Acta Poloniae Pharmaceutica – Drug Research, Vol. 68 No. 6 pp. 919–925, 2011 ISSN 0001-6837
Polish Pharmaceutical Society

PHARMACEUTICAL AND BIOPHARMACEUTICAL EVALUATION OF EXTRACTS FROM DIFFERENT PLANT PARTS OF INDIGENOUS ORIGIN FOR THEIR HYPOGLYCEMIC RESPONSES IN RABBITS

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Abstract: This study was designed to evaluate the hypoglycemic effects of different plant extracts in single and in combined formulation, in experimentally induced "diabetic rabbits". The extracts were obtained from seeds of *Syzygium jambolana*, fruits of *Momordica charantia* and leaves of *Azadirachta indica*. Treatment of diabetes with plant extracts was started at 8 days after alloxan injection. Rabbits were randomly divided into four groups, each group consisting of six rabbits. Each group of rabbits was given a dose of granules containing 200 mg/kg b.w. concentrated ethanolic extract of a plant while the fourth group was given a dose of granules consisting of combined extract of all three folk plants. Blood samples were drawn at 0, 2, 4, 8, 12, 24, 36, 48, 72 and 96 h. Serum glucose estimation was done by glucose oxidase kit method. Anti-diabetic effect was produced after 72 h in groups 1, 2 and 3 that were administered with a dose of granules of ethanolic extract of single plant but in group 4, treated with 200 mg/kg body weight of combined extract of all three plants, hypoglycemic effect was produced after 96 h. Hypoglycemic effects may be induced in rabbits by administration of extracts of various plant parts. The hypoglycemic effect produced by granules of single plant extract was more pronounced than antidiabetic effect produced by combining three extracts in a single formulation.

Keywords: rabbits, hypoglycemic effect; serum glucose levels, *Syzygium jambolana, Momordica charantia*, *Azadirachta indica*

Herbs are very effective in helping to manage elevated blood glucose (1). The traditional system of medicine prevalent in India and its neighboring countries is a repository of such well-tried herbal drugs for centuries. Some of these drugs have been scientifically tried on animals and human beings under controlled conditions and proved to be effective (2).

The present study was carried out to investigate the hypoglycemic effects of granules prepared from ethanolic extracts of *Eugenia jambolana* seeds, *Momordica charantia* fruits and *Azadirachta indica* leaves on alloxan induced diabetic rabbits.

EXPERIMENTAL

All chemicals used in the study were of analytical grade (from Merck and/or Sigma companies).

Animals

Twenty four healthy adult rabbits *(Oryctolagus cuniculus)*, weighing 1000–1500 g were purchased from a local market (Bahawalpur, Pakistan). The animals were fed green fodder and tap water *ad libitum*. The animals were acclimatized in an environment of controlled temperature (22–25°C), humidity and light/dark (12 h/12 h) cycle for one week prior to study. The instructions of the committee on ethical use of the experimental animals were strictly complied with CIOMS, 1985.

Preparation of plants material

Identification of plants

The identification of: *Syzygium jambolana,* family: Myrtaceae, *Momordica charantia*, family: Cucurbitaceae, *Azadirachta indica,* family: Meliaceae was performed in Cholistan Institute of Desert Studies at The Islamia University of Bahawalpur, Pakistan and the specimens were deposited in the herbarium; the voucher numbers are: *S. jambolana* 8066/PACL*, Momordica charantia* 2534/PACL *and Azadirachta indica* 26485/PACL, respectively.

(I) The de-pulped seeds of *S. jambolana* L. were purchased from local herbal medicine store Bahawalpur.

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These were washed, shade dried for two days and ground in an electric grinder to give coarse powder. The powder was then passed through sieve number 60 and stored in a well closed container at 25°C (3).

(II) Fresh green whole fruit of the *M. charantia* L. was obtained from the vegetable market of Bahawalpur. Completely shade dried whole fruit was powdered with an electric grinder, passed through sieve number 40 for size reduction and stored in air tight container at 25°C (3).

(III) Fresh green leaves of *A. indica* were collected, shade dried, powdered in an electric grinder and were passed through sieve number 20 and stored in a well closed container at 25°C (3).

