lines. The experimental studies of *TCR* extract on animal models (albino rats) have shown that, the *TCR* extract was an effective hypo glycaemic agent at the dose of 500mg/kg body weight in contrast to the control Dexamethasone. (Dexamethasone induced diabetes), various lipid profile test parameters were performed by treating the albino rats with Dexamethasone and two different doses of *TCR* extract .Various Blood profile tests (Hematological) parameters were performed by treating the albino rats with Dexamethasone and two different doses of *TCR* extract. The activity of the extracts increased with increasing concentrations. These substances have been described to possess various pharmacological properties such as immune stimulant, hypolipidimic and tonic, neuro-stimulant, anti-aging, anti-inflammatory activities.

Keywords: *Tephrosia calophylla* root extract; Hypo-glycaemic activity; lipid profile tests; Haematological studies

INTRODUCTION

Plants have been the eternal source of food and medicine since antiquity in all traditions and cultures. The use of plants as medicine did not remain a folk care rather it was developed in to a rational drug science even in the classical age.

The immune system plays a vital role in the defence mechanism against infectious diseases. There have been remarkable advances in the field of basic immunology during last three to four decades (Sharma P.V. Translation *et al.,* 1981). It is due to the distinction between cellular and humoral arms of immunity and recognition of cell surface phenotypes on T and B cells. The molecular mechanism of immune response mainly include, (i) immunoglobulin and T-cell receptor gene rearrangements creating diversity as well as uniqueness of the immune (ii) complexity of the MHC system and its role in the antigen presentation and restriction of effector cytotoxic cells. (iii) Signal transduction and selection of Th1 and Th2 types of cellular responses and (iv) elimination of offending agent by the effector arm of the immune response. This knowledge has helped in unravelling mysteries of various key elements of immune response (S.S. Agarwal, V.K. Singh. Immunomodulatory *et al*., 1999). Immunomodulator may be defined as a substance, biological or synthetic, which can stimulate, suppress or modulate (change) any of the components of the immune system including both innate and adaptive arms of the immune response. One of the starting points in the field of immune modulation has been the search for agents that could be used for treatment of residual cancer (Allison A.C *et al.,* 1997).

Diabetes mellitus is the most common metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism caused by insulin deficiency. It is known to Indian physician for more than 2500 years as it can be seen from the medical text such as charaka samhita and sushruta samhita. They have discussed the honey urine in detail, however the name diabetes was given by two Roman physicians Celsus and Aretaeus 1st AD in 1921. Banting and Best solved the problem of diabetes to a great extent by discovering the insulin as a therapeutic agent. Diabetes is considered as one of

* Corresponding Author
Email: adiknarayana@gmail.com
Contact: +91-9966007665
Received on: 30-11-2015
Revised on: 07-12-2015
Accepted on: 11-12-2015

the five leading causes of death in the world[14]. About 150 million are suffering from diabetes worldwide and it is almost five times more than the estimated ten years ago and this may be doubled by the year 2030 **(**Gosh R, Sharachandra KH *et al.,* 2004).

Dexamethasone is an Anti-inflammatory and Anti-allergic drug; it is a very potent and highly selective glucocorticoid, long acting. Causes marked, pituitary-adrenal suppression, but fluid retention and hypertension are not a problem. It meets any emergency with intensified potency and strikingly rapid corticosteroid action, thus ensuring speedy reversal of the situation in the patients favour. Dosage of dexamethasone usage will be preferably 4-20 mg/kg I.V. infusion or i.m. infusion or i.m.0.5-5mg/day orally. The Common Contra-Indications of dexamethasone usage are renal failure, diabetes mellitus, psychosis, osteoporosis, Pregnancy, tuberculosis and other systemic infections.

