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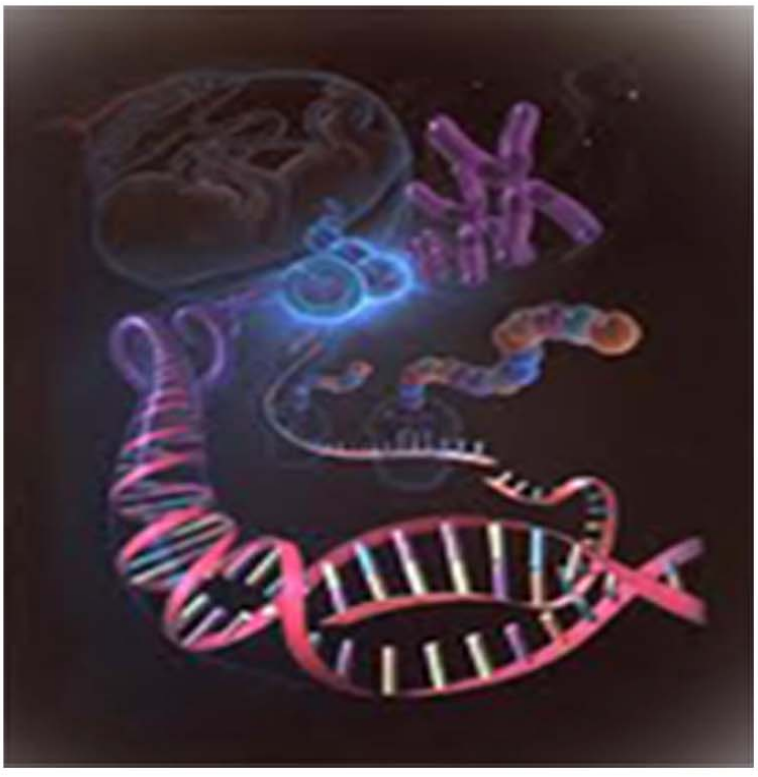

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Research Paper

HYPOGLYCAEMIC ACTIVITY OF ORALLY ADMINISTERED WOODFORDIA FRUTICOSA FLOWER EXTRACT IN ALLOXAN-INDUCED DIABETIC MICE

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Woodfordia fruticosa is a high altitude plant belonging to family Lythraceae that has been used as folkloric medicine for many ailments and diseases. In the present study, the hypoglycaemic effect of orally given flower extract of *Woodfordia fruticosa* in alloxan induced diabetic mice has been assessed. Flower extract was administered orally at variable doses in mice with or without combination with glyburide to different groups of mice (normal and alloxan treated diabetic mice). The blood glucose level, body weight, mortality rate and Oral glucose tolerance test (OGTT) was monitored in various groups. Results showed that the extract also possess a dose dependent hypoglycaemic effect and also a synergistic with drug (glyburide). The administration of extract also reduced the weight loss and mortality rate and augmented the glucose tolerance in both normal and diabetic mice. These results suggested that, the extract possess the synergistic hypoglycaemic activity.

Keywords: Antidiabetic activity, Diabetic mice, Oral glucose tolerance test, *Woodfordia fruticosa*

INTRODUCTION

Diabetes is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or insulin action. The worldwide prevalence of diabetes mellitus is estimated to be 2.8% (Sarah *et al*., 2004). The prevalence of diabetes mellitus, estimated as 285 million people in 2010, is predicted to increase to 439 million people by the year 2030. The majority of this diabetic population will emerge from developing countries (Shaw *et al*., 2010). It increases the risk of many disorders like atherosclerotic arterial disease by 2-6 folds (Sacks, 1997). The aim of therapy in diabetes is to achieve normoglycemia to prevent later microvascular complications (retinopathy, nephropathy, neuropathy and microangiopathy). Intensive therapy to achieve glycemic control has been shown to significantly diminish the risk of long-term complications (DCCT, 2002).