Preparation of ethanolic extracts

(I) One kg powdered material of *S. jambolana* seeds was macerated with 3.5 L of 95% ethanol added and soaked for 3 days. The flask was shaken for 10 min after an interval of 8 h for 3 days.

(II) 650 g of powdered material of *M. charantia* was put into a glass flask of 5 L capacity and 2.5 L of HPLC grade methanol was added and soaked for 3 days. The flask was shaken for 10 min after each interval of 8 h for 3 days.

(III) One kg of powdered material of *A. indica* leaves was macerated with 3.5 L of 95% ethanol added and soaked for 3 days. The flask was shaken for 10 min after each interval of 8 h for 3 days.

Filtration

Finally the soaked material of each plant was filtered through several layers of muslin cloth one by one for coarse filtration. The coarse filtrate was filtered through a Whatman # 1 filter paper. The filtrates so obtained were evaporated under reduced pressure at 45°C in a rotary vacuum evaporator. The process of evaporation was continued till complete evaporation of ethanol. The gummy extracts so obtained were collected in amber colored glass containers and stored in freezer at 0°C.

Preparation of formulations

Formulation I

Granules of ethanolic extract of *S. jambolana* (Formulation I) were prepared with light kaolin. Sixty grams of extract was mixed with 94 g of light kaolin and the paste was passed through sieve #10. The granules were dried at room temperature and were stored at 25°C in an oven.

Formulation II

Granules of methanolic extract of *M. charantia* (Formulation II) were prepared with light magnesium carbonate ($MgCO_3$). Hundred grams of extract was mixed with 90 g of light $MgCO_3$ and the paste was passed through sieve #10 and granules were dried at room temperature and stored at 25°C in an oven.

Formulation III

Granules of ethanolic extract of *A. indica* (Formulation III) were prepared with calcium carbonate ($CaCO_3$). Hundred grams of extract was mixed with 120 g of $CaCO_3$ and the paste was passed through sieve #10 and granules were dried at room temperature and stored at 25°C in an oven.

Formulation IV

Formulations I, II and III were mixed in the dose of 70 mg, 60 mg and 70 mg of extracts of *S. jambolana*, *M. charantia* and *A. indica*/kg b.w., respectively, to prepare Formulation IV.

Induction of diabetes in experimental animals

Rabbits were made diabetic by injecting 150 mg/kg b.w. of alloxan monohydrate in normal saline solution intravenously in the marginal ear vein to induce hyperglycemia (4). Eight days after injection, serum glucose levels of all the surviving rabbits were determined by GOD-PAP method using spectrophotometer (Shimadzu 1601). The rabbits with serum glucose levels > 200 mg/dL were considered as diabetic and were employed for study (5).

Grouping of rabbits

After one week 24 diabetic rabbits were randomly divided into four groups (A–D), each group having 6 rabbits. All the animals were given food and water *ad libitum*.

Group A was given Formulation 1, i.e., ethanolic extract of *S. jambolana* , 200 mg/kg b.w. i.e., equal to 513 mg of granules.

Group B was given Formulation II, i.e., ethanolic extract of *M. charantia*, 200 mg/kg b.w. i.e., equal to 380 mg of granules.

Group C was given Formulation III, i.e., ethanolic extract of *A. indica*, 200 mg/kg b.w. i.e., equal to 442 mg of granules.

Group D was given Formulation IV.

Collection of blood samples

After administration of granules of plant extracts, the animals were held in wooden rabbit holder; 2 mL of blood was obtained from jugular vein and serum glucose level was estimated with the help of a UV-VIS spectrophotometer (Shimadzu 1601). The samples were collected at 0, 2, 4, 8, 12, 24, 36, 48, 72 and 96 h. After collection of blood,

the pricked site of vein was pressed with cotton swab soaked with ethanol to protect the rabbit against infection and the animals were given food and water *ad libitum* just after drug administration.

Determination of serum glucose level

Normal value of serum glucose (fasting) is 75–115 mg/dL. Serum glucose was determined colorimetrically according to enzymatic colorimetric test GOD-PAP method, without de-proteinization by using kit method of RANDOX (England).

