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COMPARATIVE STUDY OF AEGLE MARMELOS, AZADIRACHTA INDICA AND GLIMEPRIDE ON BLOOD SUGAR IN EXPERIMENTALLY INDUCED HYPERGLYCEMIA IN ALBINO RATS

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ABSTRACT: INTRODUCTION: A large percentage of the global population is suffering from diabetes mellitus. Bael (Aeglemarmelos) is an Indian plant, which significantly lowers the level of blood glucose and glycosylated haemoglobin. Neem (AzadirachtaIndica), is also a well-accepted medicinal plant, grown all over the India and have hypoglycemic properties, with its leaf extract and seed oil.

OBJECTIVES: The objectives of our present study are- (1) to compare the hypoglycemic property of Aeglemarmelos, AzadirachtaIndica with Glimepride in the treatment of diabetes mellitus, and (2) to improve the quality of life of the diabetic patients and to minimize the cost of therapy. **MATERIALS &**

METHODS: About thirty (30) male albino rats of wistar strain, weighing between 100 to 200 grams were selected for the present study. Drugs used in this study was Streptozocin (STZ) and glimepride. Plant extracts used in this study was fresh leaves of Aeglemarmelos and AzadirachtaIndica. After estimation of fasting blood sugar, rats were given intraperitoneal injection of STZ to make them diabetic. Then after a series of works, hypoglycemic potential of these plant extracts and glimepride were studied. **RESULTS:** Our study showed that glimepride has maximum hypoglycemic potential. The effect of glimepride and two plant preparations, significantly decreased fasting glucose level ($P < 0.001$) with a highest reduction with glimepride. Although glimepride showed maximum fall (31.31%), effect of Aeglemarmelos was maximum among the herbals used. **CONCLUSION:** AzadirachtaIndica (Neem) and Aeglemarmelos (Bael) has significant hypoglycemic effect. These herbal medicines has also very little side effects as well as it requires no cost in few cases. So, an extensive research and developmental work should be undertaken on these herbals, so that these herbals could be proved to be boon for the diabetic population.

KEYWORDS: Diabetes, Aeglemarmelos, AzadirachtaIndica, Glimepride.

INTRODUCTION: Diabetes mellitus is a common metabolic disease around the world. A large percentage of the global population is suffering for this disorder. The disease is induced by stressful life style, fast food eating, lack of exercise and genetic make-up. Diabetes and its related complications are closely related with oxidative stress of the body.^{1,2} Oxidative stresses causes cellular and tissue damage. Uncontrolled hyperglycemia can lead to disturbances in the structure and functions of organs (Kuyvenhoven and Meinders, 1999,³ West 2000⁴). Diabetes is associated with the generation of reactive oxygen species (ROS) causing oxidative damage, particularly to heart and kidney (Mohammed et al, 1999).⁵

In humans, diabetes is mostly due to deficiency or absence or ineffectiveness of insulin, whereas in animals, it can be produced by pancreatectomy, by administration of alloxan, streptozocine, other toxins that in appropriate doses causes selective destruction of B cells of the

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pancreatic islets, drugs that inhibit insulin secretion or by administration of anti-insulin antibodies. There are some strains of mice, rats, hamsters, guinea pigs, miniature swine and monkeys that have a high incidence of spontaneous diabetes mellitus. Diabetes is characterized by polyuria, polydipsia, weight loss in spite of polyphagia, hyperglycemia, glycosuria, ketosis, acidosis and coma. In the present era, we are mainly dependent upon modern medicines which are scientifically evaluated, but they are costly and some of them are hazardous. Besides these, drug resistance and therapeutic ineffectiveness are also problems. So, scientists are now taking recourse to herbal drugs. Practitioners of these drugs claim that herbal drugs are effective, safe and economical. Our ancient literature states that there are many medicinal plants and herbs whose fruits, leaves possess anti-diabetic properties.