Tephrosia is a large tropical and subtropical genus belongs to the family Fabaceae or Leguminosae (Allen, O.N., Allen, E. K *et al.,* 1981). *Tephrosia calophylla* is a perennial undershrub found widely in Talakona of Andhra Pradesh, south India. (Madhava Chetty K, sivaji k *et al* 2008). The genus tephrosia is known to contain a wide variety of flavonoids. The compound tephlostan 7- O-methylglabranin and kaempferol-3- O-ß-D-glucopyranoside were isolated and characterized from the whole plant of *Tephrosia calophylla.* As the genus Tephrosia represents potential source of flavonoids and various biological activities (Seru Ganapaty, Guttula Veera Kantha Srilakshmi.*et al.,* 2009). According to the Ayurvedha the plant is useful as an immunomodulator and used as alternative cures for diseases of the liver, spleen, heart and blood. According to the Unani system of medicine the root is diuretic, allays, thirst, enriches blood, cures diarrhoea and is useful in bronchitis, inflammation, boils and pimples. Leaves are tonic to intestine and a promising appetizer. So the present investigation was made to study the immunomudulatory activity and hypoglycemic activity of *Tephrosia calophylla* against dexamethasone induced pancreatic damage as well as the affected blood parameters.

MATERIALS AND METHODS

Plant material

Fresh roots of *Tephrosia calophylla* were collected from Talakona forest from Andhra Pradesh and identified by Dr. Madhava chetty, Department of Botany, Sri Vankateswara University, Tirupathi, the study was conducted in 2014. The freshly collected plant materials were washed, shadow dried and then dried in hot air oven at a temperature not more than 50°C. The dried materials were coarsely powdered using an electric blender. Powdered materials (500g) were then packed in soxhlet apparatus and successively extracted with petroleum ether, chloroform, ethyl acetate and methanol. Each time before extraction with the next solvent, the powdered materials were dried in hot air oven at below 50°C. Finally extracts were concentrated in rotary evaporator at a temperature not more than 50°C and then, dried under vacuum desiccator. The concentrated crude methanol was lyophilized in to powder and used for this study.

Animals

Albino rats of either sex weighing between 175-200gms were used in the study. A bout 36 rats were procured from the King Institute, Chennai, Tamil Nadu, India, and used throughout the study. The animals were housed in the well protected cages under controlled environmental conditions with standard laboratory diet and water. The study was conducted after obtaining Institutional Animal Ethical Committee clearance (Protocol No. 1220/a/08/CPCSEA/ANCP/05).

Reagents

All the materials used for experiments were of pharmacopoeial grade. For this study, we used Glucose Oxidase and Peroxidase enzyme reagent along with chromogen 4-Aminoptyrine, phenol and EDTA, Dexamethasone and 5 % gum acacia.

1. Acute Toxicity studies

Acute oral toxicity (AOT) of *TCR* was determined using Swiss albino mice of either sex weighing 25 -30 g. The animals were fasted for 3 h prior to the experiment and were administered with single dose of methanol extract of *Tephrosiacalophylla* dissolved in 5 % gum acacia (doses ranges from 100-5000 mg/kg at various dose levels) and observed for mortality up to 48 h (short term toxicity). Based on the short-term toxicity, the dose of next animal was determined as per OECD guideline 425. The LD_{50} of the test extract was calculated using Graphical method.

2. Evaluation of Hypoglycemic activity of *TCR* extract

Preparation of *Tephrosia calophylla* root extract for experiment

Hypoglycemic effect in experimental rats was studied using freshly prepared suspension of *Tephrosia calophylla* root extract. The roots extract was weighed and suspended in 5% gum acacia.

Experimental Procedure

Normal rats of either sex weighing 175-200gms were used in the study. Here 6 groups of rats, 6 in each group were taken for the experiment and were marked as R1, R2, R3, R4, R5 and R6 for easy identification. The blood glucose levels of rats R1, R2, R3, R4, R5, and R6 were determined for a period of 24hrs, after one week 250 & 500mg/kg body weight of *Tephrosia calophylla* (*TCR*) extract as a suspension in 2% gum acacia was administered orally to 4 groups containing each six

rats. Blood samples were collected before and at 0.5, 1, 2, 3, 6, 8, 16 and 24hrs after drug administration from the retro orbital puncture of each rat and were analysed for glucose content by using Glucose Oxidase (GOD-PAP) method **(**Srinivasan K, Rama rao R *et al.,* 2007) and optical density measured by a visible spectro-photometer at wave length 520 nm (Bell RH, Hye RJ *et al.,* 1983).

