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**Original Article**

# Anti diabetic activity of aqueous extracts of leaves and stem barks of *Woodfordia fruticosa* in animal model

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## ABSTRACT

The purpose of this study was to investigate the antidiabetic potential of aqueous extract of leaves and stem barks of *Woodfordia fruticosa* in both normal and alloxan-induced diabetic rats. 200mg/kg alloxan was induced in albino rats by single intraperitoneal injection. Albino rats were divided into five group's normal control, diabetic control, test groups and standard. Aqueous extracts of leaves & stem barks of *Woodfordia fruticosa* at a dose of 200 mg/ kg were administered orally to test groups individually for antidiabetic activity. Glibenclamide (500 ug/kg) was taken as standard drug. Blood glucose level was determined on alloxan induced diabetic rats by one touch blood glucose monitoring system on 0, 1, 2, 3, 4, 5 and 6 hrs and body weight of diabetic rats were measured on 0, 2, 4, 6, 8, 10 and $12^{th}$ day. All aqueous extracts were observed significantly (P›0.05) suppress elevated blood glucose levels and increased body weight in diabetic rats. Preliminary phytochemical screening revealed that phytoconstituents tannin, terpenoids, saponins and flavonoids are present in aqueous extracts of *Woodfordia fruticosa* leaves and barks. Antidiabetic property of *Woodfordia fruticosa* may be due to the presence of phytoconstituents saponins and flavonoids, which increased the sensitivity of insulin or stimulation of secretory cells of insulin and suppress the increased blood glucose level in alloxan, induced diabetic rats. The studies indicate that the aqueous extracts of leaves and stem barks of *Woodfordia fruticosa* exhibited statistically significant antidiabetic activity in alloxan induced diabetic rats.

**KEY WORDS**: Diabetes, Antidiabetic drugs, *Woodfordia fruticosa*, Dhawai

## INTRODUCTION

Diabetes mellitus is a metabolic disorder which is characterized by chronic hyperglycemia. About 220 million people are affected by diabetes worldwide [1]. It is predicted that this number may exceed to 435 million in 2030 [2]. One of the most critical complications of diabetes is post-prandial hyper-glycemia. A large number of studies have demonstrated that oxidative stress & non enzymatic protein glycation are closely associated with the development of diabetes mellitus [3-4]. Diabetes mellitus is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid & protein metabolism [5]. Chemically induced type -1 diabetes is the most commonly used animal model of diabetes. Chemical agents which produce diabetes can be classified into three categories, and include agents that (a) specifically damage β- cell. (b) Cause temporary inhibition of insulin production and or secretion. (3) Diminish the metabolic efficacy of insulin in target tissues [6].Glucosidase inhibitors and α-amylase inhibitors are class of compounds that help in managing hyperglycemia [7]. The modern medicines such as sulphonylureas, biguauanides, thiazolidinediones and meglitinide derivatives available in market for management of diabetes which create serious side effects such as hepatotoxicity, abdominal pain, flatulence, diarrhea, and hypoglycaemia [8-9]. Herbal drugs are recommended for treatment of diabetes due to their fewer side effects, easily available and low cost. World Health Organization has also recommended the development of safe natural herbal medicines in this concern [10-11].

*Woodfordia fruticosa* (lythraceae) flowers are acrid, astringent and styptic. It is useful in the treatment of various diseases like dysentery, haemorrhoids, impaired hepatic function, fever, headache, ulcer and wounds, leucorrhoea, menorrhagia and it is also considered as a safe stimulant in pregnancy [12-13]. Some pharmacological screening was done by researchers

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on flowers of *W. fruticosa*. It is important to note that there are some reports about the antidiabetic activity of leaves and flowers have already been reported but stem barks of *Woodfordia fruticosa* antidiabetic activity has not been reported till date. Present study was carried out to identify the targets that can confirm and support the antidiabetic potential of above mentioned plant.

## MATERIALS AND METHODS

**Plant collection and identification:** The plant specimens were collected from the forests of Jashpur district of Chhatisgarh.The specimens were identified & authenticated by Botanist, from Guru Ghasidas Vishwavidyalaya, Bilaspur; C.G. The voucher specimen SLT/Med.Plant/01/2009 for *Woodfordia fruticosa* was maintained in research laboratory for further reference in SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur, C. G.

**Preparation of Plant Extracts:** The shade dried plant materials leaves and barks of *W. fruticos*a were powdered by using hammer mill and hand grinder. Preparation of the aqueous extracts of the powdered samples was separately done successively in a continuous hot soxhlet extractor using distilled water.