These fruits, leaves when consumed whole or as an extract form, along with a diabetic diet have been known to improve the glycemic control in diabetes. These medicinal plants include- Aegle marmelos (Bael), Ocimum Sanctum (Tulsi), Azadirachta indica (Neem), Momordica Charantia (Karela), Eugenia Jambalena (Jamun) and many others. Bael (Aegle marmelos) is an Indian plant, which has enormous traditional uses against various diseases and many bioactive compounds have been isolated from this plant.^{6,7} Leaves, fruits, stem and roots of this tree at all stages of maturity are used as ethnomedicines against various human ailments.⁶ The antidiabetic mode of action of Bael is of multidirectional, as the extract can significantly lower the level of blood glucose and glycosylated haemoglobin and increase the plasma insulin as well as liver glycogen in diabetic rats.⁸ The mechanism of action could be either stimulation of glucose uptake or enhancement of insulin secretion or both.⁹

Azadirachta indica (Neem) is also a well-accepted medicinal plant since a long time. This is an indigenous tree grown all over India and is attributed to have many medicinal properties. Hypoglycemic effect was observed with leaf extract and seed oil, in normal as well as alloxan induced diabetic rabbits.

Keeping these things in mind, the present study was conducted to compare the efficacy of Aegle marmelos, Azadirachta indica with Glimepiride in reducing high blood sugar levels in albino rats. Objectives of this study were- (1) To compare the hypoglycemic property of Aegle marmelos, Azadirachta indica with Glimepiride in the treatment of diabetes mellitus, and (2) To improve the quality of life of the diabetic patients and to minimize the cost of therapy.

MATERIALS & METHODS: Male albino rats of wistar strain weighing between 100 to 200 grams were selected for the present study. They were housed in cages at room temperature. The whole experiment was designed and conducted in accordance with the ethical norms approved by Institutional Animal Ethics Committee Guidelines. Drugs used in this study were Streptozocin (100 mg) Lyophilized powder (from Sigma Aldrich Chemicals Private Limited, Bangalore) and Glimepiride (1 mg) tablets (from U.S.V Limited). Streptozocin causes diabetes mellitus. Plant extracts used in this study are fresh leaves of Aegle marmelos and Azadirachta indica after a series of processings. They were administered orally through a nasogastric tube. The dose schedule of Aegle marmelos was 500 mg/kg body weight given once daily for 1 week. The dose schedule of Azadirachta indica was 500 mg/kg body weight once daily.

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INDUCTION OF DIABETES MELLITUS: The fasting blood sugar was estimated, before the application of any drug. Male albino wistar rats were given intraperitoneal injection of Streptozocin to make them diabetic. Before injecting Streptozocin, rats were deprived of food overnight and were allowed free access to water. Then STZ (streptozocin) was dissolved in 0.1 M cold Sodium Citrate buffer, which had been prepared beforehand. This solution was injected intraperitoneally into the ventral surface of the body of the animals. The dose of streptozocin injected was 50 mg/kg body weight. After that, the animals were allowed to drink 5 % glucose solution, overnight to overcome the drug induced hypoglycemia.

METHODS: The entire experimental study was carried out in six groups of albino rats. Total number of animals was thirty with each group consisting of 5 rats. The rats were kept in animal cages after properly labelling them. The groups were as follows:

1. Group A: Rats in this group were normal non-diabetic.
2. Group B: Rats in this group were diabetic controls.

These two groups, group A and B were only given normal diet. Not any extract or hypoglycemic agents were given. Normal control rats were injected with citrate buffer alone instead of streptozocin injection. The rats were, thereafter observed closely and monitored regularly to detect the appearance of diabetes. 10 days' time was allowed for the development of diabetes, within which time the diabetes developed and got stabilized in the rats. That was confirmed by high blood sugar levels as measured with the help of glucometer. The rats with fasting blood sugar in the range of >200 mg/dl were selected and used for further experiments. Thus after allowing ten days' time of stabilization of diabetes, treatment was started from 11th day of the experiment and this was considered as 0th day of the treatment. The treatment was continued for four weeks.