Evaluation of immuno modulatory activity of *TCR* extract (Haematological studies and Hypo lipidemic activity)

3. Lipid profile tests

Triglycerides are usually assayed either by chemical method or enzymatic methods. Chemical methods are cumbersome, time consuming and are reliable only in expert hands. In contrast the enzymatic methods are simple, and easy to perform. For the current research we have used an auto Analyzer for the estimation of Triglycerides in Serum by GPO /PAP method. Estimation of total cholesterol level, HDL, LDL and VLDL levels was done by using Trinder CHOD/POD method **(**Trinder P *et al.,* 1969).

4. Study of haematological parameters

Enumeration of Erythrocytes was done by diluting certain amount of blood through known proportions with a suitable diluting fluid. The diluted blood is enumerated from the number of RBC present in tube and diluted blood was calculated. Enumeration of WBC was done by taking certain amount of blood and diluted through a known proportions with a suitable diluting fluid from known volume of diluting blood the number of WBC present in one column of undiluted blood was calculated. The content of haemoglobin was estimated by using haemocytometer (Jaya shahi and Ajay singh. *et al* 2011). (Alamgeer1, Muhammad Naveed Mushtaq *et al*., 2012).

RESULTS AND DISCUSSION

1. Acute toxicity study

By graphical method it was found that the LD_{50} value of methanol extract of *Tephrosia calophylla* was 1000mg, the results were presented in table 1.

2. Hypoglycemic activity of *Tephrosia calophylla root* extract

The blood glucose levels before and after treatment with *Tephrosia calophylla* in normal rats are given in tables 2, and the plot obtained were shown in fig 1 *Tephrosia calophylla* at a dose of 500mg/kg body weight produced a maximum percentage of blood glucose reduction.

The extract at a dose of 250mg/kg body weight reduced the blood glucose level significantly ($327.6\pm8.16^{***}$) in contrast to control treated group. The extract at a dose of 500mg/kg body weight reduced the blood glucose level much predominantly ($191.9\pm8.02^{***}$) in contrast to control D.M. ($420.5\pm12.28^{\#\#}$).

PANCREAS: description for histology of pancreas

AC: AcinarCells

IL: Islets of Langerhans

NC: Necrotic Change

Normal: The normal architecture of the pancreatic tissue with lower magnification (10X) and higher magnification (40X).

Induced: Pancreas shows Necrotic changes and structural damage with lower magnification (10X) and higher magnification (40X).

Standard: Mild recovery in pancreatic tissue with lower magnifications (10X) and

T1: The regeneration of pancreatic tissue with lower magnifications (10X) and higher Magnification (40X).

T2: The regenerative changes shows pancreas similar to the normal architecture with lower magnifications (10X) and higher Magnification (40X).

3. Results of Hypolipidemic activity of *Tephrosia calophylla root* extract

Various lipid profile test parameters like Triglycerides, Total cholesterol, HDL, LDL and VLDL levels were estimated by treating the albino rats with Dexamethasone and two different doses of *Tephrosia calophylla* extract such as 250 mg/kg, 500 mg/kg and results were analysed. From the obtained results it was found that all the lipid profile parameters like Triglycerides (**$161.4\pm1.79^{**}$**), Total cholesterol (**114.9 ± 0.95**), HDL (**43.41 ± 0.28**), LDL (**60.30 ± 0.37**) and VLDL (**28.51 ± 0.57**) **levels** are significant at standard group in contrast to DM induced, control and normal groups.

4. Results of complete Blood profile test of *Tephrosia calophylla root* extract (Haematological studies)

Various Haematological test parameters like RBC, WBC, platelets and Lymphocyte count were estimated by treating the albino rats with Dexamethasone and two different doses of *Tephrosia calophylla* such as 250 mg/kg and 500 mg/kg, the results were analysed and it was found that all the haematological parameters were significantly increased at standard dose in contrast to D M induced toxicity on experimental animals at 10 mg/kg of body weight. Results were tabulated in the Table. No.4.