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Many synthetic oral hypoglycemic agents like Sulphonylureas, biguauanides, thiazolidinediones, meglitinide derivatives and α-glucosidase inhibitors are presently in use but they all have several side effects (Edwin *et al*., 2006). Most of the plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect (Malviya *et al*., 2010). This necessitates the use of herbal preparations, plant decoctions or infusions, for their little side effects, easy availability and cost effectiveness.

Hypoglyceamic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes.

Despite the availability of various classes of antidiabetic agents, diabetes mellitus remains a major cause of mortality and morbidity globally (Kokil *et al*., 2010, Roglic and Unwin 2010). As a result, there has been a considerable effort to search for more effective drugs. This has resulted in a renewed interest in research that investigates the health benefits of herbs and natural products including *Woodfordia fruticosa* in the management of diabetes mellitus.

Woodfordia fruticosa belongs to family *Lythraceae,* commonly known as Fire-flame bush and found throughout India, but abundantly in north India up to 1600 m altitude, Extracts and metabolites of this plant, particularly those from flowers possess useful pharmacological/ biological activities and chemical constituents (Das *et al*., 2007). The flowers of the plant have been used in cosmetics (Upadhye and Kumbhojkar 2001). A mixture of *W. fruticosa* powder, honey and rice water is extremely affected in diarrhoea, dysentery and piles. It can be safely used even in pregnancy. The decoction of flowers effectively quenches the excessive thirst, especially in diabetic patients (Paranjpe 2001). The flowers of this plant have various medicinal uses, possess high content of tannins and have been used as an astringent tonic in disorders of mucous membrane (Chopra *et al*., 1956, Anjaria *et al*., 2002).

The present work was premeditated with flowers as the test materials which are usually shredded or thrown away as a waste during autumn season or other reasons. Literature survey revealed that the flowers of *W. fruticosa* have not been studied for different parameters regarding hypoglycaemic and antihyperglycemic activity. Keeping above in view, the present investigation was conducted to study the effect of methanolic flower extract of *W. fruticosa* on blood glucose levels and on the oral glucose tolerance test in alloxan induced diabetic mice.

MATERIALS AND METHODS

Chemicals

Alloxan was procured from Sigma Chemical Co. (St. Louis, MO, USA), Glyburide from (Ranbaxy Pharma. Ltd., New Delhi, India) and Blood Glucose estimation kit from Bayer Health Care LLC, Ireland. All other chemicals were of analytical grade, available locally.

Plant material

Plant Material and Extract Preparation

Flowers of *W. fruticosa* collected from Himachal Pradesh, The flowers were shade dried at room temperature and reduced to coarse powder. The dried and powdered flowers were percolated four times with Methanol at room temperature. The

combined extracts were filtered (Whatmann paper), centrifuged (3200 × g, 4°C and 30 min) and concentrated under reduced pressure in a thin film evaporator at 50 ± 5°C. The paste so formed was dissolved in methanol and concentrated in thin film evaporator. Finally, the extract was completely dried under vaccum in the dessicator. The whole procedure yielded 9-10% (w/w) of the extract in terms of dried starting material.

Animals

Swiss albino mice (25-30 g) maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and water *ad libitum*, housed in the departmental animal house and were exposed to 12 h cycle of light and dark. The experimental protocol was approved by Institutional Animal Ethics Committee and care of the animals was carried out as per the guidelines of Committee For the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No- 107/99/CPCSEA-2011-34).

Acute Toxicity

Acute toxicity of *W. fruticosa* flower extract was determined as described by Malik *et al*. (2007). Mice were given a single oral dose each of 100 mg to 2000 mg/kg body weight. Animals were observed for behaviour and mortality rate. Blood was tested for various parameters (TLC, DLC, and Hb) over a period of one month. Animals were observed for mortality, signs of toxicity and abnormalities (if any) during the experimental period.

