

The Evaluation of Soybean Extracts in Alloxan-Induced Diabetic Rabbits

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Abstract: In the present study, antidiabetic potential of Soybean extracts (chloroform and alcohol) has been evaluated in alloxan-induced diabetic rabbits. Twelve healthy, male rabbits were divided into four different groups including, Normal control, Diabetic control, treated with soybean chloroform extract and soybean alcohol extract, with 3 rabbits in each group. The extracts were given orally for 24 days, 50ml extract per rabbit per day. At every 3rd day blood sample was collected, serum separated and glycemic level, total cholesterol level, urea level, uric acid level were determined by kit method. The body weight was recorded on every 6th day's interval. The data obtained revealed that soybean chloroform and alcohol extracts reduced the glucose level, 42.53% and 49.78% respectively. The treatment with these extracts also reduced the cholesterol level, urea level significantly and increased uric acid level and body weight as compared with normal and diabetic groups. Therefore, it is concluded that the soybean possess significant antidiabetic activity.

Key words: Alloxan • Rabbits • Soybean alcohol extract • Glucose • Cholesterol • Diabetes

INTRODUCTION

Diabetes mellitus is one of the main threats to human health in the 21st century because changes in human behavior and lifestyle during the last century have resulted in an increase in the incidence of diabetes worldwide. Hence, the global number of people with diabetes is estimated to rise up to 366 millions in 2030 [1,2].

More than 400 medicinal plants are present worldwide for the treatment of diabetes mellitus, while only few of them have been subjected to scientific authentication as anti-diabetic agents [3]. *Glycine max* (Soybean) belongs to the family Leguminosae or Fabaceae, which contains vegetable protein, oligo-saccharides, dietary fibre, phytochemicals (especially isoflavones) and minerals. The Food and Drug Administration (FDA) USA approved the role of soybean in reducing coronary heart disease and lowers cholesterol level. Also, it has anti-inflammatory and anti-carcinogenic effects on digestive system [4]. In Pakistan soybean is cultivated for oil extraction and therefore, it is easily available [5].

The present study was designed to screen the antidiabetic activity of the chloroform and ethanol extracts of soybean. This study was also made to investigate the effect of these extracts on different biochemical parameters.

MATERIALS AND METHODS

Animals: Adult male rabbits were purchased from local market of Hyderabad. They weighed between 850g to 1545g. They were fed on natural/plant diet and were maintained under standard animal house conditions for 4 weeks of acclimatization. They were provided with diet and water *ad libitum*. All the rabbits were kept in wooden cages.

Plant Material: The soybean seeds (*Glycine max*) were purchased from local seed dealers in Hyderabad, Sindh, Pakistan and identified by experts in Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan.

Chemical: Glucose oxidase (Centronic GmbH, Germany), uric acid, cholesterol (Human, Germany) and urea (Biomerieux, France) kits were used for performing the respected tests. Alloxan monohydrate was purchased from Sigma Chemical Company, USA. All other chemicals used were of analytical grade.

Preparation of Plant Extracts: The healthy soybean seeds were selected and kept in spirit for 5 minutes. They were dried on the filter paper and powdered in mixer grinder 50gms of the powder in 500ml of 30% chloroform and ethanol respectively mixed well and incubated for

24 hours at room temperature. The residue was removed by filtration through muslin cloth first and then through suction pump. Chloroform and ethanol were evaporated by keeping the filtrate in water bath at 80°C for 1 hour. After 1 hour, 10ml concentrated filtrate was mixed with 90ml of distilled water for chloroform extract. In case of alcohol extract 1.0g of concentrated filtrate was dissolved in 100ml distilled water and stirred at magnetic stirrer for 5 minutes before using as daily treatment dose.

Administration of Extract: Poured chloroform extract into the respective drinking troughs once in the afternoon and then free access to water when finished their extract. 50ml of alcoholic extract administered orally per rabbit once in afternoon and then free access to water.

Induction of Diabetes: For the induction of diabetes, alloxan monohydrate was dissolved in sterilized distilled water and given at a dose of 150mg/kg body weight through intravenous injection into the ear marginal vein.

Blood Sample Collection: Blood was obtained from marginal veins at the back of ear with the help of sterilized needle and sterilized syringe and transferred into the appropriate sterilized micro centrifuge tube.

Experimental Design: All rabbits were divided into different groups three in each; normal control, alloxan control, treated with chloroform extract and treated with ethanol extract. All rabbits except normal control were injected with alloxan monohydrate, dose 150mg/kg body weight. After one week the survived rabbits were screened for diabetes and fasting blood glucose above 250mg/dl were selected for the treatment. Treatment continues for 24 days, during treatment period the serum glucose concentration, serum uric acid concentration, serum urea concentration, serum cholesterol concentration and body weight were determined. After 1 week from completion of the treatment, the best selected plant extract (soybean alcoholic extract) was selected and the optimum dose was determined in 10 days time duration.