DISCUSSION OF RESULTS

As a part of our research, initially plant extractives were analysed for the yield of individual extracts and found that, the yield was abundant in Methanol rather than Pet ether, chloroform, and Ethyl acetate. Due to the high polarity of methanol most of the

Table 1: Acute toxicity of TCR extract

Dose	Log Dose	Dead/Total	% of Dead	Corrected %	Probit
100	2	1/10	10	10	3.72
500	2.698	3/10	30	30	4.48
1000	3	5/10	50	50	5.00
3000	3.4	8/10	80	80	5.84
5000	3.698	10/10	100	97.5	6.96

Table 2: Hypoglycemic activity of Tephrosia calophylla root extract

Sl.No	Group	Blood glucose (mg/dl)
1.	Normal	97.37±5.89
2.	Control treated	420.5±12.28$^{\#\#}$
3.	Standard group (P.E 500Mg/kg , p.o)	143.6±6.11***
4.	Low dose (D.M 10mg/kg s.c + P.E 250 mg/kg ,p.o)	327.6±8.16***
5.	High dose (D.M 10mg/kg s.c + P.E 500 mg/kg ,p.o)	191.9±8.02***

All values are shown as mean ± SEM and n=6; ### indicate p<0.001 when compared to normal group; *** indicate p<0.001 when compared to Control group.

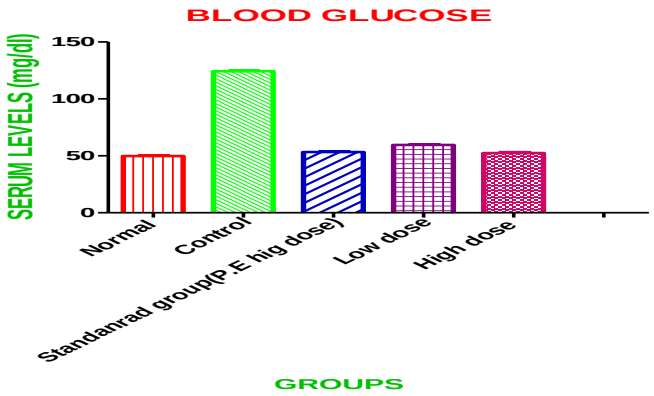


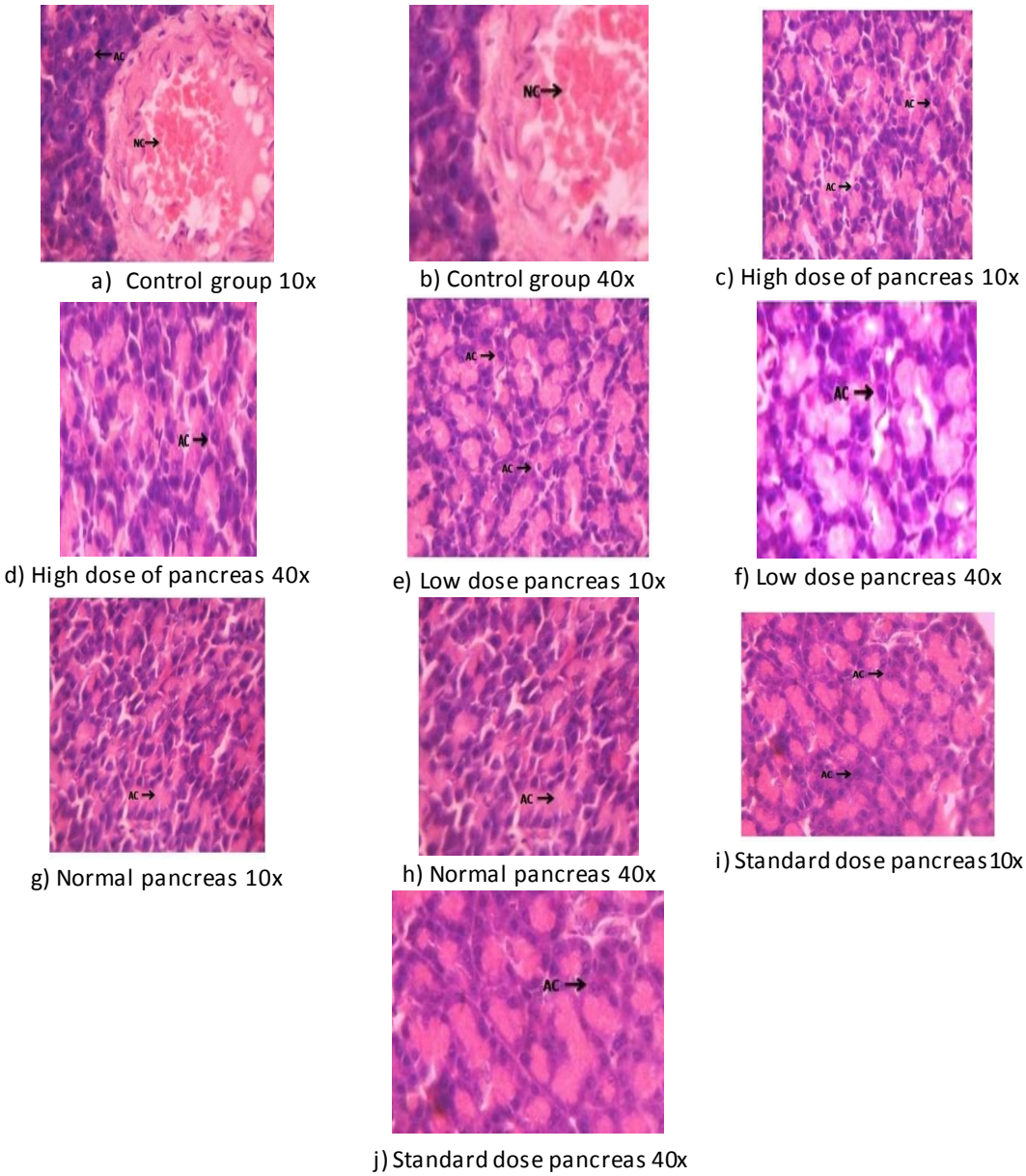
Figure 1: Effect of Methanol extract of Tephrosia calophylla on blood glucose levels