Induction of Experimental Diabetes

Experimental diabetes in mice was induced by intraperitoneal (i.p.) administration of aqueous alloxan monohydrate in acetate buffer (0.15 M, pH 4.5) in fasting mice by method of Ozbek *et al*. (2004). Total dose of alloxan (450 mg/kg b.wt.) was administered in three injections at intervals of 48 h (50 mg/kg b.wt. each time). After 48 h animals showing blood glucose level above 200 mg/dl (diabetic) were selected for study.

Animals and Experimental Groups

Diabetic Swiss albino mice of either sex were divided into six groups (n = 6) viz.:

Group I; vehicle (distilled water 10 ml/kg)

Group II; Glyburide (10 mg/kg body weight)

Group III; Extract (100 mg/kg body weight)

Group IV; Extract (400 mg/kg body weight)

Group V; Extract (800 mg/kg body weight)

Group VI; Glyburide treated (10 mg/kg body weight) and extract treated (800 mg/kg body weight).

Collection of Blood and Determination of Blood Glucose

Blood of control and experimental mice was collected from orbital sinus puncture. Blood glucose level was estimated by glucose estimation kit (Bayer Health Care LLC, Ireland).

Acute Study

Effect of flower extract on blood glucose levels in alloxan induced diabetic mice was estimated according to the method of Dunn and McLetchie (1943). The acute study involved the estimation of blood glucose levels at 0, 2, 4, 6, and 24 h after administration of extract and glyburide, with all the doses given at one time.

Sub Acute Study

The subacute study involved the repeated administration of drug for 28 days at prefixed time and blood glucose levels were estimated on 7th,

14th, 21st, and 28th days. Body weight of the mice were also noted during the study period of 28 days and represented as mean change in body weights. The death of the mice was also monitored and percentage mortality was calculated.

Oral Glucose Tolerance Test (OGTT)

Prior to commencement of experiment the animals were fasted overnight. Extract, glyburide and combination of both was administered orally and blood glucose levels were monitored. After 30 min of drug and extract administration, animals were loaded with D-glucose (2.5 mg/kg) solution and blood glucose levels were monitored at 0, 30, 60, and 120 min after glucose loading.

Statistical Analysis

All the results were expressed as Mean ± S.E.M. Data of tests were statistically analyzed using one-way ANOVA followed by Tukey's multiple range test, applied for *post hoc* analysis. A value of $p<0.001$ was considered to be statistically significant.

RESULTS

Acute Toxicity Study

Study revealed that, the extract was safe up to a dose level of 1000 mg/kg body weight. Although at higher doses no lethality was observed but the animals treated with the dose range above 1000 mg/kg b. wt. showed lethargic behaviour.

General Findings

Diabetes in rats resulted in increase urination, such that the cages had to be cleaned two to three times a day, because of the offensive smell. Water consumption by the rats also increased in all groups. These signs appeared approximately 24 h after induction of diabetes mellitus. Upon the introduction of the extract, these symptoms subsided and its offensive smell diminished such that there was no need for the daily cleaning of the cages.

Body Weight

Administration of vehicle (distilled water) in diabetic mice resulted in decrease in body weight during the period of 28 days (Table 1), while the treatment of diabetic animals with different concentrations of flower extract (100, 400 800 mg/kg, b.wt.) prevented the decrease in body weight showing the advantageous outcome of extract administration.

Table 1: Effect of Methanolic Flower Extract of *W. fruticosa* on Body Weight (g) in Alloxan-Induced Diabetic Mice

Dose(mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 28
Control	31 ± 0.32	27 ± 0.94	23 ± 1.30	21 ± 0.15	19 ± 0.12
Glyburide(10 mg/kg)	30 ± 0.54	29 ± 0.61*	31 ± 0.92*	31 ± 0.18*	31 ± 0.35*
Flower extract(100 mg/kg)	28 ± 0.65	27 ± 0.11*	26 ± 0.03*	23 ± 0.63*	20 ± 0.19*
Flower extract (400 mg/kg)	30 ± 0.81	29 ± 0.37*	29 ± 0.36*	29 ± 0.41*	29 ± 1.10*
Flower extract (800 mg/kg)	29 ± 0.26	30 ± 0.46*	30 ± 1.31*	30 ± 1.35*	31 ± 0.11*
Combination	31 ± 0.45	32 ± 0.49*	32 ± 1.21*	32 ± 1.28*	32 ± 0.78*

