Hematological study and body weight changes of streptozotocin induced diabetic wistar rats treated by hydroethanolic leaf extract of *Catharanthus roseus*

Dongare Sneha, Somkuwar AP and Dubey SA

Abstract

The present study was conducted in 50 Wistar rats, weighing around 150-200 g which was divided into five groups (T₁, T₂, T₃, T₄ and T₅). Diabetes was induced by administering Streptozotocin @ 40 mg/kg body weight in groups T₂, T₃, T₄ and T₅ whereas, T₁ group was maintained as normal control. The group T₂ was maintained as diabetic control. The group T₃ was treated with metformin (standard antidiabetic drug) at 100 mg/kg bd wt. Groups T₄ and T₅ were treated with hydroethanolic leaf extract of *C. roseus* @ 200 and 400 mg/kg b. wt. respectively. In the experiment, blood was collected on 0th, 14th and 28th day for hematological studies. Hydroethanolic leaf extract of *C. roseus* caused significant decrease in the elevated blood glucose levels of the rats from these groups on 14th and 28th day of the experiment. Hematological parameters levels altered due to the Streptozotocin induced diabetes were observed to be normal range by the treatment with *C. roseus* leaf extract @ 400 mg/kg followed by the dose rate of 200 mg/kg.

In treated groups it has been observed that the average weight were increased up to 28th day of treatment, however, this increase was less as compared to weights of normal group of rats at the same time in all the treated groups (T₄, T₃ and T₅) weights of rats were significantly increased as compared to diabetic group (T₂).

The present study is concluded that the *Catharanthus roseus* reduce the blood glucose level, improved body weight and elevated Hb and PCV to the rats.

Keywords: *C. roseus*, Streptozotocin, blood glucose, haematology, body weight and wistar rats

Introduction

Diabetes mellitus (DM) is characterized by hyperglycemia due to disturbance in the group of metabolism of carbohydrates, fat and protein, resulting from defects in insulin secretion, action or both (Georg et al., 2000 and Nyholm et al., 2000)[17, 18]. Currently there are over 150 million diabetics worldwide and this number is likely to increase due to increase in sedentary lifestyle, consumption of energy rich diet and obesity (Yajnik, 2001) [21]. In modern medicine, there is still no satisfactory effective therapy available to cure diabetes (Piedrola et al., 2001) [19]. Therefore, it has become necessary to search for an economically and therapeutically effective treatment, especially for usage in developing and under-developed countries. Many indigenous medicinal plants have been found to be useful for the successful management of diabetes (Joy and Kuttan 1999) [13].

Streptozotocin (STZ) is well known for its selective pancreatic islet cell toxicity and has been extensively used for the induction of diabetes mellitus in animals (Akhtar and Ali 1984) [2]. Streptozotocin induced diabetes is a well-documented model of experimental diabetes. Previous reported literature indicates that the type of diabetes and characteristics differ with the employed dose of STZ and animal and species used (Low et al., 1997) [14]. STZ-induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia. STZ is a pancreatic β-cell toxin that induces rapid and irreversible necrosis of β-cells (Rerup, 1970) [19]. Recently there has been a growing interest in hypoglycemic agents from natural products, especially those derived from plants. Plant sources are usually considered to be non-toxic, with fewer side effects than synthetic sources. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of organisms. Unlike primary metabolites, absence of secondary metabolites does not result in immediate death but rather in long-term impairment of the organism’s survivability, fecundity or aesthetics or perhaps in no significant change at all.
Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. Secondary metabolites often play an important role in plant defense against herbivory and other interspecies defenses.

The present study involves *Catharanthus roseus* (Apocynaceae) also known as madagascar periwinkle, is a perennial subshrub with green color simple, entire, petiolate leaves and violet pink-white or carmine red color flowers (Kokate and Purohit, 2007) [13]. It contains 150 alkaloids including vincristine vinblastine, ajmalicine, etc. (Trease and Evans, 2002) [22]. The plant has been considered due to its wide range of pharmacological activity like anti-inflammatory, antimalarial, antimitotic, antihyperensive, antifertility, antihypercholesterolemic, antimutagenic, antidiuretic, antifungal, antispasmodic, antiviral, cardio tonic, CNS depressant, antitumor, cytotoxic, antispermatic and anticancer activities. The study involves leaves of *Catharanthus roseus* for evaluating antidiabetic activity (Huxley, 1992) [9].

**Material Method**

The research work was carried out at the Department of Pharmacology and Toxicology, Nagpur Veterinary College, Nagpur. The work was aimed to study the anti-diabetic and haematology activity of hydroethanolic leaf extract of *Catharanthus roseus* on Streptozotocin induced-diabetes wistar rats. The blood glucose and hematological study carried out on 0th, 14th and 28th day of experiment. Body weight of animals measured on 0th, 7th, 14th, 21th and 28th day of experiment.