Table 3: Hypolipidemic activity of Tephrosia calophylla root extract

Sl.No	Group	T.C (IU/L)	HDL (IU/L)	LDL(IU/L)	VLDL(IU/L)	TG(IU/L)
1.	Normal	111.8±0.66	42.97±0.77	58.35±0.60	28.57±0.25	151.4±0.38
2.	Control treated	193.4±1.710.$^{\#\#\#}$	16.44±0.42$^{\#\#\#}$	91.45±0.29$^{\#\#\#}$	52.85±0.44$^{\#\#\#}$	256.7±2.59
3.	**Standard group (P.E 500Mg/kg , p.o)**	**114.9±0.95**	**43.41±0.28**	**60.30±0.37**	**28.51±0.57**	**161.4±1.79****
4.	Low dose (D.M 10mg/kg s.c + P.E 250mg/kg ,p.o)	166.2±1.85***	25.86±0.56***	74.48±0.34***	38.24±0.61***	204.4±2.26***
5.	High dose (D.M 10mg/kg s.c + P.E 500mg/kg ,p.o)	119.1±2.04***	39.55±0.31***	58.27±0.55***	30.22±0.34***	169.6±1.20***

chemical constituents of extracts would be dissolved in it and thus percentage yield was increased tremendously than other solvents. The percentage yield of *TCR* extract in methanol is 13.8%, Due to higher yield in methanol, methanolic extracts was used for further studies.

Acute toxicity study of *TCR* extract was performed in mice. It was found that the LD_{50} value of *TCR* extract was 1000mg/kg orally. Hence, 250 and 500mg/kg dose were selected for further studies. There were no adverse effects found in the experimental animals before and after administration of extracts as per schedule.



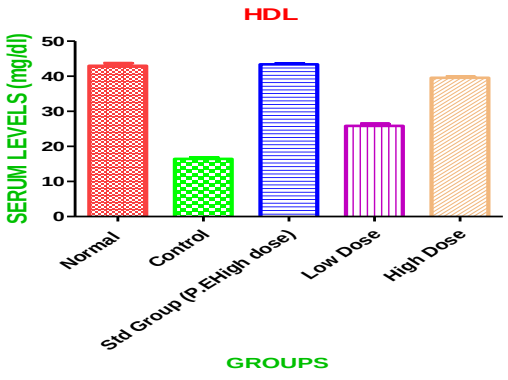
a) Control group 10x
b) Control group 40x
c) High dose of pancreas 10x
d) High dose of pancreas 40x
e) Low dose pancreas 10x
f) Low dose pancreas 40x
g) Normal pancreas 10x
h) Normal pancreas 40x
i) Standard dose pancreas10x
j) Standard dose pancreas 40x