Note: The results are presented as Mean ± S.E.M (n = 6), data were analyzed by one-way ANOVA followed by *post hoc* Tukey's multiple range test. All the values were significant ($p<0.001$*) as compared with vehicle treated group (distill water, 10 ml/kg)

Mortality Rate

Treatment of diabetic mice with vehicle only resulted in death of 50% of the total animals during the subacute study. However, the extract administration reduced the mortality rate. The extract in combination also resulted in 0% mortality.

Blood Glucose Level

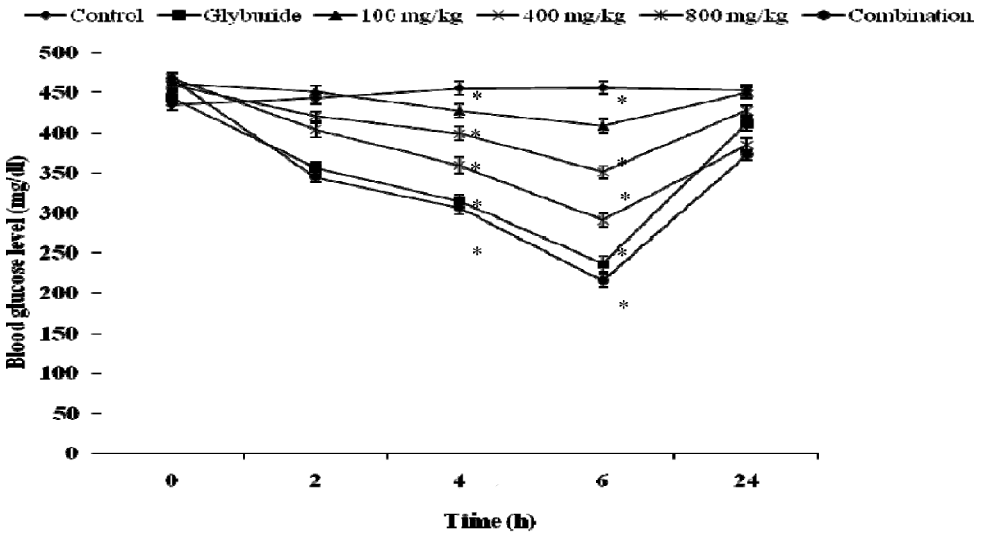
During Acute study administration of flower extract (100, 400, 800 mg/kg, b.wt.), glyburide (10 mg/kg) and the combination of both significantly reduced ($p<0.001$) the blood glucose levels at 2, 4, and 6 h. The onset of anti-hyperglycemic effect of glyburide was 2 h and that of the extract at 800 mg/kg was 4 h (Figure 1). The peak of the effect was attained at 6 h but the effect diminished at 24 h. The combination of glyburide and flower extract exerted the antihyperglycemic effect at 2 h and showed better reduction in glucose levels (26 ± 2.5%) than glyburide alone (19 ± 1.5 %).

Subacute Administration of the extract, glyburide and combination caused a significant ($p<0.001$) reduction in blood glucose level as compared to control (Figure 2). Maximum activity of the extract was observed at 800 mg/kg on 28th day. Similar pattern was observed in glyburide treated group. However, the combination of flower extract and glyburide resulted in a better and significant ($p<0.001$) response in terms of reduction in blood glucose levels. The blood glucose level on day 28th showed by the combination group was 169.97 ± 2.1 mg/dl whereas that of glyburide treated group was 183.50 ± 2.0 mg/dl.