3. Group C: STZ induced diabetic rats were treated with Aeglemarmelos leaf aqueous extract, once daily for 4 weeks (500 mg/kg body weight).
4. Group D: STZ induced diabetic rats were treated with Azadirachta indica leaf extract, once daily for 4 weeks (500 mg/kg/body weight).
5. Group E: STZ induced diabetic rats were treated with Glimepride daily (0.02 mg/kg body weight), once daily for 4 weeks.
6. Group F: STZ induced diabetic rats were treated with combination of Aeglemarmelos (500 mg/kg) + ½ dose of glimepride (0.01mg/kg body weight) once daily for 4 weeks.

ADMINISTRATION OF DRUGS: Once the diabetes had stabilized, from the 11th day of experiment, the rate of group C, D, E & F were started on hypoglycemic drugs. Diabetic rats of group C were administered Aeglemarmelos crude leaf extract at the dose of 500 mg/kg body weight orally with the help of nasogastric tube. Rats in group D were administered Azadirachta indica leaf aqueous extract at the dose of 500 mg/kg body weight orally with the help of nasogastric tube. Rats in group E were administered glimepride at the dose of 0.02 mg/kg body weight, orally once daily with the help of nasogastric tube. Rats in group F were administered combination of Aeglemarmelos and half of the dose of glimepride.

ESTIMATION OF FASTING BLOOD SUGAR: Blood samples were collected from the tail of the rats. The tail was pricked just enough to allow one drop of blood to ooze out. The Ascensia Entrust Blood

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Glucometer was made ready before hand. Once the glucometer is ready with the test strip. One drop of blood is allowed on the appropriate reaction zone of the strip. Within few seconds, the level of fasting blood sugar appears on the meter display.

Before the administration of streptozocin, blood sugar level of all the rats were taken. At 2 days intervals fasting blood sugar of all streptozocin treated rats were measured till blood sugar level was stabilized. In 10 days, it was stabilized, then rats having fasting sugar level > 200 mg/dl were selected for the study. From 11th day diabetic rats were treated daily with hypoglycemic agents orally once. It was counted as 0 day. Before administration of hypoglycemic agents, fasting blood sugar was measured. Then, at 1 week interval fasting blood sugar were measured. So, five times fasting blood sugar level estimation was done, including 0 day of all rats.

RESULTS:

Groups	Mean Fasting Blood Sugar in mg/dl				
	0 day	1 week	2 week	3 week	4 week
A	79.6+3.55	83.0+2.09	77.4+2.18	64.0+4.23	82.4+3.35
B	281.8+2.53	286.0+1.97	286.6+1.88	286.4+1.12	282.8+2.51
C	283.2+3.15 P>0.5	279.6+3.08 P<0.5	249.0+5.79 P<0.001	214.0+1.69 P<0.001	205.0+1.84 P<0.001
D	280.6+3.23 P>0.5	275.4+2.58 P<0.001	248.0+2.98 P<0.001	232.6+2.78 P<0.001	222.2+2.51 P<0.001
E	284.2+3.81 P>0.5	278.0+3.88 P<0.01	226.0+4.30 P<0.001	206.4+2.73 P<0.001	195.0+1.84 P<0.001
F	279.0+1.16 P<0.5	270.2+0.66 P<0.001	235.6+2.16 P<0.001	210.6+1.33 P<0.001	198.0+2.47 P<0.001

Table 1: FASTING BLOOD SUGAR LEVELS IN VARIOUS GROUPS ON DAY 0, 1 WEEK, 2 WEEK, 3 WEEK AND 4 WEEK (Each group has 5 rats)

Values are mean + SE, n=5, (P values when compared with diabetic control).

Table-1 shows values of fasting blood sugar in various diabetic groups of rats on day 0, 1 week, 2 week, 3 week and 4 week. The fasting blood sugar values were compared with that of normal diabetic control rats.