Collection of serum sample

After centrifugation, blood serum was taken and used for determination of glucose level. Enzyme reagent (2.5 mL) was taken in test tube and 25 µL of blood serum was added and stirred on vortex mixer for 1 min. In other test tube, 2.5 mL of enzyme reagent was taken and 25 µL of standard solution was added. Both test tubes were incubated in water bath at 37°C for 10 min. Absorbance of standard and sample at 500 nm was measured spectrophotometrically against reagent blank within 60 min.

Statistical analysis

Data were subjected to statistical analysis for standard errors of the means and analysis of variance and plotted graphically using Slide Write computer software. The figures were transformed to MS-paint file before importing to manuscript.

RESULTS AND DISCUSSION

Diabetes mellitus can be induced readily in different animals by chemical or surgical methods. Alloxan and streptozotocin are the most extensively used agents because their diabetogenic dose usually is about 1/4th of the lethal dose (6). These drugs selectively destroy β cells without affecting α cells of islets of Langerhans of pancreas. There was a different response on serum glucose level with time by the administration of extract from different plant parts within group and among the groups. More interestingly, the individual animal showed somewhat unique response of its own rising and falling trends of serum glucose levels, so the results of the individual rabbit as monitored during the experimental period are discussed here. However, for the comparison of performance of experimental extracts, the curve fit plot has been utilized to describe the response of a group as a whole.

Formulation I

The value of serum glucose level of rabbit 1 was 505 mg/dL at 0 hour. There was pronounced decrease in serum glucose level, i.e., 115 mg/dL at 12 h, which at 24 h increased to 316 mg/dL and remained constant up to 72 h. It further decreased to 154 mg/dL at 96 h. The value of serum glucose level of rabbit 2 was 447 mg/dL at 0 hour and it increased to 548 mg/dL at 4 h and remained constant up to 12 h. There was marked decrease in serum glucose level i.e., 289 mg/dL at 24 h and remained constant up to 48 h but decreased remarkably i.e., 184 mg/dL and 168 mg/dL at 72 and 96 h, respectively. The value of serum glucose level of rabbit 3 was 530 mg/dL at 0 h and decreased to 415 mg/dL at 12 h which remained constant up to 48 h. It further decreased to 381 mg/dL and 255 mg/dL at 72 and 96 h, respectively. The value of serum glucose level of rabbit 4 was 555 mg/dL at 0 h and remained constant up to 8 h. It decreased to 336 mg/dL at 12 h and continued to decrease gradually up to 72 h. It suddenly decreased to 162 mg/dL at 96 h. The value of serum glucose level of rabbit 5 was 220 mg/dL at 0 h and there was gradual increase up to 24 h but abruptly increased to 418 mg/dL and gradually decreased up to 96 h. The value of serum glucose level of rabbit 6 was 224 mg/dL at 0 h and remained constant up to 12 h and suddenly increased to 417 mg/mL at 36 h and then gradually decreased to 109 mg/dL at 96 h.

A curve fit plot for mean blood glucose concentrations in the rabbits administered with *S. jambolana* seed extract 200 mg/kg b.w. at different time intervals is presented in Figure 1. There was an overall decrease in serum glucose levels in all the cases of administrations. However, the glucose concentration increased up to 8 h, after 12 to 96 h glucose concentration decreased. This might be due to the stress or reflex activated some mechanisms which increased glucose level up to 8 h. The effect of drug on reduction of serum glucose concentration was found to be insignificant ($p > 0.05$) at 2, 4, 8, 12, 24, 36, and 48 h but was found to be significant ($p \leq 0.05$) at 72 and highly significant at 96 h when compared with zero hour.

Medicinal herbs used in indigenous medicines for the management of diabetes mellitus contain both organic and inorganic constituents. Some of these inorganic trace elements such as zinc, chromium, vanadium, potassium, and sodium possess antidiabetic properties. It can be concluded that inorganic constituents might play an important role in the antidiabetic effect of *S. jambolana* seeds (7). It was reported that ethanolic extract of *S. jambolana* seeds exhibited significant reduction in glycosylated hemoglobin (Hb) in both mild and severe diabetic rabbits. The extract was not only effective

in lowering blood glucose level but also caused significant increase in plasma insulin levels which could correct other essential metabolic alterations (8). It might also be possible that the ethanolic extract increased the sensitivity of insulin in diabetic rabbits because it showed favorable effect in mild diabetic rabbits having adequate though reduced levels of plasma insulin. It might be possible that the *S. jambolana* seeds extract contain active principles like ellagic acid and jambolin that increase the liver and muscle glycogen contents by glycogenesis and thus decreased the blood glucose levels.