Oral Glucose Tolerance test (OGTT)

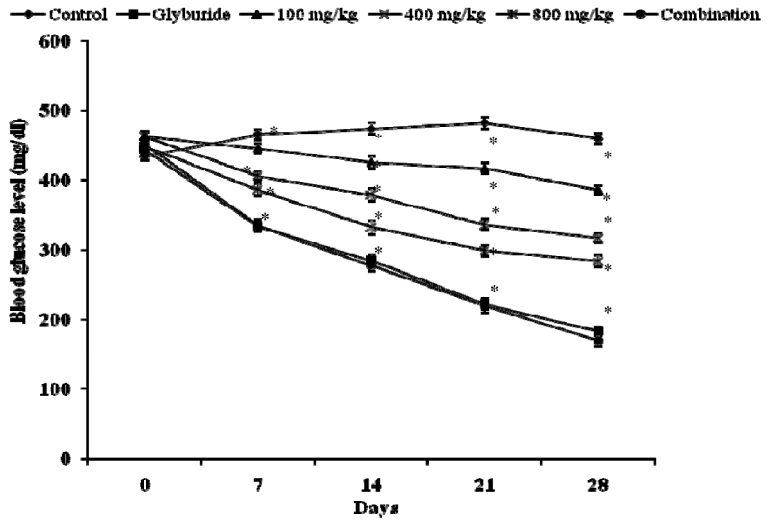
Administration of glucose (2.5 g/kg) increased the blood glucose levels significantly ($p<0.001$) after the 30 min of glucose loading in diabetic mice (Figure 3) and normal mice (Figure 4). Extract, glyburide and combination of extract and glyburide

Figure 1: Effect of Acute Treatment of Methanolic Flower Extract of *Woodfordia Fruticosa*, Glyburide and Combination (Flower Extract and Glyburide) on Blood Glucose Level in Alloxan-induced Diabetes in Mice



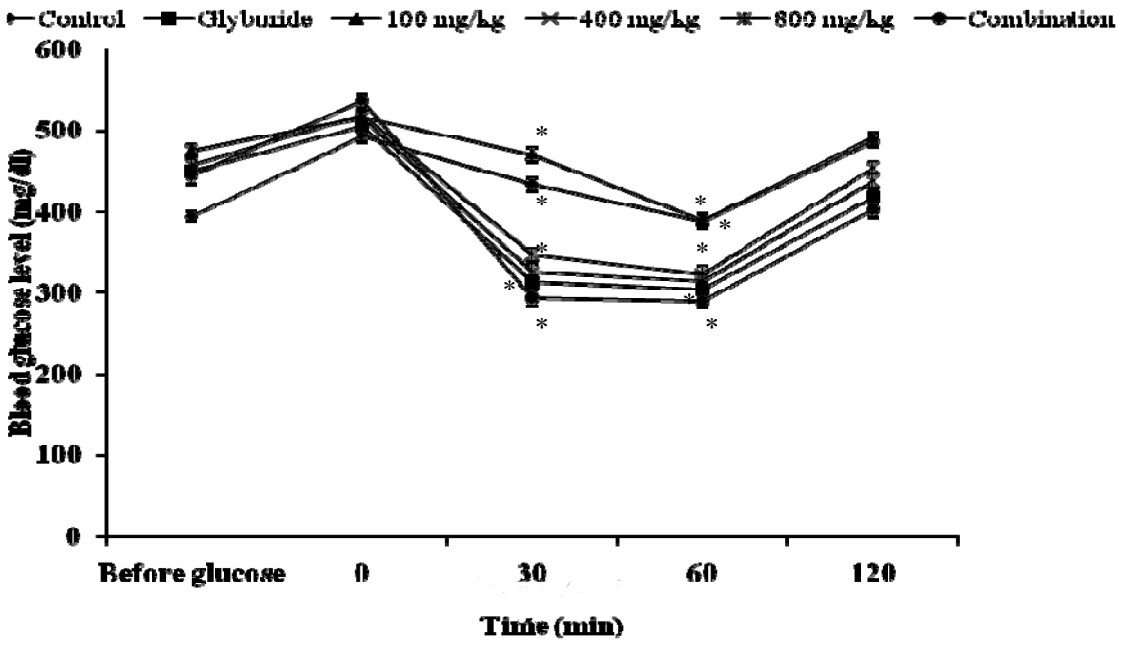
Note: Blood glucose levels were assessed at regular interval of 0, 2, 4, 6 and 24 h hour after administration of extract at different concentration, glyburide and combination. The results are presented as Mean ± S.E.M (n = 6). $p<0.001$* comparison with untreated control