Diabetic Control (B): In this group of Streptozocin induced diabetic control rats, fasting blood glucose at 0 day was 281+2.53 mg/dl, on 1 week 286.0+1.97 mg/dl, on 2 week 286.6+1.88 mg/dl, on 3 week 286.4+1.12 mg/dl and at the end of 4 weeks fasting blood sugar was 282.8+2.51 mg/dl.

Diabetic + Aeglemarmelos (C): On 0 day – On 0 day, the fasting blood sugar level was 283.2+3.15. The values were not significant.

On 1 week – On 7th day administration of aqueous extract of Aeglemarmelos fasting blood sugar became 279.6+3.08 mg/dl. This value was also not significant statistically.

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On 2 week – On 2 week administration of aqueous extract of Aeglemarmelos, fasting blood sugar became 249.0+5.79 mg/dl. This value was found to be statistically highly significant as compared to control group.

On 3 week – At the end of 3rd week of continuous treatment with Aeglemarmelos blood sugar decreased to 214.0+1.69 mg/dl. The value was found to be highly significant.

On 4 week - At the end of 4th week, the fasting blood sugar was 205.0+1.84 mg/dl. This value when compared with 4 week value in normal diabetic control rats, was found to be highly significant.

Diabetic + Azadirachta indica (D): On 0 day – On 0 day, the fasting blood sugar was 280.6+3.23 mg/dl. This value was not statistically significant.

On 1 week – After 1 week treatment with aqueous extract by Azadirachta indica at dose of 500 mg/dl, the blood glucose level was 275.4+2.58 mg/dl. It was highly significant as compared to normal diabetic control group.

On 2 week – After 2 week treatment, the blood glucose level came down to 248.0+2.98 mg/dl. This value was found to be statistically highly significant.

On 3 week – When rats of this group were treated with aqueous extract of Azadirachta indica daily once, at the end of 3 week, fasting blood glucose reading was 232.6+2.78 mg/dl. This value was found to be highly significant statistically.

On 4 week – At the end of 4 week, the reading was 222.2+2.51 mg/dl. When it was compared with the value of normal diabetic rats, it was highly significant statistically.

Diabetic + Glimepride (E): On 0 day – In the glimepride treated group of rats, the 0 day fasting blood sugar was 284.2+3.81 mg/dl. It is not significant.

On 1 week – When rats were treated with glimepride for 1 week, the fasting blood sugar was 278.0+3.88 mg/dl. This value is significant statistically.

On 2 week – After 2 week treatment with glimepride, the reading on glucometer was 226.0+4.30 mg/dl. This figure is highly significant as compared to control diabetic group of rats.

On 3 week – At the end of 3 week, the fasting blood glucose level is 206.4+2.73 mg/dl. This value is also found to be highly significant as compared to normal diabetic control.

On 4 week – After 4 week treatment with glimepride for 4 weeks once daily, fasting blood glucose was 195.0+1.84 mg/dl. This value was found to be highly significant. The dose of glimepride administered was 0.02 mg/kg body weight.

Diabetic + Combination of Aeglemarmelos + ½ dose glimepride (F): On 0 day – In this group of rats, 0 day fasting blood sugar was 279.0+1.16 mg/dl. It is not significant.

On 1 week – With the combination of Aeglemarmelos and glimepride treatment, fasting blood sugar became 270.2+0.66 mg/dl, which is highly significant.

On 2 week – After 2 week treatment, the fasting blood sugar was 235.6+2.16 mg/dl. It is highly significant statistically.

On 3 week – After 3 week treatment, the fasting blood sugar of this group of rats decreased to 210.6+1.33 mg/dl. It is also highly significant.

On 4 week – At the end of 4 weeks, fasting blood sugar became 198.0+2.47 mg/dl, which is a highly significant figure statistically when compared to normal diabetic control.