**Phytochemical screening:** The dried leaves powder and stem barks powder (100gm) were extracted successively with distal water using soxhlet extractor (48 hrs separately for both powder). Finally the extracts were concentrated under vacuum pressure and it is subjected to various phyto chemical tests for identification of different chemical constituents present in their respective extracts. Phytochemical tests revealed that tannin, terpenoids, saponins and flavonoids are present in this plant extract.

**Animals:** Albino Wistar rats (200-250 gm) were obtained from the animal centre of SLT Institute of Pharmaceutical Sciences, Bilaspur; G.G.V., C.G. Animals were housed in groups of five in polypropylene cages at room temperature of 25±1°C with free access to food and water ad libitum. Pharmacological activities were carried out in accordance with the rules governing the use of laboratory animals as accepted internationally and guide lines set by the institute of animal ethical committee (IAEC).

**Experimental protocol**: All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC); Registration no is 994/a/Go/06/CPCSEA of SLT Institute of Pharmaceutical Sciences, Bilaspur; C.G, constituted under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical guidelines were strictly followed during all the experiments.

**Preparation of test sample:** All the aqueous extracts of leaves and stem barks of *W.fruticosa* were suspended in 5% Tween 80 separately by using pestle and mortar.

**Statistical analysis:** All values are expressed as Mean ± SEM. The data obtained were statistically analysed by one way analysis of variance (ANOVA) followed by Dunnett's comparison test. $P < 0.05$ was considered to be statistically significant. The above mentioned statistical analysis was carried out using "Graph pad Instat" for Windows XP, Graph Pad Software, San Diego California USA.

**Acute oral toxicity:** Healthy Albino Wister rats (200-250 gm) of either sex were selected for study. These animals were starved and orally fed the extracts of leaves and stem barks of *W.fruticosa*, in increasing dose level of 50, 100, 250, 500, 1000, 2000 and 5000 mg/kg body weight. Rats were observed for 24 hrs for any lethality [14-15]. No lethality and toxicity was observed in study.

**Determination of antidiabetic activity:**

**Alloxan induced diabetic rats:** The Albino Wister rats were divided into five different groups of six animals each. Albino rats were fasted for overnight and optimum care was exercised to avoid carophagia. Diabetes induced by a single intraperitoneal injection of alloxan dissolved in a freshly prepared 0.15 M sodium acetate buffer (PH 4.5) at a dose of 200 mg/kg body weight [16]. Aqueous extracts of leaves & stem barks *of W. fruticosa* were used individually for their antidiabetic activity in albino rats. Non diabetic control group received normal saline while diabetic control received alloxan and vehicle and test groups received extracts.

Rats were divided into the following five groups; each group contained six albino rats.

Group 1: Normal control

Group 2 (Diabetic control): Diabetic rats received 0.5 ml of 5% tween 80.

Group 3 (Standard): Diabetic rats received 0.5 ml of 5% tween 80 containing glibenclamide (500 ug/kg).

Group 4 (Test group): Diabetic rats received aqueous extract of *W.fruticosa* leaves at a dose of 200 mg/kg in 0.5 ml of 5% tween 80.

Group 5 (Test group): Diabetic rats received aqueous extract of *W.fruticosa* stem barks at a dose of 200 mg/kg in 0.5 ml of 5% tween 80.

Blood was collected from the tail. The assessment of blood glucose was carried out on 0, 1, 2, 3, 4, 5 & 6 hr after oral administration of extracts and effect of body weight of albino rats were observed and recorded on days of 0, 2, 4, 6, 8, 10 and 12$^{th}$ day.

