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# Comparative efficacy of four Ayurvedic antidiabetic formulations in alloxan—induced diabetic rabbits

**Md. Rafeeuddin, N. Venkat Rao*, S. M. Shanta Kumar and J. Bheemachari**

Department of Pharmacology, V.L. College of Pharmacy, RAICHUR—584 103 (India)

## Abstract

A number of polyherbal formulations are widely marketed claiming to be very effective in diabetes and other associated diseases. But, the documented reports authenticate that, though there are numerous traditional plants reported to have hypoglycemic properties, many of them proved to be not very effective in lowering blood glucose levels in severe diabetes. Emphasizing these perplexing reports, present study was planned to evaluate the efficacy of four ayurvedic antidiabetic formulations for their antihyperglycemic and antihyperlipidemic effects in alloxan-induced diabetic rabbits. Out of four formulations (A, B, C and D) studied formulation– A exhibited extremely significant antihyperglycemic activity for satisfactory duration and also showed a significant activity as an antihyperlipidemic agent compared to other three and tolbutamide. This study provides a ready reference for the selection of an appropriate formulation in the clinical practice and hence effective rational therapy; the overall theme of health sciences.

**Key Words:** Antihyperglycemic, antihyperlipidemic, ayurvedic formulation, diabetes, efficacy, serum lipids.

## Introduction

Herbal formulations have been used by the majority of Indians since ancient times. In recent years, there has been an increased inclination towards the herbal formulations due to the trend towards the natural sources and a healthy life style. Moreover, the complexity, side effects and costly treatment associated with the allopathic medicines have caused both the health care practitioners and the majority of world populations to turn towards alternative therapies, more likely towards the herbal medicines (Ranjit 1992), since, these systems are believed to be free from side effects and affordable.

Ayurvedic formulations are used to treat a wide variety of diseases including diabetes mellitus described as "madhumeha" in ayurvedic texts. Diabetes mellitus is a heterogeneous metabolic disorder, characterized by altered carbohydrate, lipid and protein metabolism. It has been estimated that, about 1.3 % of the world population suffer from this disease. But, most of the hypoglycemic agents and hypolipidemics used in allopathic practice to treat diabetes mellitus and hyperlipidemia are reported to have side effects in long term use (Khan and Shechter 1991, Speight 1987). Hence, there is the need to search for effective and safe drugs for these ailments. Pharmaceutical research across the world shows that, natural products are potential sources of novel molecules for drug development (Marles and Farnsworth 1995).

In this regard, a number of herbal formulations are marketed, claimed to be useful in diabetes

*Corresponding author: nvenkatrao123@rediffmail.com

and other associated diseases. However, there are reports that, though numerous traditional medicinal plants are reported to have hypoglycemic properties, many of them proved to be not very effective in lowering blood glucose levels in severe diabetes (Nagarajan *et al.* 1987). Hence, there is need to explore herbal medicines in the context of modern science and validate accordingly. In the last decade, WHO had passed many resolutions vis-à-vis improving the quality and efficacy of plant drugs (Subramoniam 2001). Documented reports suggest the presence of hypoglycemic, antihyperlipidemic and other activities of plant's materials used in the 4 marketed herbal formulations A, B, C, D (Bhattachary 1995, Chattopadhyay 1998, Babu and Srinivasan 1997, Dwivedi 1996, Thompson 1990, Expert panel 1993, Aiman 1970, Shaila *et al.* 1997, Dubey *et al.* 1993, Mukharjee and Mukharjee 1987, Goyal *et al.* 1999, Dixit *et al.* 1986, Ponnachan *et al.* 1993 and Srinivasan 1999). Hence, in the present study, the efficacy of the four marketed ayurvedic antidiabetic formulations for their antihyperglycemic and antihyperlipidemic activities in alloxan-induced diabetic albino rabbits was studied. The results and conclusions of the study provide a ready reference for the selection of an appropriate formulation in the clinical practice and hence effective rational therapy.