Statistical Analysis: Data were calculated as mean values and then final value minus initial value, then divided by initial value. The answer was multiplied by 100.

RESULTS

The results of the effect of soybean chloroform and alcohol extracts on serum glucose, cholesterol, urea, uric acid levels and body weight have been shown in Figures 1-5. The effect of different concentrations of soybean alcohol extract has been shown in Figure 6. Figure 1 shows that there was a significant reduction of glucose level after extract administration as compared with diabetic control group. The soybean chloroform and alcohol extracts lowered blood glucose level, showed maximum reduction of 42.53% and 49.78% respectively, on 21st day. Marked increase in serum cholesterol was observed in diabetic control group as compared with the normal group Figure 2. Treatment with soybean extracts lowered the serum cholesterol level and 18.86% and 22.65% reduction was observed at 21st day, by soybean chloroform and soybean alcohol extract respectively.

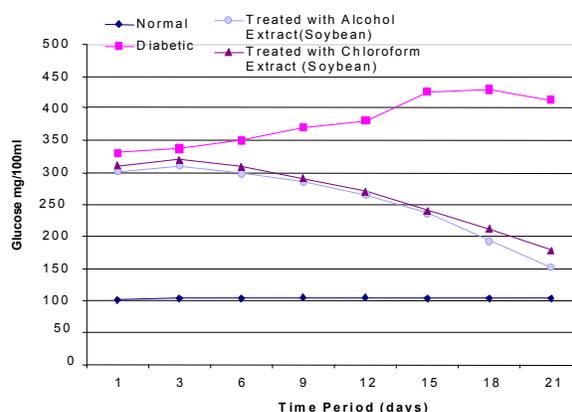


Fig. 1: Effect of Soybean Extracts on Serum Glucose Concentration of alloxan-induced diabetic rabbits

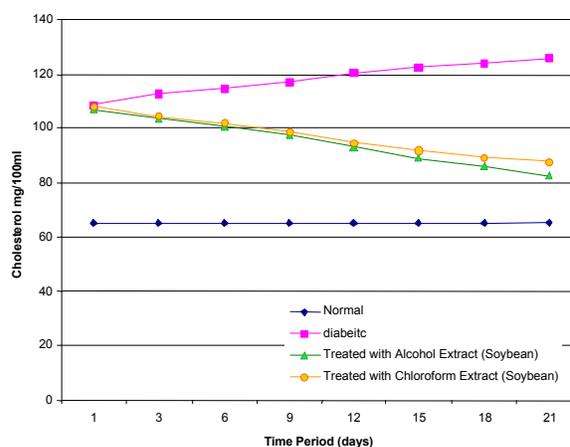


Fig. 2: Effect of Soybean Extracts on Serum Cholesterol Concentration of alloxan-induced diabetic rabbits

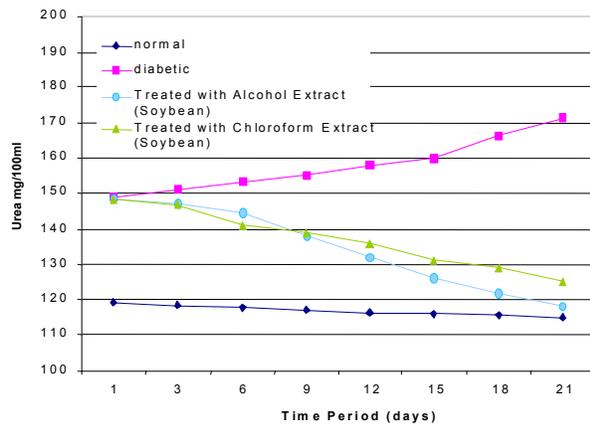


Fig. 3: Effect of Soybean Extracts on Serum Urea Concentration of alloxan-induced diabetic rabbits

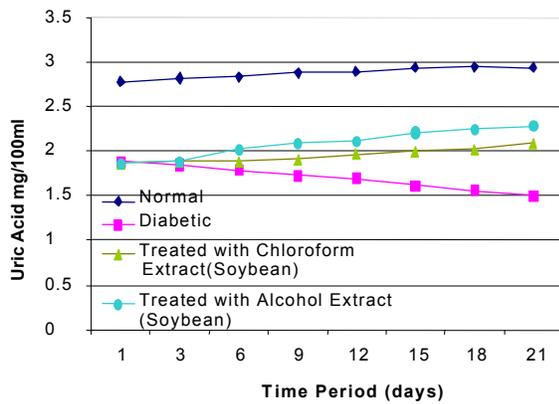


Fig. 4: Effect of Soybean Extracts on Serum Uric acid Concentration of alloxan-induced diabetic rabbits