## RESULTS AND DISCUSSION

Diabetes mellitus is a very common chronic disease. It is well documented that alloxan induce diabetes is damaging the insulin secreting cells of the pancreas leading to hyperglycaemia [17]. Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissue is the fundamental mechanism underlying hyperglycemias in the diabetic state [18]. The experiment reveals that leaves and barks aqueous extract of *W.fruticosa* exhibit decrease in the elevated blood glucose levels significantly. It is found, that before treatment of alloxan blood sugar level of all the groups of normal rats were shown 76-82 in zero hrs to 6 hrs (Table 1, fig 1). After treatment of alloxan blood glucose levels were increased slowly with the increasing of hrs. Blood sugar levels indicated continuous increase in both test groups and diabetic control groups respectively as the hour's time passed except normal control group (Table 2& fig 2). Effect of aqueous extract of leaves and stems bark of *W.fruticosa* was observed that blood sugar levels lowering on albino rats significantly (P›0.05) as compared to glibenclamide (Table 3 & fig 3). Body weights of diabetic rats were decreased as compared to normal albino rats. Study exhibited that after the treatment of aqueous extract of *W.fruticosa* on diabetic albino rat's body weight increased significantly as the days went on (Table 4 & fig 4). Phytochemical screening revealed that chemical constituents' tannin, terpenoids, saponins and flavonoids are found in *W.fruticosa*. Literature revealed that some phytoconstituents such as tannin, terpenoids, saponins, glycoside and flavonoids are having antihyperglycemic property [19-23]. Flavonoids are the effective constituents, which have been used in clinical treatment of diabetes to improve the sensitivity of insulin [24]. Saponins are phytoconstituents, which inhibit carbohydrate digestive enzymes and stimulating of insulin secretion [25]. The effect of aqueous extract of *W.fruticosa* on the body weight of diabetic rats may increase the sensitivity of insulin or stimulating of insulin secretion and prevent the tissue damage. Antidiabetic property of *W.fruticosa* may be due to the presence of flavonoids or saponins which can increase the sensitivity and stimulation of insulin secreting cells to secrete insulin of the pancreas and lowering the blood glucose levels in diabetic rats.

## CONCLUSION

In conclusion, the aqueous extract of the leaves and stem barks of *W. fruticosa* at the dose of 200 mg/kg was found to be effective in alloxan induced diabetic rats. And plant can be a future potential medicine for the treatment of diabetes, thus it supports the claim of traditional use of the plant for treatment of diabetes.

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**Table 1: Observation of blood glucose level in normal rats**

| Group | Blood glucose level (mg/dl) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0 h** | **1h** | **2h** | **3h** | **4h** | **5h** | **6h** |
| Control (normal saline) | 80.04± 1.42 | 80.12± 2.04 | 80.74± 2.60 | 80.09± 2.56 | 80.89± 2.06 | 80.78 ±2.08 | 80.92 ±2.48 |
| Diabetic rats (tween 80) | 79.56± 2.73 | 79.09± 1.32 | 79.14± 2.36 | 79.45± 2.81 | 79.04± 2.82 | 79.08 ±2.83 | 79.12 ±2.85 |
| Diabetic rats + Standard (glibenclamide) | 76.98± 2.78 | 77.01± 2.81 | 76.99± 2.30 | 76.98± 2.63 | 77.02± 2.62 | 77.00 ±2.85 | 76.98 ±2.80 |
| Diabetic rats +Aq ext of *W. fruticosa* leaves | 79.82± 2.08 | 79.85± 2.12 | 79.89± 2.13 | 79.90± 2.09 | 79.56± 2.85 | 80.01 ±2.41 | 79.99 ±2.06 |
| Diabetic rats +aq ext of *W.fruticosa* barks | 80.52± 2.95 | 80.56± 1.98 | 80.82± 1.82 | 80.62± 2.43 | 80.75± 2.86 | 80.69 ±2.89 | 80.70 ±2.92 |

Values are expressed Mean ± SEM.

**Table 2: Observation of blood glucose level after intraperitoneal treatment of alloxan in albino rats**

| Group | Dose | Blood glucose level (mg/dl) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 h | 1h | 2h | 3h | 4h | 5h | 6h |
| Control | 200mg | 80.42± 2.36 | 80.89± 3.65 | 79.56± 2.45 | 81.67± 3.76 | 80.43± 3.72 | 81.82 ±2.62 | 80.39 ±2.80 |
| Diabetic rats (tween 80) | 200mg | 79.82 ±2.08 | 80.03± 1.03 | 81.38± 1.85 | 98.48± 3.58 | 119.42 ±3.62 | 126.62±2.85 | 155.69±3.29 |
| Diabetic rats + Standard (glibenclamid) | 200mg | 76.54± 2.84 | 81.38± 2.89 | 86.56± 3.50 | 95.98± 3.82 | 120.84 ±4.08 | 129.09±3.11 | 165.52±2.34 |
| Diabetic rats +Aq ext of *W. fruticosa* leaves | 200mg | 79.25± 2.03 | 82.92± 2.94 | 89.42± 4.35 | 99.45± 3.49 | 134.09 ±2.95 | 145.08±2.82 | 169.65±2.74 |
| Diabetic rats +aq ext *W.fruticosa* barks | 200mg | 80.50± 3.18 | 82.24± 2.99 | 92.32± 3.29 | 112.45 ±2.56 | 128.98 ±3.98 | 148.52±2.08 | 198.82±2.98 |

Values are expressed Mean ± SEM.