Streptozotocin (STZ) was dissolved in citrate buffer (pH - 4.5) and injected intraperitoneally @ 40 mg/kg body wt. to induce diabetes in rats. Diabetics Rats were divided into 4 groups T1, T2, T3 and T4 each group comprising of 10 rats with equal sex ratio. Additional group T1 comprising of 10 non-diabetic rats having equal sex ratio involved in the design as a normal control group. Group T2 received Streptozotocin @ 40 mg/kg body wt. i.p. Group T3 received metformin @ 100 mg /kg body wt. referral standard drug and T4&T5 were treated with *C. roseus* @ 200, 400 mg /kg body wt. respectively.

The blood glucose levels were measured on 0th, 14th and 28th day by commercial available glucose estimation strip. The blood was collected on 0th, 14th and 28th days of the experiment. Blood was collected by retro bulbar method in glass vials containing 1% ethylene diamine tetra acetic acid (EDTA) for hematological estimation and in clot activator tube for biochemical estimation. One week after administration of the streptozotocin to the rats from the treatment groups T2, T3, T4, and T5 blood collections was considered as the 0th day collection.

The collected blood samples in 1% EDTA were subjected to hematological studies such as hemoglobin concentration (Hb), using Sahli’s Method (acid hematin) and Packed Cell Volume (PCV) by micro tube method as mentioned by Benjamin (1965) [4].

**Result and Discussion**

**Blood glucose levels**

In the present study rat blood glucose levels were estimated by digital glucometer individually and also using biochemical kits supplied by M/S using auto analyzer model as early as possible after blood collections.

The blood glucose levels obtained by both the method were almost equal with minor variation and hence, the values obtained by using the digital glucometer were included the present study.

The fig. 1 depicts the mean blood glucose levels on 0th, 14th and 28th day of the rats from the groups T1, T2, T3, T4 and T5. The blood glucose levels of the group T1 differ significantly with other group T2, T3, T4 and T5. The mean blood glucose values of groups T1, T2, T3, T4 and T5 on 0th day were the blood glucose levels in 100.5 ± 3.65, 320 ± 1.5, 327 ± 1.66, 325 ± 2.8 and 318.5 ± 1.82 mg/dl respectively. The values in group T2 to T5 did not differ significantly, it indicating development of diabetes in all the rats belonging to these groups.

The mean blood glucose values of groups T1, T2, T3, T4 and T5 on 14th day were 99.45 ± 2.28, 332 ± 2.1, 225 ± 4.03, 281 ± 2.22and 220.64 ± 3.87 mg/dl respectively. This indicates that the standard reference antidiabetic drug metformin was found to be more effective when administered daily at the dose rate of 100mg/kg b.wt. Than hydroethanolic leaf extract of *C. roseus* at the dose of 200 mg/kg b.wt. However, at the dose rate of 400 mg / kg hydroethanolic leaf extract of *C. roseus* was found to be more effective than standard reference antidiabetic drug metformin. The blood glucose levels of all treatment groups when compared with each other reveals that the group T5 shows good improvement in the blood glucose levels (220.64 ± 3.87mg/dl) than the groups T1 (225 ± 4.03mg/dl) and T2 (281 ± 2.22mg/dl).

In comparison to the 14th day, the 28th day of treatment, rats in different groups showed blood glucose level which were maintained in group T1, little increase in diabetic control (T2), significantly decreased in std.drug treated group (T4) and also significant decrease in *C. roseus* treated groups T1& T5. The mean blood glucose values of groups T1, T2, T3, T4 and T5 on 28th day was 99.26 ±1.05, 348 ± 3.14, 139 ± 1.33, 166.58 ± 2.45, and 137.49 ± 2.51 mg/dl respectively. From these findings it is observed that on 28th day there is no significant variation in the blood glucose levels of T1 (139 ± 1.33 mg/dl) and T5 (137.49 ± 2.51mg/dl) and was significantly lower than the blood glucose of T4 (166.58 ± 2.45 mg/dl). This is indicative that the treatment with treatment with leaf extract of *C. roseus* (400mg/kg) was as effective as metformin (100mg/kg) in controlling the blood glucose levels of the diabetic rats.

Streptozotocin is responsible for an autoimmune process that results in disruption of the langerhans islet beta cells. Which results in the toxicity of beta cells with emergence of clinical diabetes within 2-7 day or even upto one week depending upon the dose of Streptozotocin.

In the present study streptozotocin @ 40 mg/ kg body wt was used intraperitoneally in rats to produce diabetes. And rats showed increase in blood glucose levels clinically more than 300 mg/dl after a period of one week indicated development of clinical diabetes.