It might be possible that *S. jambolana* like *Gymnema sylvestre* enhanced endogenous insulin production, possibly by pancreatic β cells regeneration or repair because of higher insulin levels in the serum (9). Usually, the crude extracts take longer time to get absorbed from the stomach and/or intestines while sulfonylureas and other oral antidiabetics are pure chemical entities and produce hypoglycemia relatively quickly (10). Therefore, the effect of active principle on reduction of serum glucose level was observed in later stage i.e., after 72 h of drug administration. The changes in serum glucose level reflect that there might be more than one mechanism by which glucose level was decreasing.

Formulation II

Similar fall and rise trend of serum glucose level was seen in the case of administration of Formulation II. The value of serum glucose level of rabbit 1 was 496 mg/dL at 0 h, it remained constant up to 8 h and suddenly decreased to 318 mg/dL at 12 h and then gradually decreased up to 48 h. It suddenly increased to 344 mg/dL at 72 h then abruptly decreased to 158 mg/dL at 96 h. The value of serum glucose level of rabbit 2 was 444 mg/dL at 0 h. It abruptly decreased to 131 mg/dL and then gradually increased to 163 mg/dL at 12 h. It again suddenly decreased to 95 mg/dL at 24 h and remained constant up to 48 h and then gradually increased to 141 mg/dL at 96 h. The value of serum glucose level of rabbit 3 was 354 mg/dL at 0 h, suddenly decreased to 225 mg/dL at 2 h, but abruptly increased to 494 mg/dL at 4 h and then gradually decreased to 180 mg/dL till 96 h. The value of serum glucose level of rabbit 4 was 452 mg/dL at 0 h and then gradually decreased to 191 mg/dL till 48 h and increased suddenly to 295 mg/dL at 72 h. It again remarkably decreased to 155 mg/dL at 96 h. The value of serum glucose level of rabbit 5 was 255 mg/dL at 0 h and remained constant till 72 h and suddenly decreased to 176 mg/dL at 96 h. The value of serum glucose level of rabbit 6 was 235 mg/dL at 0 hour and remained constant up to 24 h but decreased to 119 mg/dL at 36 h and then gradually to 100 mg/dL till 96 h.

The curve fit plot (Figure 1) for mean blood glucose concentrations in the rabbits administered with *M. charantia* seed extract (200 mg/kg b.w.) at different time intervals showed the trend of glucose concentration decrease up to 96 h. This showed that drug was effective from the beginning. The effect of drug on reduction in blood glucose concentration was found to be insignificant ($p > 0.05$) at 2, 4, 8, and 12 h but was found to be significant ($p = 0.05$) at 24, 36, 48, and 72 h and highly significant at 96 h when compared with 0 h.

The active components of M. *charantia* are thought to be charantin, vicine and polypeptide-P (an unidentified insulin-like protein similar to bovine insulin). Theoretical mechanisms for reduction of serum glucose include increased insulin secretion, tissue glucose uptake, liver muscle glycogen synthesis, glucose oxidation and decreased hepatic gluconeogenesis. Studies in alloxan-induced diabetic rabbits have suggested hypoglycemic effects (11).

It might be possible that hypoglycemic activity of *M. charantia* may be mediated through stimulation of insulin release, stimulation of enzymes responsible for glucose uptake and utilization and/or inhibition of intestinal absorption of glucose. It might be possible that *M. charantia* extract could not produce the expected higher hypoglycemic effects by the presence of some other substances in the ethanolic extract which interfere with hypoglycemic effect.

The cells of diabetics fail to recognize and respond to insulin as they once did, leading to elevated blood sugar. Insulin prompts cells to take in the glucose by increasing the permeability and thus increase glucose utilization. It might be possible that the extract of *M. charantia* like cinnamon makes cells much more sensitive to insulin, thus increasing glucose metabolism, a process in which cells convert blood glucose to energy (12).