**Table 3: Effect of aqueous extracts of *W. fruticosa* leaves and barks on alloxan induced albino rats.**

| **Group** | **Dose** | **Blood glucose level (mg/dl)** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **0 h** | **1h** | **2h** | **3h** | **4h** | **5h** | **6h** |
| Control | | 80.62± 2.56 | 78.32±3.82 | 81.54± 2.59 | 80.42±2.62 | 79.62± 3.41 | 81.64± 3.84 | 80.45 ±3.12 |
| Diabetic rats (tween 80) | | 270.42± 3.51 | 269.06±2.54 | 271.54 ±2.62 | 268.62± 2.68 | 270.82 ±2.62 | 269.28 ±3.68 | 268.92 ±3.85 |
| Diabetic rats + Standard (glibenclamid) | 500ug | 271.68± 3.26** | 238.52±3.62** | 206.62 ±2.58** | 173.18± 3.68** | 152.65 ±2.52** | 134.37 ±2.90** | 103.29 ±2.89** |
| Diabetic rats +Aq ext of *W. fruticosa* leaves | 200mg | 268.57±2.82** | 255.42±2.85* | 234.63 ±2.69** | 216.16± 3.57** | 201.30 ±3.64** | 190.52 ±2.56** | 176.52 ±3.56** |
| Diabetic rats +aq ext of *W.fruticosa* barks | 200mg | 278.62±3.31** | 248.28±2.63** | 223.52 ±4.24** | 212.52± 3.20** | 194.39 ±3.27** | 185.92 ±2.41** | 162.38 ±2.54** |

Values are expressed Mean ± SEM, **P‹0.01,*P<.05, ns-P›0.05

**Table 4: Effect of aqueous extracts of *W. fruticosa* leaves and barks on % loss of body weight in alloxan induced diabetic rats.**

| **Group** | **Dose** | **% loss in body wt** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **0 day** | **2 day** | **4 day** | **6 day** | **8 day** | **10 day** | **12 day** |
| Control | | 25.85± 2.52 | 29.54 ±2.06 | 36.32 ±2.57 | 38.82± 2.64 | 40.12± 2.62 | 43.53± 2.85 | 43.87± 3.65 |
| Diabetic rats (tween 80) | | 21.86± 3.50 | 34.32± 3.52 | 39.36± 2.56 | 46.53± 2.49 | 58.52± 3.64 | 62.56± 2.59 | 69.53± 2.83 |
| Diabetic rats + Standard (glibenclamide) | 500ug | 23.64±2.64 | 27.53± 2.62 | 32.62± 4.82 | 39.61± 3.62 | 45.62± 2.64 | 49.42± 3.48 | 62.70± 3.85 |
| Diabetic rats +Aq ext of *W. fruticosa* leaves | 200mg | 20.85±2.36 | 25.54± 3.58 | 29.52± 3.42 | 33.84± 3.06 | 48.53± 3.92 | 54.36± 2.82 | 60.52± 2.94 |
| Diabetic rats +aq ext *W.fruticosa* barks | 200mg | 25.63±2.85 | 28.20± 2.53 | 34.24± 2.48 | 39.42± 3.52 | 45.48± 3.59 | 51.85± 3.80 | 58.49± 2.43 |

Values are expressed Mean ± SEM.

