WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 12, 1094-1102. Research Article ISSN 2277– 7105

ANTIHYPERGLYCEMIC AND HEAMATOLOGICAL EFFECT OF COMBINATION OF *BOUGAINVILLEA SPECTABILIS* AND *CATHARANTHUS ROSEUS* LEAVES ON ALLOXAN-INDUCED DIABETIC WISTAR RATS

Firdous Majeed[1]*, Hakim Mudasir Maqsood[2] and Kusum Singh[1]

[1]Department of Zoology, Institute of Basic Science, Bundelkhand University, Jhansi, (U.P), India.

[2]Division of Animal Biotechnology, Faculty of Vety. Science & Animal Husbandry, SKUAST, Kashmir, India.

Article Received on
12 August 2017,
Revised on 03 Sep. 2017,
Accepted on 25 Sep. 2017
DOI: 10.20959/wjpr201712-9777

***Corresponding Author**
Firdous Majeed
Department of Zoology,
Institute of Basic Science,
Bundelkhand University,
Jhansi, (U.P), India.

ABSTRACT

Bougainvillea spectabilis and *Catharanthus roseus* leaves are known to have medicinal properties. Antihyperglycemic property and other beneficial effects of their plant extract were evaluated in Alloxan-induced diabetic Wistar rats. A total of 24 mature rats weighing 200±10g were used for this study. They were divided into four groups of 6 rats each. Group I was conserved as normal control. Group II, III and IV were made diabetic by intraperitoneal injection of Alloxan. After being confirmed as diabetic, Group II was conserved as diabetic control, Group III was administrated plant extract (*Bougainvillea spectabilis* and *Catharanthus roseus*) at 300 $mgkg^{-1}$ body weight and Group IV was administrated standard drug, Glibenclamide at 5 $mgkg^{-1}$ body weight for defined time intervals. The blood glucose level and heamatological parameters viz. red blood and white blood cells and some of their functional indices were evaluated in diabetic treated groups on 7^{th}, 14^{th} and 21^{th} day after daily oral administration of plant extracts and were compared with the respective controls. The result of study revealed that the plant extract at 300 $mgkg^{-1}$ body weight exhibited significantly hypoglycemic activity at defined interval and had beneficial effect on all studied heamatological parameters viz. RBC, WBC, Hb, PVC, SGOT and SGPT.

KEYWORDS: *Bougainvillea Spectabilis*, *Catharanthus Roseus*, Diabetes, Heamatology, Wistar Rat.

INTRODUCTION

Diabetes mellitus is caused by lack of insulin secretion by the pancreas (type I diabetes) or by insufficient insulin secretion to compensate for decreased sensitivity to the effects of insulin (type II diabetes). (Guyton and Hall, 2006). Patients with diabetes experience significant morbidity and mortality from microvascular (retinopathy, neuropathy, nephropathy *etc*) and macrovascular complications *viz*. heart attack, stroke and peripheral vascular disease (Halder *et al*., 2003 and Merlin *et al*., 2005). The population of humans suffering from this disorder is increasing at epidemic rate and about 366 million people are likely to become diabetic by the year 2030 (Wild *et al*., 2004). Management of diabetes is still a challenging task. According to recommendations by the WHO expert committee on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become important (Viturro *et al*., 1999). Medicinal plant extracts may have adjuvant effects to existing antidiabetic agents. Many compounds isolated from these plants are used in combinational therapy for diabetes (Srivastava *et al*., 2012).

Bougainvillea spectabilis belonging to the family Nyctaginaceae. The genus *Bougainvillea* of flowering plants native to South America from Brazil west to Peru and south to southern Argentina. The leaves of this genus are reported to have medicinal properties *viz.* antiviral, antibacterial, anti-inflammatory, larvicidal and antifertility potential (Joshi *et al*., 1984; Andrea *et al*., 1997 and Umamaheswari *et al*., 2008). The alcoholic extract of the leaf has been reported to possess hypoglycemic effect and has been used for the management of diabetes mellitus. The hypoglycemic principle of the leaf extract has been isolated and named Pinitol (Bates *et. al*., 2000). Despite the isolation of the hypoglycemic principle, the crude extract is still being used for the management of diabetes mellitus in some local settings today.

