Influence of Hydroalcoholic Extract of *Cinnamomum Cassia* on Anti-Diabetic Effect of Glibenclamide, Metformin Alone and Their Combination

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Summary

Cinnamomum cassia is commonly known as cinnamon and it has anti-diabetic activity, but its influence in diabetic patients under treatment is not clear. Hence aim of this work is to find out the influence of hydroalcoholic stem bark extract of *Cinnamomum cassia* (HAECC) on anti-diabetic effect of Glibenclamide, Metformin alone and their combination. The influence of HAECC (285.71 & 666.66 mg/kg), Glibenclamide (1 mg/kg), Metformin (300 mg/kg) and their combinations (2-12 groups, n=6) were evaluated diabetic rats by estimating fasting blood glucose levels on initial, 1st, 3rd, 7th, 14th and 21st day of the treatment. On 21st day blood and liver samples were collected and other parameters were estimated. Combination therapy does not produce any synergistic effect but blood glucose level was very significantly ($p < 0.01$) reduced from 1st day, came to normal on 21st day of the treatment. In addition, significant reduction was observed in cholesterol (27.1-47.4%), SGOT (38.8-63.6%) and SGPT (7.6-40%). Significant increase in protein (13.7-30.4%) and HDL cholesterol (44.4-108.3%) indicates reduction in drug treated rats. The HAECC has potential anti-diabetic action in alloxan induced diabetic rats. Combination of HAECC with anti-diabetic drugs does not produce any significant hypoglycaemic effects.

Key words: HAECC, Metformin, Glibenclamide, diabetes and serum glucose.

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Introduction

The Increasing worldwide incidence of diabetes mellitus in adults is a global public health burden. It is predicted that by 2030, India, China and United States will have the largest number of people with diabetes[1]. The WHO estimated that 19.4 million individuals were affected by this deadly disease in India in 1995, it is likely to go up to 57.2 million by the year 2025. Diabetes mellitus is a chronic disorder of carbohydrate, fat and protein metabolism. Deficiency in insulin secretory response leads to impaired carbohydrate (glucose) use results in hyperglycemia which is a characteristic feature of diabetes mellitus. The oral hypoglycemic agents are used in the treatment of diabetes that is associated with insulin resistance and cannot be managed by non pharmacological means. Sulfonylureas and Biguanides are the two classes of drugs which are in general use. Sulfonylureas act mainly by increasing insulin secretion by the pancreatic islets and may reduce insulin resistance directly or indirectly by reducing the effects of elevated glucose and free fatty acids[2]. Biguanides increase the receptor and post receptor action within the tissues which are targeted by insulin but not by increasing insulin secretion.

Interest in cinnamon as potentially useful treatment for diabetes began with the discovery almost 20 years ago cinnamon's insulin-sensitizing properties. *Cinnamomum cassia* (Nees) is a bark belonging to the family Lauraceae. In folk medicine it is given that C*innamomum cassia* is used as treatment to cure many diseases. Cinnamon is one of the traditional folk herbs used in Korea, China and Russia for diabetes mellitus[3]. Cinnamic aldehyde[4], cinnamic acid[5], tannin[6] and methylhydroxychalcone polymer (MHCP) are its main componets[7]. Qin *et al.,* (2003) have recently reported that cinnamon extract decreases blood glucose in Wistar rats. Jarvull-Taylor's research also showed that cinnamon increases the insulin sensitivity and glucose uptake in adipocytes[8]. But the influence of the crude drug in diabetic patients who are under treatment is not clear. Hence, the present study is planned to find out the Influence of alcoholic extract of *Cinnamomum cassia* on anti-diabetic effect of glibenclamide, metformin and their combination.

Glibenclamide is an oral hypoglycemic agentwhich is widely used for the treatment of diabetes mellitus. It produces hypoglycemic effect primarily by stimulating insulin secretion from β cells of pancreatic islets[9]. Metformin, a member of the biguanide class, reduces blood glucose in patient with diabetes mellitus. This drug has been reported to exert its effect primarily on the liver by inhibiting gluconeogenesis and reducing hepatic glucose output. Metformin has also been reported to increase glucose uptake in skeletal muscles and adipose tissue[10]. As the effect of metformin is peripheral, no insulin secretion is stimulated. Hyperinsulinemia and insulin-resistance induced by some of the antihyperglycemic drugs are not seen with metformin. Because of this, metformin is considered to be a safe and effective drug for the treatment of type 2 diabetes[11, 12].

Drugs are used to prevent, diagnose, treat or cure many diseases or disorders. However, they must be used safely with precaution to ensure that they are safe and effective. Many drugs undergo interaction with the body in different ways, like with our daily diet or lifestyle, which has great significant impact on a drug's ability to show its effects which may be enhanced or decreased.