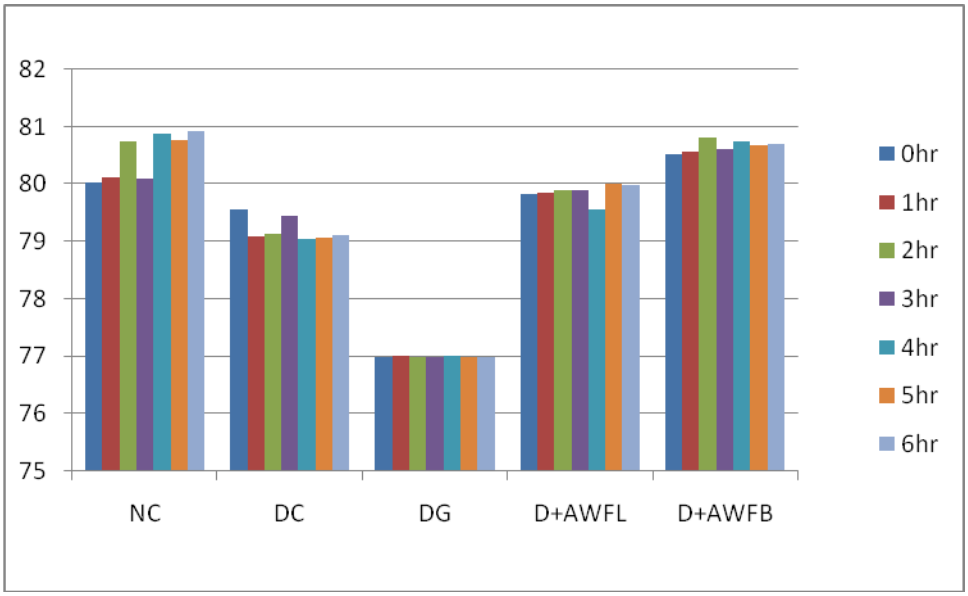


Fig 1: Graphical representation of blood glucose level before treatment of alloxan in albino rats. Notes- (NC-normal control, DC- Diabetic control, DG- Diabetic& glibenclamide, D+AWFL- Diabetic& Aqueous extracts of *W.fruticosa* leave, D+AWFB- Diabetic& Aqueous extracts of *W.fruticosa* bark.

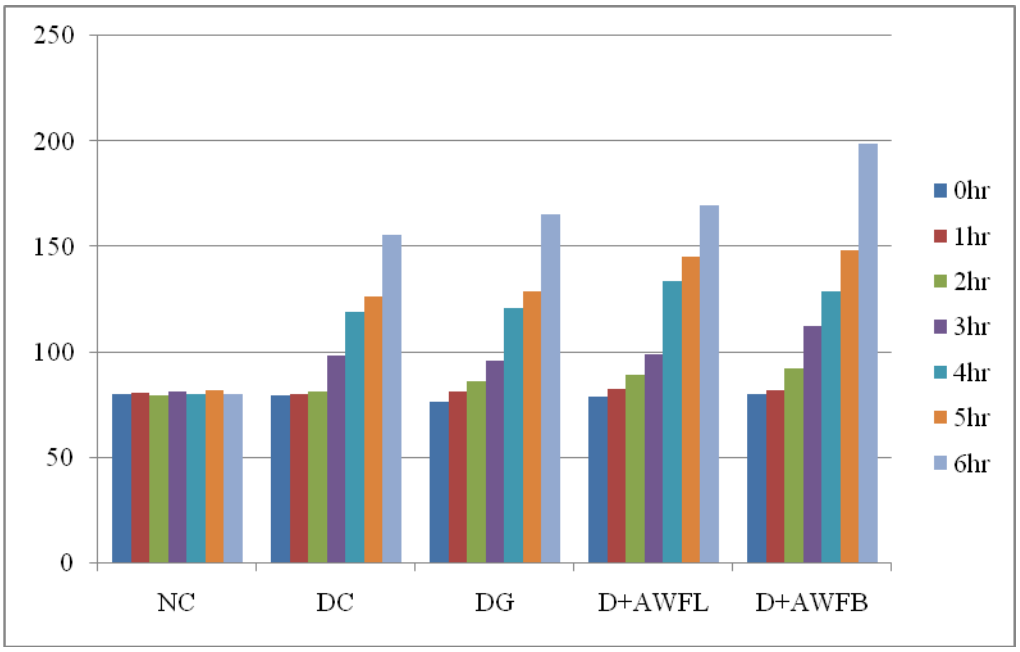


**Fig 2: Graphical representation of blood glucose level after intraperitoneal treatment of alloxan in albino rats.** Note-(NC-normal control, DC- Diabetic control, DG- Diabetic& glibenclamide, D+AWFL- Diabetic & Aqueous extracts of *W.fruticosa* leave, D+AWFB- Diabetic & Aqueous extracts of *W.fruticosa* bark,.