Catharanthus roseus is known with various names (Madagascar periwinkle; *Vinca rosea; Lochnera rosea*) in India and all over world. *Catharanthus roseus* contains more than 400 known alkaloids, some of which are approved as antineoplastic agents to treat leukemia, Hodgkin's disease, malignant lymphomas, neuroblastoma, rhabdomyosarcoma, Wilmer's tumor and other cancers. Its vasodilating and memory enhancing properties have been shown to alleviate vascular dementia and Alzhemlizer's disease (Hindmarch *et al*., 1991 and Fishhof

et al., 1996). Water decoction of the leaves and /or the whole plant is used as household remedy for diabetes in several countries *viz*. Brazil, Cook Islands, Dominica, England, Jamaica, Pakistan, Taiwan and West Indies (Don, 1999).

The major objective of this investigation was to study the antihyperglycemic activity and heamatological alterations due to the methanolic extract of combination of *Bougainvillea spectabilis* and *Catharanthus roseus* leaves (1:1) in Alloxan-induced diabetic rats.

MATERIALS AND METHODS

Collection of Plant and Preparation of Extract

The leaves of *Bougainvillea spectabilis* and *Catharanthus roseus* were collected during the month of March and April 2013 from Bundelkhand University Campus, Jhansi, U.P. Medicinal were subsequently authenticated and identified in the Ayurveda Research Institute Jhansi, U.P. The freshly collected leaves were air dried under shade at room temperature for 7 days. Drying the plant materials were pounded separately using mortar and pestle into smaller particles and grounded to fine powder using an electric blender. The powdered material of the both plants in the ratio of 1:1 was extracted with methanol in a Soxhlet apparatus and evaporated to dryness yielding a semisolid crude extract. This crude extract was weighted and used in preparation of doses.

Experimental Animals

The study was conducted in sexually mature, male albino rats of Wistar strain (200±10g), purchased from DRDE (Defense Research Development Establishment) Gwalior. Prior to study, the ethical clearance was obtained from the Institutional Animal Ethical Committee (BU/Pharm/IAEC/12/34) New Delhi. All the protocols and the experiments were conducted in strict compliance with the ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals were acclimatized to the experimental room at temperature of 25-30˚C, controlled humidity conditions (50-55%) and 12 hours light and 12 hours dark cycle. They were fed with rat pelleted diet (Amrut Feeds, Pranov Agro Ltd. Sangli) and water *ad-libtum*.

Induction of diabetes

Diabetes was induced in rats by single intraperitoneal injection of Alloxan (Alloxan monohydrate, CDH, Bombay Ltd.). Alloxan monohydrate was dissolved in ice-cold physiological saline (0.9% NaCI) to constitute a 10% (w/v) solution and a dose of 100 $mgkg^{-1}$

b.wt. of rat was selected to induce diabetes. The fasting blood glucose level of rats was measured after 72 hours of Alloxan injection using One Touch® glucometer (Lifescan, Johnson & Johnson, California). The rats with effective and permanent elevated blood glucose levels (above 200 $mgdl^{-1}$) were selected for the study.

Experimental Design

The research work was carried out for 4 weeks. In the first week diabetes was induced in rats and rats were allowed to acclimatize to laboratory environment. The following 3 weeks were investigational period with crude methanolic extract of *Bougainvillea spectabilis* and *Catharanthus roseus* leaves. Twenty-four rats were grouped into four groups of six rats each, in following experimental design.

Group I: Normal control.

Group II: Diabetic control.

Group III: Diabetic; will receive plant extract at $300mgkg^{-1}$ b.wt.

Group IV: Diabetic; will receive standard drug, Glibenclamide at $5mgkg^{-1}$ b.wt.

After daily administration of doses for the intervals of 7, 14 and 21 days, anesthesia was given to animals by using diethyl ether. The blood samples were collected from each rat by puncturing the optical vein of rat eye *i.e.* Retro-Orbital Plexus with the help of capillaries. The collected blood samples were analyzed for following parameters.

Clinical Chemistry

(a) Blood Sugar (Accu-Chek Glucometer).

(b) SGOT and SGPT (Reitman & Frankel Method, 1957),

Haematocellular Components

(c) Red Blood Cell Count (Naubaur's Chamber).

(d) White Blood Cell Count (Naubaur's Chamber).

(e) Haemoglobin Percentage (Sahli's Apparatus).

(f) Packed Cell Volume (Wintrobes and Landsberg, 1935).