However, the gradual decrease in serum urea level was observed and, 15.602% and 20.38% reduction was noticed at 21st day of treatment with soybean chloroform and soybean alcohol extracts respectively Figure 3. Serum uric acid level was lower in diabetic control group as compared with the normal group while treatment with soybean chloroform and alcohol extracts increased the uric acid level by 11.94% and 23.12% respectively Figure 4. The effect was more prominent in soybean alcohol extract treated group. Figure 5 shows the effect of soy bean extracts on body weight of alloxan induced diabetic rabbits. The diabetic control group exhibit gradual weight loss as compared with the normal group while weight gain was observed in soybean extracts treated groups. 18.18% and 20% weight gain was noticed in soybean chloroform and alcohol extracts treated groups.

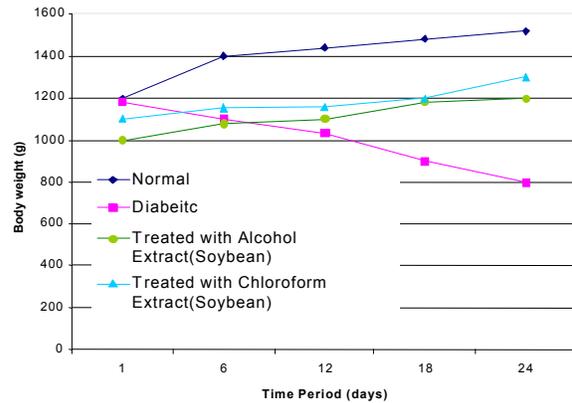


Fig. 5: Effect of Soybean Extracts on Body Weight of alloxan-induced diabetic rabbits

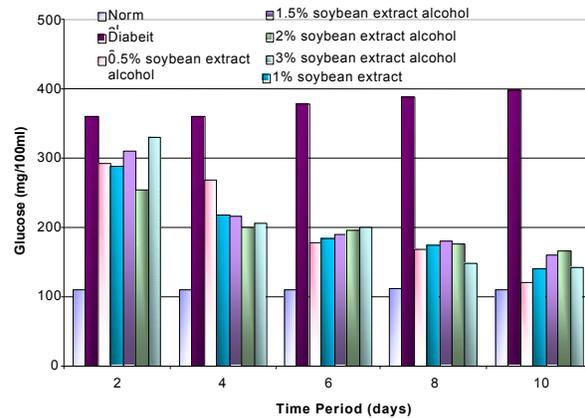


Fig. 6: Effect of different concentrations of Soybean Alcohol Extract on Glucose Level of alloxan-induced diabetic rabbits

Treatment with different concentrations of soybean alcohol extracts showed reduction in glucose level and maximum reduction of 57.31% was noticed with 3% soybean alcohol extract treatment Figure 3.

DISCUSSION

Alloxan acts as a cytotoxin for beta-cells of the islet of langerhans, causes diabetes by inducing cell necrosis [6,7]. The Reactive Oxygen Species mediates the cytotoxic action with the increase in cytosolic calcium concentration, leading to rapid beta-cells destruction [8]. This results into decreased insulin secretion and elevated blood glucose level [9]. This experimental study reveals that alloxan-treated rabbits received soybean extracts showed lower blood glucose level as compared to the diabetic control group may be due to the possibility that

few of beta cells are still surviving and stimulated by extract component (s), releasing insulin.

The extracts exhibited significant reduction of serum cholesterol level in alloxan-treated rabbits. The abnormal high concentration of serum lipids in the diabetic subject is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots [10]. Maintenance of serum cholesterol profile indicates that phytoestrogens and saponins in soybean may exert their role in maintenance [11-13].

Diabetes causes disturbance in renal function so that the blood urea level is elevated. Extract-treated group exhibited reduction in serum urea level [14] that indicates that extract may exert its effect on renal function.

Uric acid is one of the main antioxidant present in the body and diabetes causes reduced levels of uric acid [15]. The extract reflects the antioxidant potential as it reduced the oxidative stress and increase in uric acid level.

Proteolysis, lipolysis and acute fluid loss during diabetes pave the way for weight loss [16]. The weight gain in extract treated groups reflects the correction of body metabolism. Results reveal that the soybean alcohol extract exhibits the antidiabetic activity in a dose dependent manner.

In conclusion, this study indicates that the soybean extracts possess significant antidiabetic activity along with antihypercholesterolemic activity. It also has antioxidant potential for oxidative stress produced by diabetes. The active principle (s) in the extracts may have better performance isolated and purified form.

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