Balamurugan et al. (2003) used STZ @ 40 mg/k to produce diabetes in rats STZ @ 60 mg/ kg have been used by Akbarzadeh (2007) [1] and Braslau et al. (2007) [4]wherea spalanisamy et al. (2011) used STZ @ 45 mg / kg, Furman (2015) used at @ 65 mg /kg and saleh et al. (2013) used STZ @ 55 mg /kg to produce clinical diabetes in rats.

The rats treated with hydroethanolic leaf extract of *C. roseus* @ dose rate of 200 & 400 mg/kg b.wt. Cause significance decreased in blood glucose level. On 28th day in the present study *C. roseus* @ 400 mg/kg showed better results as compared to metformin @ 100 mg / kg b.wt. Similar results have been quoted by waleed et al. (2015) [25] were in the observed rats receiving *C. roseus* @ 100 mg/ kg and 200 mg
/kg b.wt. could reduced the elevated blood glucose level better than that of metformin @ 100 mg/kg b.wt.

Manoharan et al. (2018) reported antidiabetic effects C. roseus against alloxon induced diabetes in wistar rats @ 300 mg/kg b.wt. and claimed that C. roseus leaves have potent antidiabetic effect. C. roseus leaves improved the diabetes which might be due to stimulating insulin secretions from by surviving pancreatic beta cells as well as enhance utilization of glucose by hepatic & extrahepatic tissue of the diabetic rats as stated by manoharan et al. (2018).

**Hemoglobin (Hb) Levels (g/dl)**

The fig. 3 depicts the mean hemoglobin levels of the groups T1, T2, T3, T4 and Tson 0th, 14th and 28th day of the experiment. The mean hemoglobin values of groups T1, T2, T3, T4 and Tson 0th day were 13.18 ± 0.23, 10.11 ± 0.10, 10.12 ± 0.02, 10.3 ± 0.06, 10.28 ± 0.07 g/dl respectively. There is significant decrease in the mean hemoglobin levels of groups T2, T3, T4 and T5 at 5% level of significance as compare to T1 on 0th day of experiment.

The mean hemoglobin values of groups T1, T2, T3, T4 and Tson 14th day were 13.43 ± 0.20, 9.36 ± 0.07, 12.25 ± 0.07, 12.69 ± 0.19 and 14.23 ± 0.04 g/dl respectively. There is significant difference in the mean hemoglobin levels of all the groups T1, T2, T3, T4 and T5 at 5% level of significance. The treatments with the hydroethanolic leaf extract of C. roseus at the dose of 400 mg/kg improved the lowered hemoglobin levels of the diabetic rats and brought the levels comparable to that in control group (T1) rats. The treatments with the metformin (100 mg/kg) and the hydroethanolic leaf extract of C. roseus (200mg/kg) also significantly improved the hemoglobin levels of the diabetic rats and the levels were close to normal.

The mean hemoglobin values of groups T1, T2, T3, T4 and Tson 28th day were 13.51 ± 0.15, 9.02 ± 0.04, 12.83 ± 0.08, 12.80 ± 0.12 and 14.52 ± 0.08 g/dl respectively. There is significant difference in the mean hemoglobin levels of all the groups T1, T2, T3, T4 and T5 at 5% level of significance. The treatments with the hydroethanolic leaf extract of C. roseus at the dose of 400 mg/kg increased the lowered hemoglobin levels of the diabetic rats and the effect was such that the hemoglobin levels were found to be more than the normal levels. The treatments with the metformin (100 mg/kg) and the hydroethanolic leaf extract of C. roseus (200mg/kg) also significantly improved the hemoglobin levels of the diabetic rats, which were near to the normal levels.

The above results indicate that the hemoglobin levels decreased significantly in the diabetic rats and are corresponding to the findings of Emmanuel et al. (2010) and Kemasari et al. (2011). The decreased level of hemoglobin observed in diabetic rats might be due to increased formation of glycosylated hemoglobin. It has been reported that subjects the total hemoglobin levels are much lower than normal, as stated by Kemasari et al. (2011).

The standard drug Metformin (100 mg/kg) treated rats of group T1 showed the significant improvement in the hemoglobin levels. However, the treatments with hydroethanolic leaf extract of C. roseus at the dose of 400 mg/kg to the groups T2 showed significant increase in the hemoglobin levels of the diabetic rats as compared to the treatments with metformin (100 mg/kg) and the hydroethanolic leaf extract of C. roseus (200 mg/kg) to the group T1 and T3 respectively. The hydroethanolic leaf extract of C. roseus has improved the hemoglobin levels of the diabetic rats.