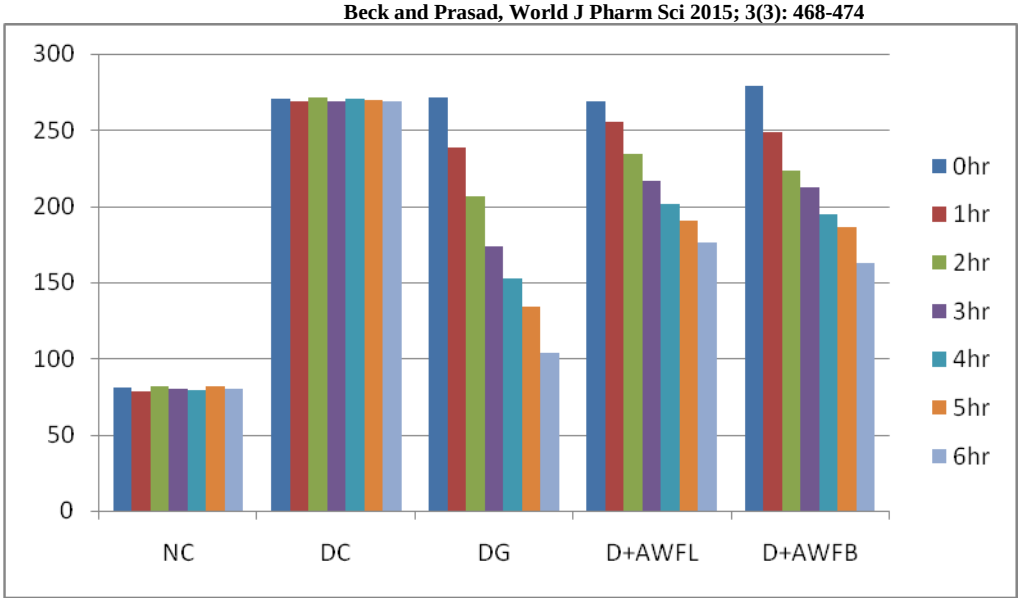


**Fig 3: Graphical presentation of blood glucose level after treatment of aqueous extracts of *W.fruticosa* leaves and barks on alloxan induced albino rats.** Note-(NC-normal control, DC- Diabetic control, DG- Diabetic& glibenclamide, D+AWFL- Diabetic& Aqueous extracts of *W.fruticosa* leave, D+AWFB- Diabetic & Aqueous extracts of *W. fruticosa* bark.

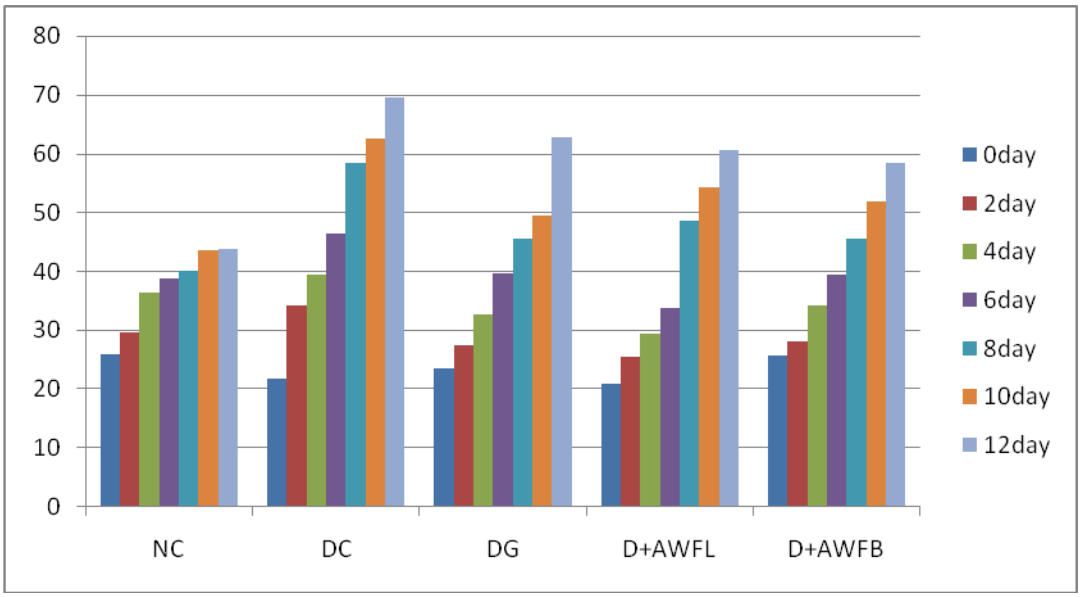


**Fig 4: Graphical presentation on aqueous extracts of *W. fruticosa* leaves and barks on % loss of body weight in alloxan induced diabetic rats.** Note-(NC-normal control, DC- Diabetic control, DG- Diabetic& glibenclamide, D+AWFL- Diabetic& Aqueous extracts of *W.fruticosa* leave, D+AWFB- Diabetic& Aqueous extracts of *W.fruticosa* bark.

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