Materials and Methods

Plant material

The stem barks of Cinnamomum cassia were procured commercially from Ananthakesari, Bangalore. The barks were authenticated by Mr. M Nijagunaiah, Taxonomist, Department of Botany, Bangalore University, Bangalore. A voucher specimen of bark has been deposited in department of pharmacognosy, Acharya & B.M Reddy College of Pharmacy, Bangalore.

Experimental Animals

Male Albino wistar albino rats weighing (150-250 g) were obtained from Raghavendhra enterprises, Bangalore, Karnataka and housed three animals per cage with paddy husk as bedding in our institution (**Regd. No: 997/c/06/CPCSEA**). Animals were housed at temperature of 25±2 ^{o}C, relative humidity of 30-60% and 12:12 h light and dark cycle was followed. The animals had accessed to feed and purified water *ad libitum*.

Extraction

The dried powdered stem barks of *Cinnamomum cassia* was extracted (for 6 h by heating) with hydroalchol in soxhlet apparatus. The extract was concentrated to dryness. The yield of the hydroalcholic extract of *Cinnamomum cassia* was found to be 12.12 % w/w.

Experimental design

Experimental design and treatment schedule

Male Albino wistar rats weighing (130-250 g) were fasted for overnight before challenging with single subcutaneous (s.c.) injection of alloxan monohydrate, freshly prepared and injected within 5 min of preparation to prevent degradation at a dose of 110 mg/kg.

Diabetic rats were divided into eleven groups including diabetic control and one normal group consisting of normal healthy rats each group consisting of six animals as follows. Group 1: Normal control, Group 2: Diabetic control, Group 3: Glibenclamide (1 mg/kg), Group 4: Metformin (300 mg/kg), Group 5: HAECC (285.71 mg/kg), Group 6: HAECC (666.66 mg/kg), Group 7: HAECC (285.71 mg/kg + glibenclamide (1 mg/kg), Group 8: HAECC (666.66 mg/kg) + glibenclamide (1 mg/kg), Group 9: HAECC (285.71 mg/kg + metformin (300 mg/kg), Group 10: HAECC (666.66 mg/kg) + metformin (300 mg/kg), Group 11: HAECC (285.71 mg/kg + glibenclamide (1 mg/kg) and metformin (300 mg/kg), Group 12: HAECC (666.66 mg/kg) + glibenclamide (1 mg/kg) and metformin (300 mg/kg). Blood glucose levels were estimated on initial, 1st, 3rd, 7th, 14th and 21st day of the treatment. On the 21st day of treatment overnight fasted rats were sacrificed blood samples and liver were collected for biochemical estimations.

Estimation biochemical parameters

Serum glucose, cholesterol, HDL cholesterol, total protein and transaminases are estimated by GOD/POD method with help of clinical chemistry analyzer (Metro Lab, 1600 DK-R) by using glucose liquid stable reagent (Swemed Diagnostics, Bangalore).

Statistical analysis

Statistical analysis was performed using graphpad prism 5 software. The values were analyzed by one way analysis of variance (ANOVA) followed by Dennett's. All the results were expressed as mean ±SD for six rats in each group. P-Values <0.05 were considered as significant.

Results

Effect of HAECC, Glibenclamide, Metformin and their combination on serum glucose

We studied the effects on blood glucose after administration of HAECC 285.71 mg/kg was insignificant on 1st and 3rd day and found to be significant (P <0.05) on 7th till 21st day of treatment. Anti-hyperglycemic effect of HAECC 666.66 mg/kg was significant (P <0.05) from 1st day and very significant (P <0.01) from 3rd till 21st day treatment and glucose levels almost came to normal by 21st day. HAECC produced dose dependent anti-diabetic effect.

The anti-hyperglycemic effect was insignificant on the 1st day of the treatment by HAECC 285.71 mg/kg in combination with Glibenclamide but showed significant (P <0.05) effect on 3rd day, very significant (P <0.01) from 7th till 21st day and glucose levels almost came to normal by 21st day. HAECC 666.66 mg/kg in combination with Glibenclamide produced very significant (P <0.01) anti-hyperglycemic effect from 1st till 21st day of treatment and glucose levels almost came to normal by 14th day. Combination of both the doses of HAECC when given with Metformin produced significant (P <0.05) anti-hyperglycemic effect on the 1st day and very significant (P <0.01) from 3rd till 21st day of the treatment. The anti-hyperglycemic effect of HAECC-285.71, 666.66 mg/kg in combination with Glibenclamide and Metformin was very significant (P <0.01) on 1st, 3rd, 7th, 14th and 21st day of the treatment respectively and glucose levels almost came to normal by 21st day. Results are shown in figure-1.