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DISCUSSION: Our present study shows that glimepride has maximum hypoglycemic potential. The effect of glimepride and two preparations, i.e. Azadirachta leaf extract & Aeglemarmelos leaf extract significantly decreased fasting glucose level ($P < 0.001$) with a highest reduction by glimepride (31.31%). Aeglemarmelos leaf extract produced a significant decrease in blood glucose level in diabetic rats. The reduction observed was gradual. Similar gradual reduction was observed by Azadirachta indica leaf extract. In 1 week, after stabilization of diabetes, blood sugar level started falling, which was gradual and maximum at the end of 4 weeks. Diabetic control group did not show any significant rise or fall in blood sugar as compared with treated group. At the end of 4 weeks, Azadirachta indica showed 20.81% fall and Aeglemarmelos showed 27.71% fall in fasting blood glucose. Although, glimepride showed maximum fall (31.31%), effect of Aeglemarmelos was maximum among the herbals used.

A significant hypoglycemic effect was also observed by feeding neem oil to fasting rabbit. Recently, hypoglycemic effect was observed with leaf extract and seed oil in normal as well as alloxan induced diabetic rabbits (Khosla et al, 2000).¹⁰ As suggested by Sharma et al (1983),¹¹ the hypoglycemic action of Azadirachta indica may partly be due to extra pancreatic sites of action, i.e by increased peripheral utilization or by direct metabolic effect on tissues, particularly in liver. Nimbidin present in neem may be responsible for significant hypoglycemic effects because oral administration of nimbidin has shown hypoglycemic effect in fasting rabbits.

When leaf extract of Aeglemarmelos were given in dose of 500 mg/kg body weight once daily in diabetic rats, then at the end of 4th week 27.61% reduction in the fasting blood glucose was seen. Diabetes was produced by STZ (50 mg/kg body weight) intraperitoneally. Aeglemarmelos leaf extract enhances the regeneration of B cells, which are partially destructed by STZ as suggested by Paulose et al (1996).¹² This effect was gradual and was maximum at the end of 4 weeks. Some extra pancreatic actions may be responsible which causes reduced hepatic uptake of indigenous insulin. All these mechanisms may be responsible for the hypoglycemic action of Aeglemarmelos. In some studies, oral as well as intraperitoneal administration of the aqueous extract of bael fruit also exhibited hypoglycemic effect against streptozocin induced diabetic rats.⁸ In a number of pre-clinical trials, it has been found that the methanolic, alcoholic and aqueous extracts of bael leaves have antidiabetic action. These extracts significantly decreased the serum glucose level, improved the ability to utilize the external glucose load and increased the plasma insulin levels in artificially induced diabetic animal models.¹³⁻¹⁷

When combination of Aeglemarmelos (500 mg/kg body weight) and glimepride (0.02 mg/kg body weight) were given, then the decrease in fasting blood sugar was 29.03%, which is little less than glimepride, but more than Aeglemarmelos. So, combination therapy with herbal and oral hypoglycemic drug (Synthetic), is much more effective than herbal alone, when synthetic drug is used in its half dose. So, it will certainly reduce the side effects of glimepride and the additive effect will be produced at lesser cost.

CONCLUSION: The use of oral hypoglycemic agents (glimepride) is not free from side effects. The use of herbal medicine has little side effects, as well as it requires no cost in few cases. This study showed a significant hypoglycemic effect of Azadirachta indica (Neem) and Aeglemarmelos (Bael). Considering the economic resource constraints and cheapness of these plant products, the present study was conducted to investigate the efficacy of these herbal products on blood glucose level in diabetic rats.

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RECOMMENDATIONS: As the global scenario is now changing towards use of non-toxic plant products having traditional medicinal use, development of modern drugs from neem and bael should be emphasized. An extensive research and development work should be undertaken on these herbals, so that these herbals could be proved to be boon for the diabetic population. A drug development programme can be developed through extensive investigations of the bioactivity of various compounds, their mechanisms of action, pharmacotherapeutics, toxicity, standardization, and clinical trials.

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