Figure 2: Effect of Subacute Treatment of Methanolic Flower Extract of *Woodfordia Fruticosa*, Glyburide and Combination (Flower Extract and Glyburide) on Blood Glucose Level in Alloxan-induced Diabetes in Mice



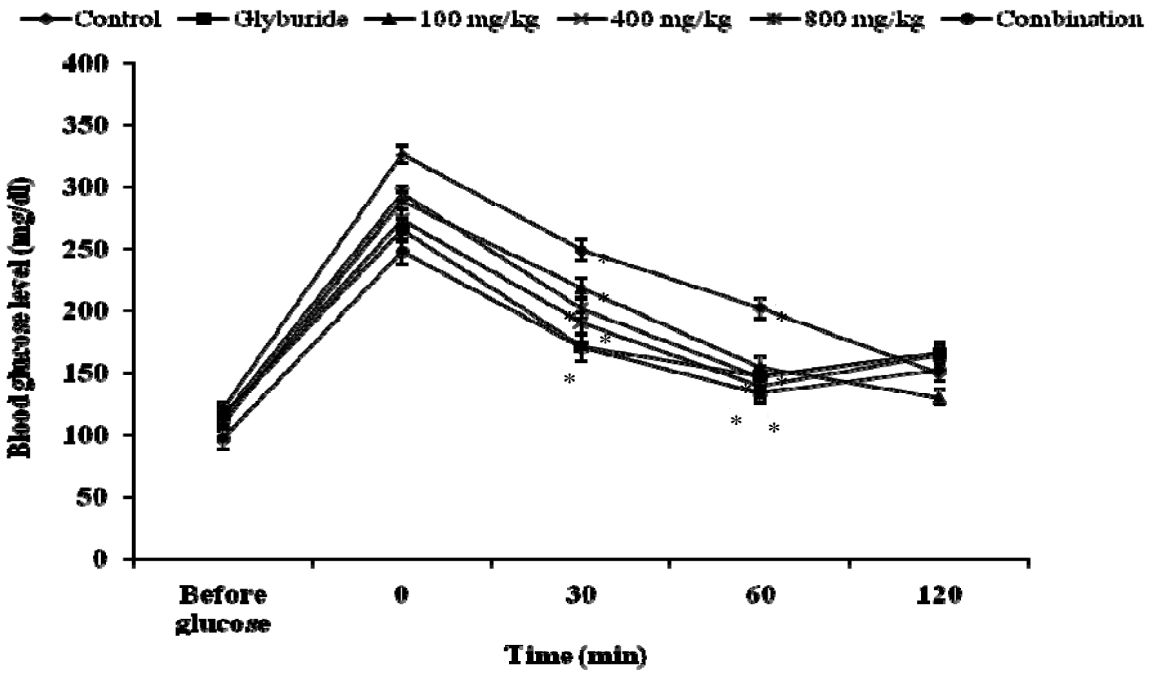
Note: Blood glucose levels were assessed on day 0, 7, 14, 21 and 28 after simultaneous administration of extract at different concentration, glyburide and combination. The results are presented as Mean ± S.E.M (n = 6). $p<0.001$* comparison with untreated control.