Statistical analysis

The results were expressed as Mean ± S.E. significance of differences compared to the control was determined using student's t-test.

RESULTS AND DISSCUSSION

The blood glucose levels of four groups of experimental albino rats at the end of defined treatment durations are shown in table I. During daily co-administration of methanolic extract of *Bougainvillea spectabilis* and *Catharanthus roseus* leaves at a dose of 300 $mgkg^{-1}$ b.wt. and standard drug, Glibenclamide at a dose of 5 $mgkg^{-1}$ b.wt. for 7, 14 and 21 days respectively, the blood glucose level decreased significantly as compared to the diabetic control group. The reduction is blood glucose levels of diabetic rats treated with combination of *Bougainvillea spectabilis* and *Catharanthus roseus* extract for was 41.54% while reduction in blood glucose levels of diabetic rats treated with standard drug Glibenclamide was 54.09%, compared with their respective diabetic controls at the end of 21 days($p<0.05$). Jawla *et al.*, (2011) reported the similar results by the administration of the root bark of *Bougainvillea spectabilis* at different doses and intervals. Highest hypoglycemic activity was observed with root bark extract at 100 $mgkg^{-1}day^{-1}$ after 7 days.

In the present study, RBC count, Hb% and PCV decreased significantly due to administration of Alloxan in albino Wistar rats as compared to normal control group. The occurrence of anemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins (Oyedemi *et al*., 2011). Oxidation of these proteins and hyperglycemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC (Arun *et al*., 2002). During daily co-administration of methanolic extract of *Bougainvillea spectabilis* and *Catharanthus roseus* leaves at a dose of 300 $mgkg^{-1}$ b.wt and standard drug, Glibenclamide at a dose of 5 $mgkg^{-1}$ b.wt. for 7, 14 and 21 days respectively, the RBC count, Hb% and PCV increased significantly as compared to the diabetic control group and recouped towards the normal group(Table III).

WBC play vital role in body's immune system and protect the body from invading foreign substances. In the present study, WBC count increased significantly due to administration of Alloxan in albino Wistar rats as compared to normal control group. During daily co-administration of methanolic extract of *Bougainvillea spectabilis* and *Catharanthus roseus* leaves at a dose of 300 $mgkg^{-1}$ b.wt. and standard drug, Glibenclamide at a dose of 5 $mgkg^{-1}$ b.wt. for 7, 14 and 21 days respectively, the WBC count decreased significantly as compared to the diabetic control group (TableII). The present study revealed the WBCs count in diabetic group showed a significant increase. This increase in WBCs count may be due to the increased hemopoitic activity as a result of the hemolysis of RBCs in diabetic rats and may be

a response to stressful condition after antigen (Alloxan) injection (Sovneyl *et al*., 1990). Furthermore, the increase in WBC in the diabetic group is ameliorated by the co-administration of *Bougainvillea spectabilis* and *Catharanthus roseus* extract. Therefore, the plant extract could reduce the destructive effect of Alloxan-induced diabetes. This indicates that the treatment with plant extract affects the defense mechanism and immune response to inhibit the inflammation resulting from Alloxan-induced diabetes (Shakoori *et al*., 1992).

Alanine amino transferases are ectoplasmic enzymes found in very low concentration in the liver which are released into plasma following hepatocellular damage (James *et al.*, 2007). SGOT and SGPT level increased significantly due to administration of Alloxan in albino Wistar rats as compared to normal control group. During daily co-administration of methanolic extract of *Bougainvillea spectabilis* and *Catharanthus roseus* leaves at a dose of 300 $mgkg^{-1}$ b.wt. and standard drug, Glibenclamide at a dose of 5 $mgkg^{-1}$ b.wt for 7, 14 and 21 days respectively, the SGOT and SGPT level decreased significantly as compared to the diabetic control group and recouped towards the SGOT and SGPT level of normal control group (Table III).

Table. I: Effect of combination of *Bougainvillea spectabilis* and *Catharanthus roseus* extract on Blood Glucose Levels of diabetic albino rats.

Blood Glucose (mg dl^{-1})				
Duration of Treatment	**Normal Control**	**Diabetic Control**	**Diabetic + Plant Extract (300$mgkg^{-1}$ b.wt.)**	**Diabetic + Glibenclamide (5$mgkg^{-1}$ b.wt.)**
7 DAYS	93.83±1.49	256.33±1.24*	190.00±2.83*	141.83±2.45*
14 DAYS	94.33±1.12	261.50±0.93*	164.83±3.96*	126.83±0.86*
21 DAYS	94.16±0.43	267.30±1.53*	138.66±3.07*	106.50±3.12*

Values are given as Mean ± SE of 6 animals in each group.*Values are statistically significant at $p<0.05$.