## Materials and Methods

All the four formulations used in the study were purchased from the retail chemists and available in the form of tablets and the composition of each formulation as it appeared on the label is as follows:

*FORMULATION – A*

*Powders and Bhasmas Curcuma longa* (100 mg), *Eugenia jambolana* (50 mg), *Swertia chirata* (50 mg), *Shilajit shuddha* (25 mg), *Trivang Bhasma* (25 mg).

*Mass Extracts: Cassia auriculata* (100 mg), *Emblica officinalis* (100 mg), *Enicostemma littorale* (50 mg), *Gymnema sylvestre* (50 mg), *Pterocarpus marsupium* (50 mg), *Tinospora cordifolia* (50 mg) and *Melia azadirachta* (25 mg).

*FORMULATION – B*

*Salacia oblonga* Wall (150 mg), *Tinospora cordifolia* Miers (50 mg), *Emblica officinalis* Gaertn (50 mg), *Curcuma longa* Linn (50 mg) and *Gymnema sylvestre* R.Br. (200 mg).

*FORMULATION – C*

*Gymnema sylvestre* (100 mg), *Eugenia jambolana seed* (120 mg), *Emblica officinalis dried fruit* (60 mg), *Curcuma longa dried rhizome* (60 mg), *Casearіana esculenta* (20 mg), *Enicostemma littorale* (20 mg) and *Asphaltum* (30 mg).

*FORMULATION – D*

*Enicostemma littorale* (33.33 mg), *Phllanthus amarus* (33.33 mg), *Eugenia jambolana* L (33.33 mg), *Melia azadirachta* (33.33 mg), *Terminalia arjuna* (33.33 mg), *Aegle marmelos* (133.33 mg), and *Asphaltum* (66.66 mg).

*Drugs and Reagents*—Tolbutamide was a gift sample from M/s. Albert David, Mumbai. Glucose kit (Dr.Reddy's Labs, Hyderabad), Autoenzyme Cholesterol kit, HDL-Cholesterol kit (Accurex Biomedical Pvt. Ltd., Thane) and other reagents were procured from the regular store supplies.

*Experimental animals*—the study protocol was duly approved by the Institutional Animal Ethical Committee and was conducted in accordance with the National Institute of Health (NIH) guidelines, at an institution, approved by the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA). New Zealand white rabbits of either sex weighing between 1.5 to 2.0 kg were randomly selected. The animals were caged individually in similar environmental conditions and were categorized into six groups (n=6).

*Induction of Diabetes*—the rabbits were injected with freshly prepared alloxan monohydrate 150 mg/kg through subcutaneous route. Blood glucose levels were estimated after 24 h and 48 h to confirm the

development of diabetes. The diabetic rabbits exhibiting persistent blood glucose levels in the range of 320-340 mg/dl were selected to determine the efficacy of the formulations. Alloxan was used since it produces hyperglycemia, hypercholesterolemia and elevated serum lipid peroxidases levels (Cross *et al.* 1987) which is the appropriate model for the present study.

*Experimental Procedure*—all the animals were fasted for 18 hours and given water *ad libitum.* After 18 h of fasting, "zero" hour blood samples were collected from all the animals of each group and then the drug was administered orally to each group as follows. The dose of different formulations was selected by extrapolating the human dose to the animal dose as described by Laurence and Bacharach (Laurence and Bacharach 1964). Since this dose also did not show significant antihyperglycemic activity, the doses were raised to one and half times the animal dose and administered orally by suspending individually in 2% w/v acacia suspension in distilled water.

| | |
|---|---|
| GROUP – I: | 2% Acacia suspension (control) |
| GROUP – II: | Formulation – A (141.75 mg / 1.5kg) |
| GROUP – III: | Formulation – B (105 mg / 1.5 kg ) |
| GROUP – IV: | Formulation – C (86.1 mg / 1.5 kg ) |
| GROUP – V: | Formulation – D (105 mg / 1.5 kg ) |
| GROUP – VI: | Tolbutamide (40 mg/kg), (positive control) |