In diabetic rabbits, the plant drug is unlikely to act indirectly by stimulating the release of insulin since alloxan-treatment causes permanent destruction of the β cells (13). It is, therefore, conceivable that the hypoglycemic principle(s) in the extract of *M. charantia* also exert a direct effect in the diabetic rabbits, probably by a mechanism similar to insulin.

Although the precise mechanism of action remains to be fully elucidated, preliminary evidence suggests that *M. charantia* may help to stimulate

insulin release or possibly glycogen synthesis in the liver. Additionally, the plant is believed to contain several antidiabetic principles. For instance, insulin like protein, known as insulin-p or polypeptide-p, has been extracted from *M. charantia* fruit and has demonstrated hypoglycemic effects when injected subcutaneously into type I diabetics. *M. charantia* also contains charantin, a mixed steroid compound isolated through alcohol extraction, which was found to be a more potent hypoglycemic agent than tolbutamide (1).

Formulation III

The value of serum glucose level of rabbit 1 was 496 mg/dL at 0 hour and it remained constant up to 12 h but abruptly decreased to 131 mg/dL at 24 h and remained constant till 96 h. The value of serum glucose level of rabbit 2 was 403 mg/dL at 0 h and increased to 525 mg/dL at 2 h and then gradually decreased to 402 mg/dL at 48 h. It again decreased abruptly to 148 mg/dL at 72 h and there was slight increase to 207 mg/dL at 96 h.

The value of serum glucose level of rabbit 3 was 274 mg/dL at 0 h and remained constant up to 48 h. It suddenly decreased to 148 mg/dL at 72 h and remained constant up to 96 h. The value of serum glucose level of rabbit 4 was 246 mg/dL at 0 h, which increased suddenly to 427 mg/dL at 8 h and then gradually decreased till 96 h. The value of serum glucose level of rabbit 5 was 361 mg/dL at 0 h and gradually increased to 515 mg/dL at 36 h but suddenly decreased to 312 mg/dL at 48 h then gradually decreased to 120 mg/dL at 96 h. The value of serum glucose level of rabbit 6 was 229 mg/dL at 0 h and gradually increased to 520 mg/dL at 36 h and then gradually decreased to 186 mg/dL at 96 h.

The glucose concentration increased up to 36 h but after 36 to 96 h glucose concentration decreased. This increase may be due to the stress or reflex mechanisms that increased glucose level up to 8 h. The effect of drug on reduction in blood glucose concentration was found to be insignificant ($p > 0.05$) at 24, 36, 48 and 72 h but was found to be significant ($p < 0.05$) at 8, 12, and 96 h when compared with 0 h.

Possible mechanism of hyperglycemic effects of *A. indica* leaf extract which persisted up to 24 h may be due to reduction in peripheral utilization of glucose and glycogenolytic effect due to blockage of epinephrine action by the extract in diabetic rabbits (14). The drug administration was started after 8 days of alloxan-induced diabetes and a significant fall in the blood glucose levels was observed after 72 h with *A. indica* leaf extract. The study showed that *A. indica* leaf extract produced a marked decrease in blood glucose level after 48 h. The antidiabetic effect increased gradually and was observed to be maximal at 96 h. The hypoglycemic effect may be due to increased release of insulin from β cells of pancreas similar to that observed after sulfonylureas administration. However, it was reported that the sulfonylureas do not decrease blood glucose in alloxan-induced diabetic animals. In contrast, parental administration of insulin is well known to produce hypoglycemia in normal as well as alloxan-induced rabbits (15). In this study with *A. indica*, the fall in blood glucose was observed in the alloxan-induced rabbits. This indicated that the mechanism of hypoglycemia produced by *A. indica* may be similar to that of insulin. Insulin increases the conversion of glucose into glycogen to store glucose as glycogen. This may be brought about by stimulating the enzyme, glycogen synthetase, which catalyzes glycogen formation and inhibits the enzyme glycogen phosphorylase, which catalyzes glycogen breakdown into glucose L-phosphate (16). It is concluded that *A. indica* may have beneficial effects in established diabetes mellitus, and it may also delay or prevent the onset of disease.