Table. II: Effect of combination of *Bougainvillea spectabilis* and *Catharanthus roseus* extract on Heamatological parameters of diabetic albino rats.

Groups	**Duration of Treatment**	**Heamatological Parameter**			
		RBC(million mm^{-3})	**Hb (g dl^{-1})**	**PCV (%)**	**WBC (1000 mm^{-3})**
Normal Control	**7 Days**	8.8±0.2	14.1±0.04	40.2±0.1	8.6±0.2
	14 Days	8.9±0.4	14.3±0.11	40.5±0.3	8.7±0.3
	21 Days	8.7±0.2	13.9±0.05	40.4±0.1	8.5±0.2
Diabetic Control	**7 Days**	7.2±0.5	11.2±0.02*	33.4±0.4*	16.6±1.1*
	14 Days	7.0±0.1*	10.8±0.03*	32.9±0.1*	16.5±0.4*
	21 Days	6.9±0.2*	10.5±0.12*	32.8±0.2*	16.7±0.2*

Diabetic+ Plant Extract (300mgkg^{-1} b.wt.)	**7 Days**	8.5±0.2	11.5±0.30	37.2±0.2	15.0±1.0
	14 Days	9.1±0.3*	13.7±0.50*	39.5±0.2*	13.2±1.2*
	21 Days	10.1±0.2*	15.4±0.20*	42.5±0.1*	11.0±1.1*
Diabetic+ Glibenclamide (5mgkg^{-1} b.wt.)	**7 Days**	7.9±0.5	11.3±0.50	35.4±0.3	16.2±1.0
	14 Days	8.4±0.2	12.4±0.20	38.7±0.5*	15.3±0.7
	21 Days	9.0±0.1*	13.9±0.10*	40.3±0.4*	13.2±0.5*

Values are given as Mean ± SE of 6 animals in each group.*Values are statistically significant at p<0.05.

Table. III: Effect of combination of *Bougainvillea spectabilis* and *Catharanthus roseus* extract on SGOT and SGPT of diabetic albino rats.

Groups	**Duration of Treatment**	**Liver Enzymes**	
		SGOT(IU L^{-1})	**SGPT(IU L^{-1})**
Normal Control	7 Days	42.52±1.5	35.08±2.9
	14 Days	44.15±1.8	34.01±2.7
	21 Days	40.00±0.9	33.05±1.5
Diabetic Control	7 Days	69.61±20*	53.25±2.5*
	14 Days	72.50±1.8*	51.25±2.3*
	21 Days	70.12±1.5*	49.15±2.0
Diabetic+ Plant Extract (300mgkg^{-1} b.wt.)	7 Days	51.15±0.5	45.31±1.7
	14 Days	45.52±1.2*	41.01±1.3
	21 Days	40.02±0.6*	37.25±1.2*
Diabetic+ Glibenclamide (5mgkg^{-1} b.wt.)	7 Days	50.34±1.0	42.15±2.1
	14 Days	44.20±1.1*	37.21±1.8*
	21 Days	39.42±1.0*	30.44±1.8*

Values are given as Mean ± SE of 6 animals in each group.*Values are statistically significant at p<0.05.

CONCLUSION

India is a developing country with a large portion of people suffering from diabetes mellitus and its complications. As large section of the population dwell with poor health infrastructures. The high costs for therapeutic treatment has compelled to look for alternative and cost effective methods to minimize complications associated with diabetes. Therefore, medicinal plants and their products are more convenient for the treatment of diabetes mellitus due to their easy availability, low cost, minimum side effects and greater acceptance amongst the users. This study indicated use of methanolic extracts of combination of *Bougainvillea spectabilis* and *Catharanthus roseus* leaves exhibited significant antihyperglycemic effect and also possessed beneficial effects on heamatological parameters in diabetic rats.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Zoology, Bundelkhand University Jhansi for providing necessary laboratory facilities to carry out this research work.