Figure 2: Effect of TCR extract on pancreas at various levels of treatment (Dexamethasone induced diabetes)

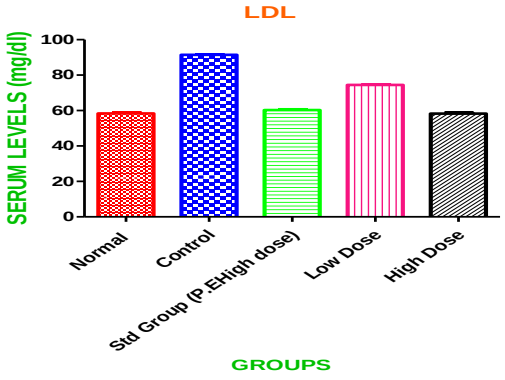
Table 4: Effect of Tephrosia calophylla on blood parameters in experimental animals.

Sl.No	Group	RBC	WBC	PLATE LETS	LYPHOCYTES
1.	Normal	5.85±0.02	4.83±0.02	2.44±0.04	26.12±0.21
2.	Control treated	7.47±0.18$^{###}$	3.3±0.12	0.89±0.007	57.60±0.39
3.	**Standard group (P.E 500Mg/kg , p.o)**	**5.86**±0.04	**5.5**±0.04***	**2.32**±0.11	**34.32**±1.03***
4.	Low dose (D.M 10mg/kg s.c + P.E 250 mg/kg ,p.o)	5.81±0.05***	4.19±0.06***	1.39±1.91***	46.19±0.79***
5.	High dose (D.M 10mg/kg s.c + P.E 500 mg/kg ,p.o)	5.29±0.16***	4.6±0.27***	1.91±0.02***	32.38±0.38***

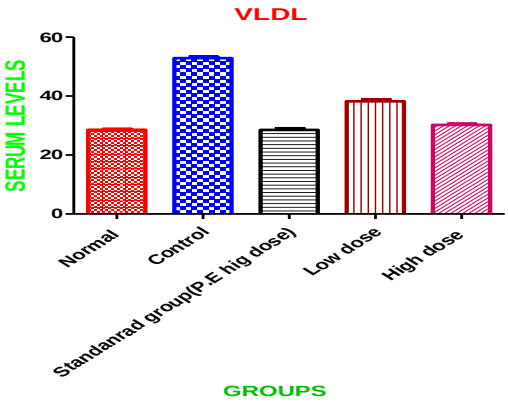
The *TCR* extract has produced hypoglycaemic action in a dose dependent manner within 250mg/kg body weight and 500mg/kg body weight represents the direct or indirect action on blood sugars. The sustained hypoglycaemic action produced by these two doses indicates that the extract may contain compounds which are slowly metabolised into its active form and may be responsible for its prolonged lowering of