*Serum analysis* —The blood samples were collected from the marginal ear vein at 0, 2, 4, 6, 8, 12, 24, 30, 36 and 48 h and blood glucose levels were analyzed by using GOD/ POD method (Trinder 1969). The serum triglycerides (Tiffany *et al.* 1974), high density lipoprotein (Burstein N, 1970), and total cholesterol (Allian 1974) levels of all the groups were estimated and compared with the control group. The antihyperglycemic activity of all the four formulations at the time interval 't' was calculated as the percentage of blood glucose reduction at that time with respect to basal blood glucose level. The anti-hyperglycemic activity results are outlined in Table 1 and the anti-hyperlipidemic activity is graphically depicted in Figure 1.

*Statistical Analysis*—the values are reported as mean ± SEM. The data was analyzed by One-Way Analysis of Variance (ANOVA), followed by Tukey-Kramer multiple comparisons. $p$ values lower than 0.05 were considered statistically significant.

## Results

From the results outlined in the Table 1 and depicted graphically in the Figure 1 the following can be deduced.

Out of four formulations A, B, C and D studied, formulations B, C and D exhibited the onset of action around 12 h after administration, whereas, the formulation A showed an earlier onset of action compared to the other three, around 8$^{th}$ h. The formulations B and C exhibited the peak antihyperglycemic effect at 24$^{th}$ h, by reducing the blood glucose levels by 50.03±1.87% and 49.68±1.11% respectively, which is less than that of tolbutamide. But the formulation D showed the peak effect a little later than the other two by reducing the blood glucose level to 55.92±2.16% at 30$^{th}$ h, which is almost comparable with that of tolbutamide (56±1.37%) at the 4$^{th}$ h. Out of the four formulations studied, formulation A exhibited the peak effect at 24$^{th}$ h by reducing the blood glucose level by 67.43±1.22%, which is extremely significant and higher than that exhibited by the tolbutamide. The duration of action of the formulations B and C were between 18-24 h, whereas that of formulation A was more than 28 h.

Similarly out of the four formulations, 'C' exhibited very significant reducing effect on all serum lipids except HDL-cholesterol. On the other hand, 'A' showed the very significant effect on reducing total cholesterol and significant effect on triglycerides, LDL-cholesterol, VLDL-cholesterol and HDL-cholesterol. The formulation 'B' showed the significant effect only on triglycerides and VLDL-cholesterol. On the contrary, the formulation 'D' exhibited no satisfactory reduction of none of the lipids studied.