REFERENCES

1. Andrea, B.; Letizia, P.; Fabiola, O.; Paola, V.; Luigi, B. and Battelli, M.G. New ribosome inactivating proteins with polynucleotide adenosine glycosidase and antiviral activities from Basella rubra L. and Bougainvillea spectabilis wild. Planta, 1997; 203: 422-429.
2. Arun, G.S. and Ramesh, K.G. Improvement of insulin sensitivity by perindopril in spontaneously hypertensive and Streptozotocin-induced diabetic rats. Indian J. Pharmacol., 2002; 34: 156-164.
3. Bates Sarah, H.; Robert Jones, B. and Cliord Bailey, J. Insulin-like effect of pinitol. British Journal of Pharmacology, 2000; 130: 1944-1948.
4. Don, G. Catharanthus roseus. Medicinal plants of the world edited by: Ross 14. Totwa. New Jersey, Human press, 1999; 109-18.
5. Fischhof, P.K.; Moslinger Gehnayr, R.; Herrmann, W.M.; Friedmann, A.; and Russmann, D.L. Therapeutic efficacy of vinacamine. Neuropsychology, 1996; 34(1): 29-35.
6. Guyton, C. Arthur and Hall, E. John Text Book of Medical Physiology. 11th ed. Elsevier Saunders, Philadelphia, Pennsylvania, 2006; 398-399.
7. Halder, N.; Joshi, S. and Gupta, S.K. Lens aldose reductase inhibiting potential of some indigenous plants. J. Ethnopharmacol., 2003; 86: 6-12.
8. Hindmarch, I.; Fuchs, H.H.; and Erzigkeit, H. Efficacy and tolerance of vinpocetine in ambulant patients referring from mild to moderate organic pyschosyndromes. Int. Clin. Psychopharmacol., 1991; 6(1): 367-372.
9. James, S.A.; Bilbiss, L. and Muhammad, B.Y. Comparative study of the hypoglycemic and biochemical effect of Catharanthus roseus and Chlorpamide (Diabenese) on Alloxan-induced diabetic rats. Biokemistri., 2007; 17(2): 149-156.
10. Jawla Sunil; Kumar, Y. and Khan M.S.Y. Hypoglycemic potential of Bougainvillea spectabilis root bark in normal and Alloxan-induced diabetic rats Pharmacologyonline, 2011; 3: 73-87.
11. Joshi, D.D.; Mujumdar, A.M. and Narayan, C.R. Anti-inflamanatory activity of Bougainvillea spectabilis. India Journal of pharmaceutical Science, 1984; 86: 187-188.
12. Merlin, T.; Con, T.; Richard, M. and George, J. Anaemia in diabetes: An emerging complication of microvascular disease. Curr. Diab. Rev., 2005; 107: 1-26.

13. Oyedemi, S.O.; Yakubu, M.T. and Afolayan, A.J. Antidiabetic activities of aqueous leaves extract of Leonotis leonurus in Streptozotocin-induced diabetic rats. J. Med. Plant Res., 2011; 5(1): 119-125.
14. Shakoori, A.R.; Butt, U.; Riffat, R. and Aziz, F. Toxic effects of short-term oral administration of alloxan on the blood and liver of rabbits. Punjab Univ. J. Zool., 1992; 13: 26.
15. Srivastava, S.; Vijay Kumar Lal; Kamlesh Kumar Pant Polyherbal formulations based on Indian medicinal plants as antidiabetic phytotherapeutics. Phytopharmacology, 2012; 2(1): 1-15.
16. Svenyl, J.F.; Yamamato, H.; Fujimoto, S. and Kusuda, R. Lymphomyeloid cells, susceptibility to erythrodermatitis of Carp and bacterial antigens. Dev. Comp. Immunol., 1990; 14: 185-220.
17. Umamaheswari, A.; Shreevidya, R.; and Nuni, A. In vitro Antibacterial activity of Bougainvillea spectabilis leaf extract. Advan. Biol. Res., 2008; 2: 01-05.
18. Viturro, C.; Molina, A. and Schmeda-Hischmann, G. Free radical scavengers from Mutisia friesiana (Asteraceae) and Sanicula graveolens (Apiaceae). Phytother Res., 1999; 13: 422-424.
19. Wild, S.G.; Roglic, A.; Green, R.; Sicree, R. and King, H. Global prevalence of diabetes: estimated for the year 2000 and projection for 2030. Diabetes Care, 2004; 27(5): 1047-1053.