Quercetin – a flavonoid, has been isolated from leaf extract of *A. indica*, which was found to reduce the hyperglycemia in streptozotocin diabetes (17). The hypoglycemic action of *A. indica* may partly be due to extra pancreatic sites of action i.e., by increased peripheral glucose utilization or by direct metabolic effect on tissues particularly on liver. It is also well known that the chemical constituents isolated from the plant can vary according to humidity, chemical constituents of soil, climate, and geography.

The possible mechanism of antihyperglycemic effect of *A. indica* leaf extract might be that the extract blocks significantly ($p < 0.05$) the inhibitory effect of serotonin on insulin secretion mediated by glucose (18). *A. indica* treatment might increase the incorporation of glucose into the protein components of these tissues. The hypoglycemic activity of *A. inidica* may occur in a slow and steady manner in rabbits with alloxan-induced diabetes. The extract seemed to be less potent than oral hypoglycemic agents and showed its effect after 72 h.

The late effect might be due to slow absorption of antihyperglycemic active principle from intestine. Usually, the crude substances take longer time to get absorbed from the stomach and/or intestines while sulfonylureas and other oral antidiabetics are pure chemical entities and produce hypoglycemia relatively quickly (10).

Formulation IV

The value of serum glucose level of rabbit 1 was 466 mg/dL at 0 h; it remained constant up to 8 h but remarkably decreased to 280 mg/dL and 117 mg/dL at 12 and 24 h, respectively, and remained constant till 96 h. The value of serum glucose level of rabbit 2 was 478 mg/dL at 0 h and suddenly decreased to 290 mg/dL but abruptly increased to 509 mg/dL at 4 h and remained constant till 72 h but suddenly decreased to 148 mg/dL at 96 h. The value of serum glucose level of rabbit 3 was 584 mg/dL at 0 h and gradually decreased to 266 mg/dL up to 48 h and then remained constant till 96 h. The value of serum glucose level of rabbit 4 was 242 mg/dL at 0 h and suddenly increased to 510 mg/dL at 8 h but abruptly decreased to 235 mg/dL at 12 h, which remained constant till 96 h. The value of serum glucose level of rabbit 5 was 354 mg/dL at 0 h, which gradually increased up to 72 h but suddenly decreased to 196 mg/dL at 96 h. The value of serum

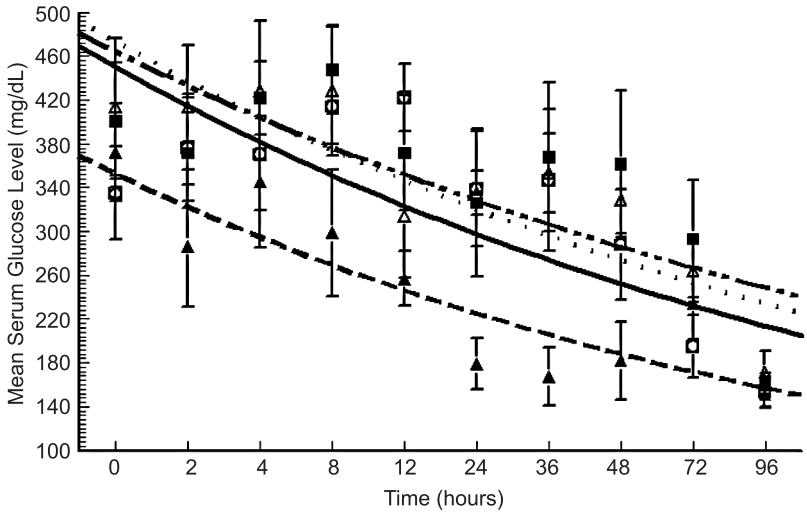

Figure 1. Curve fit plot for the comparison of values of different formulations administered in the oral dose of 200 mg/kg b.w. each. △ = Formulation I (*Syzygium jambolana* seed extract); ▲ = Formulation II (*Momordica charantia* fruit extract); □ = Formulation III (*Azadirachta indica* leaf extract); ■ = Formulation IV (combination of all three Formulations in doses of 70 mg, 60 mg and 70 mg of extracts/kg b.w., respectively

Table 1. Comparative effects of individual formulations in the diabetic rabbits.