**Packed Cell Volume (PCV) Levels (%)**

The fig. 4 depicts the mean PCV levels of the groups T1, T2, T3, T4 and Tson 0th, 14th and 28th day of the experiment. The mean PCV values of groups T1, T2, T3, T4 and Tson on 0th day were 39.55 ± 0.69, 30.34 ± 0.32, 30.37 ± 0.07, 30.90 ± 0.20 and 30.85 ± 0.22% respectively. There is significant difference in the mean PCV levels of the group T1 and groups T2, T3, T4 and Tson 5% level of significance. The levels of PCV of the groups T2, T3, T4 and T5 does not varied significantly. There is significant decline in the mean PCV levels of the groups T2, T3, T4 and Tson 0th day of the experiment than the group T1 (Normal Control).

The mean PCV values of groups T1, T2, T3, T4 and Tson 14th day were 40.29 ± 0.62, 28.10 ± 0.21, 36.77 ± 0.22, 38.09 ± 0.59 and 42.71 ± 0.13% respectively. There is significant difference in the mean PCV levels of the groups T1, T2, T3, T4 and Tson 5% level of significance. There is significant improvement in the mean PCV levels of the diabetic rats treated with the hydroethanolic leaf extract of C. roseus (400mg/kg) followed by treatments with the metformin (100 mg/kg) and the hydroethanolic leaf extract of C. roseus (200mg/kg).

The mean PCV values of groups T1, T2, T3, T4 and Tson 28th day were 40.55 ± 0.47, 27.08 ± 0.14, 38.5 ± 0.25, 38.41 ± 0.37 and 43.58 ± 0.24% respectively. There is significant difference in mean PCV levels of the groups T1, T2, T3, T4 and Tson 5% level of significance. The treatments with the hydroethanolic leaf extract of C. roseus 400 mg/kg increased the lowered PCV levels of the diabetic rats and were found to be higher than the normal levels. The treatments with the metformin (100 mg/kg) and the hydroethanolic leaf extract of C. roseus (200 mg/kg) to the groups T1, T2, T3, T4 and Tson 5% level of significance. However the effect was less than that of the treatments with the hydroethanolic leaf extract of C. roseus 400 mg/kg.

The above results suggest that the PCV levels get significantly declined in the diabetes. The standard drug Metformin treated rats of group T1 were made significant improvement in the PCV levels on consecutive period of days of the experiment. The diabetic rats from the groups T2 and T3 treated with hydroethanolic leaf extract of C. roseus at the dose of 400 mg/kg and 200 mg/kg respectively showed significant increase in the PCV levels of the diabetic rats than the rats from the normal group T1 and diabetic group T2 on 28th day of the experiment.

**Body Weight**

As shown in fig. 2, there was a constant increase in body weights of rats from normal group (T1). Whereas in diabetic control group of rats (T2) there was a consistent decrease in weight from its initial weight of 156.33 ± 0.61 to 138.83 ± 3.49 on 28th days of study. In treated groups it has been observed that the average weight were increased up to 28th day of treatment, however, this increased was less as compared to weights of normal group of rats at the same time in all the treated groups (T3, T4 and T5) weights of rats were significantly increased as compared to diabetic group (T2) from the results obtained it is evident that the weight loss in this diabetic rats was due to emaciation, in appetite as a diabetic complications on stated by Braslasu et al. (2007).

As per the results obtained in the present study metformin @ 100 mg/kg body weight not only controls the loss of weight but also caused gain in the weights from its 162.41 to 171.16 ± 0.74. The groups of rats under study treated with C. roseus @ 400 mg/kg and 200 mg/kg showed comparable
results with the standard drug metformin and could present the loose in body weight and also there was an increase in weights of rats from 164.16±2 to 172.66±1.25 and from 161.66±2.78 to 169±2.52 respectively. The improvement in the body weight of the diabetic rats with the treatment of the hydroethanolic leaf extract of *C. roseus* is in agreement with the observations made by Nayak (2006) [17], Jayanthi *et al.* (2010) [10] and Hikmah *et al.* (2015) [8].

**Conclusion**

The blood glucose levels were increased in the rats due to streptozotocin induced diabetes and were decreased by the treatment with hydroethanolic leaf extract of *C. roseus* at the dose rate of 400 mg / kg followed by 200 mg/kg as used in the present study. This indicates that the hydroethanolic leaf extract of *C. roseus* possess the antidiabetic property. The hematological parameters (Hb & PCV) and body weights of rats were altered due to streptozotocin induced diabetes and corrected to normal with the treatment of *C. roseus*.

**Fig 1-2:** Mean Blood Glucose Levels by digital glucometer observed in different groups (mg/dl) and Mean Body weight observed in different groups (gms/week)

**Fig 3-4:** Mean Haemoglobin Levels observed in different groups (g/dl) and Mean Packed Cell Volume Levels observed in different groups (%)

**References**