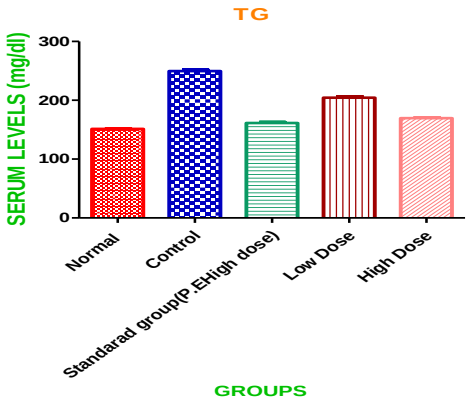
Effect of TCR on HDL levels



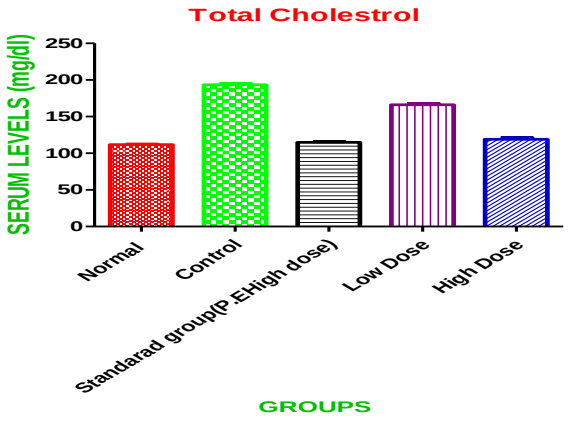
Effect of TCR on LDL levels



Effect of TCR on VLDL levels



Effect of TCR on TG levels



Effect of TCR on total cholesterol levels

Figure 3: Results of Lipid profile test of Tephrosia calophylla root extract: (Hypolipidemic activity of Tephrosia calophylla)

blood sugar. The blood glucose levels before and after treatment with *TCR* extract in normal rats and the plot obtained *TCR* extract at a dose of 500mg/kg body weight produced a maximum percentage of blood glucose reduction.

The extract at a dose of 250mg/kg body weight reduced the blood glucose level significantly (327.6±8.16***) in contrast to control treated group. The extract at a dose of 500mg/kg body weight reduced the blood glucose level much predominantly (191.9±8.02***) in contrast to control D.M. (420.5±12.28$^{\#\#}$).

The *TCR* extract was tested for immunomodulatory activity by assessing its potency on lipid profile parameters like total cholesterol, HDL, LDL, TG, VLDL levels and also for various hematological parameters like RBC, WBC, Platelets, lymphocytes and leucocytes concentrations/counts. From this study it was found that *TCR* extract was more effective in controlling lipid parameters and hematological parameters at normal levels at a dose of 500mg/kg body weight when compare to 250mg/kg body weight.

SUMMARY AND CONCLUSION

In current research *TCR* extract has revealed that, since the plant belongs to the family of Fabaceae it