Figure 3: Effect of Treatment of Methanolic Flower Extract of *Woodfordia fruticosa*, Glyburide and Combination (Flower Extract and Glyburide) on Blood Glucose Level in OGTT in Diabetic Mice



Note: Blood was collected and assessed for blood glucose levels at 0. 30, 60, and 120 min after loading of glucose in diabetic mice. The results are presented as Mean ± S.E.M (n = 6). $p<0.001$* comparison with untreated control.

Figure 4: Effect of Treatment of Methanolic Flower Extract of *Woodfordia Fruticosa*, Glyburide and Combination (Seed Extract and Glyburide) on Blood Glucose Level in OGTT in Normal Mice



Note: Blood was collected and assessed for blood glucose levels at 0. 30, 60, and 120 min after loading of glucose in diabetic mice. The results are presented as Mean ± S.E.M (n = 6). p<0.001* comparison with untreated control.

produced significant (p<0.001) increase in glucose threshold within 30 min of glucose loading.

DISCUSSION

Numerous plants have been used traditionally to treat diabetes and some of them have been proven scientifically and reported to be hypoglycaemic agent. The hypoglycaemic activity in these plants is assumed to be due to the active principles present. Compounds like polysaccharides (Tomoda *et al*., 1985), flavonoids, terpenoids, tannins (Reher *et al*., 1991) and alkaloids (Karawya and Wahab 1984) etc. have been reported to be responsible for hypoglycemic effect.

Ethanolic extract of *W. Fruticosa* flower has been reported to possess antidiabetic potential (Verma *et al*., 2012). The antihyperglycemic activity of aqueous leaf extract of *W. fruticosa* has also been reported (Cisse *et al*., 2000) and was found to be comparable to known antihyperglycemic agent glibenclamide. *Woodfordia fruticosa* is used in the Indian systems of medicine like Ayurveda, Siddha and Unani (Weersaooriya and Yatawara, 2002). Plant constituents like tannins and flavonoids are well known for their antioxidant and hepatoprotective activities (Alex *et al*., 2004, Hesham *et al*., 2002). The flowers of *Woodfordia fruticosa* contain substantially high amount of tannins, flavonoids and some polyphenolic compounds, that show antioxidant and hepatoprotective properties (Shome *et al*., 1981).

In the earlier finding it has been postulated that the flower extract of *Woodfordia fruticosa* helpful in reducing the blood glucose level (Verma *et al*., 2012), but the studies lacked in detailed effect of *W. fruticosa* flower extract on acute and subacute administration. Our study is an attempt to know more about the effect of *W. fruticosa* on acute, subacute and OGTT pattern in normal and diabetic mice. The acute toxicity showed that animals treated with higher doses exhibited lethargic behaviour, but there was no mortality. Moreover, the body weight and spleen weight did not show any abnormal rise. The weight of lymphoid organ is a good indicator of toxicity. Thus the extract apparently is safe for use. In the current study, the hypoglycemic activity of MEWF was evaluated in alloxan induced diabetic mice. Significant ($p<0.001$) reduction in blood glucose level was observed at 4^{th} h and maximum reduction occurred at 6^{th} h of extract treatment in acute study at the dose range of 800 mg/kg. The combination of glyburide (10 mg/kg, b.wt.) and extract (800 mg/kg) resulted in significant reduction ($p<0.001$) at 2 h which was higher than that of glyburide treatment alone. The more pronounced effect of the extract may be due to the limited or compromised action of insulin in diabetic condition and on the contrary more due to the anti-hyperglycemic principles present in the extract. The effect of MEWF on blood glucose may be due to chemical constituent such as tannins, terpenoids, saponins and flavonoids reported to have antihyperglycemic effect (Matsudha *et al*., 2002; Kambouche *et al*., 2009; Sharma *et al*., 2008). Various compounds such as hecogenin, lupeol, oleanolic acid, ursolic acid, quercetin have also been isolated from *W.fruticosa* which enhance the importance of this plant.