No.	Time (h)	Formulation I mg/dL, Average ± SEM	Formulation II mg/dL, Average ± SEM	Formulation III mg/dL, Average ± SEM	Formulation IV mg/dL, Average ± SEM
1	0 (control)	413.500 ± 62.288	372.667 ± 44.615	334.833 ± 42.456	401.167 ± 53.236
2	2	413.167 ± 56.388	286.667 ± 56.034	376.667 ± 49.051	371.667 ± 49.995
3	4	428.667 ± 63.348	345.167 ± 59.623	370.833 ± 51.335	421.833 ± 33.678
4	8	428.333 ± 58.915	298.667 ± 57.998	414.333 ± 34.124*	447.667 ± 40.739
5	12	314.167 ± 56.611	256.833 ± 24.804	422.333 ± 30.747*	371.667 ± 52.257
6	24	334.833 ± 20.005	178.833 ± 23.514*	338.667 ± 52.408	326.167 ± 67.103
7	36	353.167 ± 36.289	167.167 ± 26.564*	347.000 ± 64.499	368.333 ± 68.126
8	48	329.167 ± 31.593	182.000 ± 35.555*	288.000 ± 50.147	361.500 ± 67.054
9	72	263.833 ± 28.938*	234.667 ± 33.05*	195.167 ± 28.387	293.333 ± 53.906
10	96	171.5 ± 19.47**	151.667 ± 11.871**	153.167 ± 14.409**	159.667 ± 11.431**

Values are the mean ± SEM; n = 6. If a value of p = 0.05 compared with control, * = significant, ** = highly significant.

glucose level of rabbit 6 was 283 mg/dL at 0 h, which gradually decreased to 224 mg/dL up to 24 h but suddenly increased to 496 mg/dL at 36 h and remained constant up to 48 h, then abruptly decreased to 145 mg/dL at 96 h.

As shown in Table 1 and Figure 1, in rabbits administered with *S. jambolana* (70 mg/kg), *M. charantia* (60 mg/kg) and *A. indica* (70 mg/kg) b.w., the trend of glucose concentration decreased at 2 h but increased from 4 to 8 h and after 8 to 96 h glucose concentration again decreased. This increase in glucose concentration at 4 to 8 h might be due to the stress or reflex mechanisms activated and glucose level increased up to 8 h. The effect of drug on reduction in blood glucose concentration was found to be insignificant ($p > 0.05$) at 2, 4, 8, 12, 24, 36, 48, and 72 h but was found to be highly significant ($p = 0.05$) at 96 h when compared with 0 h.

Usually, the crude substances take longer time to get absorbed from the stomach and/or intestines while sulfonylureas and other oral anti diabetics are pure chemical entities and produce hypoglycemia relatively quickly. Therefore, the effect was produced after 72 h.

According to ayurvedic texts, a combination of substances is used to get the enhanced desired action and eliminate unwanted side effects. These ingredients may aid absorption of active principles responsible for hypoglycemic actions and protective actions on kidneys (19). Contrarily, in present study, combination of three extracts showed fewer efficacies than the individual extract, so there are some possibilities to be considered.

The ingredients may antagonize the effects of each others. Secondly, the less concentration in Formulation IV may be responsible for not to elicit the required response in the diabetic rabbits. These may result in less hypoglycemic effect.

Moreover, when the extracts were administered individually in the dose range of 200 mg/kg b.w., certainly, the active principles were in higher quantities in case of single dose of the extract as compared when combined dose of three extracts was used. Although the combined dose contains the dose of extract of 200 mg/kg b.w., the quantity of active principles was definitely reduced.

CONCLUSIONS

Hypoglycemic effects may be induced in rabbits by the administration of extracts of various plant parts but a comprehensive study of chemical nature, its mode of action, standardization of dose, administration interval, possible side/adverse effects etc. must be carried out before going to a full length exploitation of any plant extract.

The active principles have been found neither synergistic nor potentiating the activity of others so the extent of the interference within the mixture or combined application must be worked out.

Hypoglycemic or otherwise any deleterious effect(s) of various plant extracts with different dose levels should be worked out especially with higher doses of administrated extracts.

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Received: 14. 10. 2010