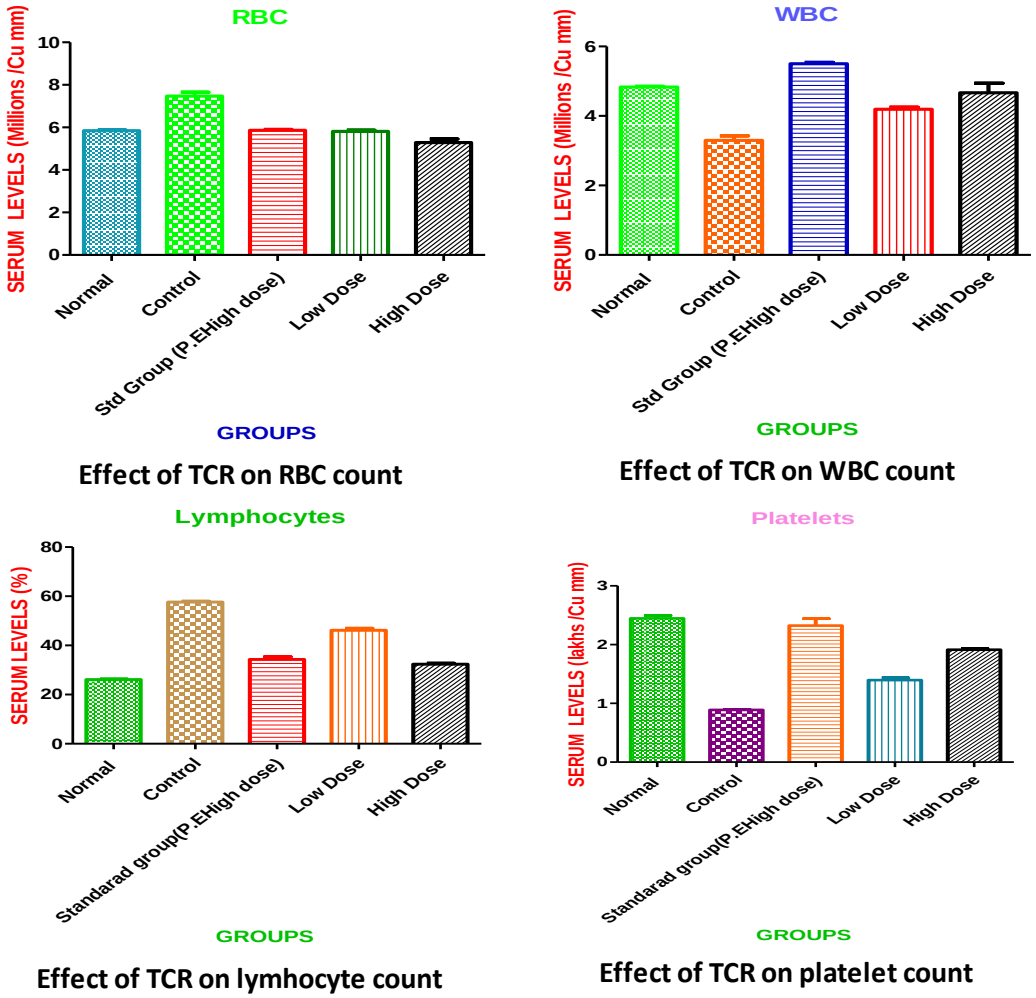


Figure 4: Results of complete Blood profile test of Tephrosia calophylla root extract: (Haematological studies)

was very rich in phenolic and polyphenolic compounds. As they possess these chemical principles they are effective against several diseases.

TCR extract contains a wide variety of flavanoids and iso-flavanoids. Acute toxicity studies of *TCR* extract has revealed that it was safer to be use at a concentration of 1000mg/kg in experimental animals. Hypoglycemic activity of *TCR* extract has shown that, it was effective against hypoglycaemia in experimental animals at the concentrations of 250mg/kg and 500 mg/kg of body weight. The *TCR* extract was tested for immunomodulatory activity by assessing its potency on lipid profile parameters like total cholesterol, HDL, LDL, TG, VLDL levels and also for various hematological parameters like RBC, WBC, Platelets, lymphocytes and leucocytes concentrations/counts. From this study it was found that *TCR* extract was more effective in controlling lipid parameters and hematological parameters at normal levels at a dose of 500mg/kg body weight when compare to 250mg/kg body weight.

REFERENCES

Alamgeer1, Muhammad Naveed Mushtaq1, Sajid Bashiz..*et al.,* Hypoglycemic and hematological effects of aqueous extract of *Thymus serpyllum* Linn. in alloxan-induced diabetic rabbits. African Journal of Pharmacy and Pharmacology Vol. 6(40), pp. 2845-2850, 29 October, 2012.

Allen, O.N., Allen, E. K. The Leguminoseae; *the University of Wisconsin Press, Wisconsin*; 1981: pp 645–649.

Allison A.C. Immunological adjuvants and their modes of action. *Arch, Immunol. Ther, Exp*. Vol.45; 1997: pp14-17.

Bell RH, Hye RJ, Animal models of diabetes mellitus: physiology and pathology. J. Surg. Res. 35; 1983: pp 433.

Gosh R, Sharachandra KH, Rita S, Thokchom IS. Hypoglycemic activity of *Ficus hispida* (bark) in normal and diabetic albino rats. Indian journal of pharmacology. 36; 2004: pp 222.

Jaya shahi and Ajay singh. et al., Effect of bioactive compounds extracted from Euphorbious plants on hematological and biochemical parameters of *channa punctatus* Rev. Inst. Med. Trop. Sao Paulo53(5):259-263, September-October, 2011

Madhava Chetty K, sivaji k, Tulasi Rao. Flowering Plants of Chittor District - Andhra Pradesh, India. 1st ed, Students Offset Printers. Tirupathi; 2008: pp 102.

S.S. Agarwal, V.K. Singh. Immunomodulatory; A Review of studies on Indian Medicinal plants and synthetic peptides, Part 1. *PINSA* B65; 1999: PP 179-204.

Seru Ganapaty, Guttula Veera Kantha Srilakshmi. Cytotoxic benzil and Coumestan derivatives from *Tephrosia calophyll.* Phytochemistry. Vol. 70; 2009: pp 95-99.

Sharma P.V. Translation; Charaka Samhita. 1st edition, Vol.II; 1981.

Srinivasan K, Ramarao R. Animal models in type 2 diabetes research. An overview; Indian J. Med. Res, 125; 2007: pp 451.

Trinder P., Ann. Clinical. Biochem. 6; 1969: pp 24.