Glyburide is a second generation oral sulfonylurea antidiabetic agent which is used as a diet adjunct to lower the blood glucose levels in diabetic patients. Glyburide act by stimulating the pancreatic islet cells, which result in secretion of insulin. Sulfonylurea acts by binding to and blocking the ATP-sensitive K^+ channel. The drugs thus be similar to the physiological secretagogues like glucose, leucine, and also lower the conductance of this channel. Reduced K^+ conductance causes membrane depolarization and an influx of Ca^{2+} through the voltage sensitivity Ca^{2+} channel. Prolonged administration of glyburide produces extra pancreatic effect and thus contributes to hypoglycemic activity (Shah *et al*., 2006). The results of both acute and subacute study hypothesized the late onset and prolonged duration of the action of extract may be due to the improved pancreatic cytoarchitecture.

Body weight is a sensitive indicator that reflects the state of health of experimental animals and decrease in body weight correlates with defects in body metabolism that is due to toxicity (Heywood, 1983). One of the parameters to consider the amelioration of diabetic state is to ascertain the effect of treatment on the body weight (Al-Attar and Zari, 2010). In diabetes mellitus, deranged glucagon-mediated regulation of cyclic AMP formation in insulin deficiency leads to accelerated proteolysis (Rajasekaran *et al*., 2005). Since structural and tissue proteins contribute to 30 to 40% of total body weight, the excessive breakdown of tissue proteins due to diminished insulin response as well as the unavailability of carbohydrate for energy metabolism in diabetes mellitus results in decreased body weight (Zurina *et al*., 2010). Body weight holds one of the key in evaluating the effectiveness of an antidiabetic treatment (Al-Attar

and Zari, 2010). In the present study, treatment on diabetic rats with *MEWF* showed decrease in body weight loss, which indicates the prevention of muscle tissue damage and protein wasting that is due to hyperglycemic condition, suggesting the potential of *MEWF* in ameliorating diabetic state in Alloxan induced diabetic mice. Moreover, the extract alone or in combination with glyburide protected the weight loss induced by alloxan, which are in corroboration with the earlier studies from other plant extracts (Badole *et al*., 2006). Administration of the extract also reduced the mortality rate from 50 to 33.3% when the dose was given at 400mg/kg, b.wt. Moreover, there was no mortality when the dose of the given extract was at 800mg/kg. Thus, it was apparent that when drug was not administered, progression of diabetes resulted in mortality of mice whereas the extract treatment resulted in reduction of mortality or even no mortality.

The protective effect against diabetes induced weight loss is supported by earlier studies (Swanton-Flatt, 1989). Subacute treatment of extract for 28 days also resulted in reduction of blood glucose level. In the oral glucose tolerance test (OGTT), the doses increased the tolerance of glucose suggesting increased peripheral utilization of glucose in both diabetic as well as non-diabetic mice.

The antihyperglycemic effects observed in alloxan induced and OGTT can be attributed to several mechanisms. The antihyperglycemic activity exhibited by the extract could be through glucose/insulin metabolism and/or by enhancing the peripheral insulin sensitivity (Talpur *et al*., 2002) or by enhancing insulin release by the islets of Langerhans (Gray and Flatt, 1998). However, the exact mechanism for the hypoglycaemic activity shown by the flower extract of *W. fruticosa* is not known.

CONCLUSION

Our results imply that flower extract of *W. fruticosa* contain principles that possibly exert multiple actions involving different mechanisms in exerting hypoglycemic and antihyperglycemic effects. The plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates.

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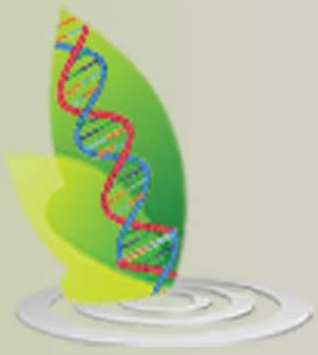

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