**Figure 1.** Change in serum lipid levels (mg%) with Ayurvedic antidiabetic formulations in diabetic rabbits

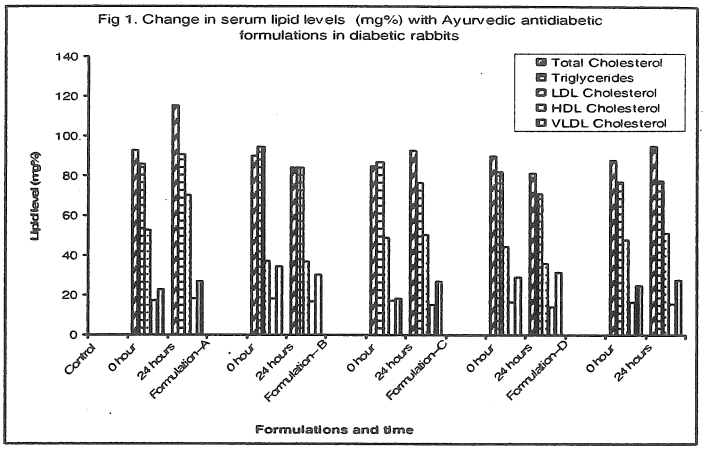


**Table 1.** Antihyperglycemic effect of four Ayurvedic formulations in diabetic rabbits

| Percent blood glucose reduction with different formulations and Tolbutamide [Values are mean ± SEM of 6 replicates] | | | | | |
|---|---|---|---|---|---|
| Time in hours | Formulation A | Formulation B | Formulation C | Formulation D | Tolbutamide (40mg/kg p.o.) |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 5.63±0.45 | 3.87±0.98 | 4.30±2.21 | 2.91±1.70 | 40.68±1.83 |
| 4 | 12.09±0.44** | 12.71±0.09** | 8.69±1.58 | 5.05±1.63 | 56.00±1.37 |
| 6 | 17.54±0.76** | 18.85±2.03*** | 10.96±1.65 | 7.36±1.86 | 51.39±0.37 |
| 8 | 27.62±0.74*** | 24.56±0.41*** | 12.50±2.01 | 11.28±2.09 | 39.63±2.44 |
| 12 | 44.24±2.79*** | 28.59±1.51 | 27.98±0.73 | 29.87±1.45 | 25.10±1.80 |
| 24 | 67.43±1.22*** | 50.03±1.87 | 49.68±1.11 | 42.61±3.47 | 14.99±1.57 |
| 30 | 64.43±0.81*** | 39.90±1.19 | 39.57±0.47 | 55.92±2.16*** | 12.09±1.75 |
| 36 | 43.13±4.20*** | 25.84±0.75 | 20.58±3.52 | 29.66±3.58 | 7.94±2.08 |
| 48 | 6.26±4.61 | 4.57±0.65 | 3.49±1.31 | 5.02±1.77 | 1.19±0.79 |

Values are mean ± SEM, determined before and after treatment at different time intervals.
** Very significant at $P< 0.01$
*** Extremely significant at $P< 0.001$

## Discussion

Diabetic patients suffer from several complications such as retinopathy, nephropathy, atherosclerosis, infections etc. The documented reports indicate that, the incidence of atherosclerosis is high in diabetics (Kannel and Mc Gee 1979). However, no single formulations are available in allopathic practice to treat both diabetes and hyperlipidemia simultaneously. Multi-drug therapy used in the management of these ailments may lead to hazardous drug interactions and side effects in the long run (Khan and Shechter 1991, Speight 1987). On the other hand, there are several polyherbal formulations in the ayurvedic system which are very much useful in the management of these two ailments and are believed to be free from side effects. The concept of efficacy in phytotherapy is based on the mixture of substances contained in the medicinal plants and the polyherbal formulations composed of more than one herbal ingredient have specific time proven therapeutic values (Latha 1993).

In the present study, among the four formulations, formulation A exhibited extremely significant antihyperglycemic effect. It showed the onset of action relatively early (8h) and the duration of action was for a satisfactory period (i.e.>28 hours), whereas, the formulation B showed a weak antihyperglycemic effect, compared to formulation D. The formulations B, C and D showed the onset of action around 12 hours and duration of action for 18–24 hours, which is less than that of A. Formulation C, exhibited a weak antihyperglycemic effect compared to the other three. Based on their antihyperglycemic efficacy and duration of action the four formulations can be ranged in the order of: A > D > B > C.

Similarly, the formulations C and A exhibited highly significant antihyperlipidemic activity on serum lipid profiles compared to others. Between formulations B and D, formulation 'B' showed significant effect only on triglycerides and VLDL-cholesterol. But their effect on total cholesterol and LDL-cholesterol was not quite significant. Depending upon their antihyperlipidemic efficacy the four formulations can be arranged in the following order: C > A > B > D.

When antihyperglycemic effect of all the four formulations were compared among themselves and also with that of tolbutamide, "formulation-A" was found to be superior. It also exhibited optimum antihyperlipidemic activity compared to the control and others. It is very difficult to point out an ingredient responsible for these favorable responses. According to Ayurvedic texts, substances are used in combination in order to get the enhanced desired action and to eliminate unwanted side effects (Nadkarni 1982). The present study was based on single dose administration of the formulations. Indeed, diabetics need treatment for life. Hence, further studies demanding long term treatment with these formulations is under consideration, which would provide more information about the possible mechanism involved in the action of these formulations. Simultaneously, the study also demands for clinical trials in human volunteers, in order to understand their relevance to human subjects.

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