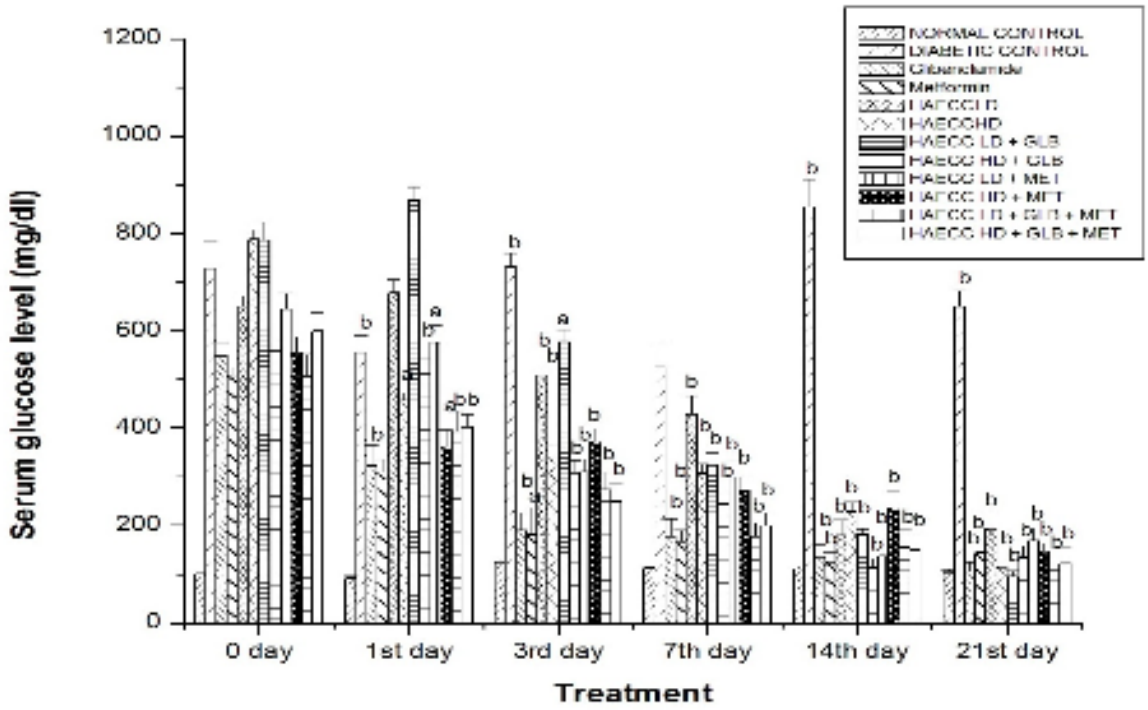

Figure 1: Effect of HAECC, Glibenclamide, Metformin and their combination on serum glucose levels in diabetic rats. Each bar represents the mean ± SEM (n=6), *P < 0.05, **P < 0.01, All treatment groups are compared against diabetic Control and diabetic control compared against normal control (HAECC- Hydroalcholic extract of *Cinnamomum cassia*, GLB-Glibenclamide, MET-Metformin, LD-lowdose(285.71mg/kg), HD-highdose (666.66mg/kg).

Effect of HAECC, Glibenclamide, Metformin and their combination on biochemical parameters in diabetic rats

Cholesterol

Significantly reduction of serum cholesterol was 27.13 and 29.52 after daily treatment of diabetic rats with HAECC-285.71, 666.66 mg/kg respectively. HAECC-285.71, 666.66 mg/kg with Glibenclamide reduced serum cholesterol by 38.01 and 47.44% respectively. HAECC-285.71, 666.66 mg/kg with Metformin reduced serum cholesterol by 40.78 and 31.91% respectively. HAECC - 285.71, 666.66 mg/kg with Metformin and Glibenclamide reduced serum cholesterol by 41.17 and 36.68% respectively. Results are shown in table-2.

HDL cholesterol

Significantly increase of serum HDL cholesterol was 44.40 and 55.48 after daily treatment with HAECC-285.71, 666.66 mg/kg respectively. Combination of HAECC-285.71, 666.66 mg/kg with Glibenclamide increase serum HDL cholesterol by 101.05 and 64.84% respectively. Combination of HAECC-285.71, 666.66 mg/kg with Metformin increase serum HDL cholesterol by 53.81 and 52.59% respectively, Combination of HAECC-285.71, 666.66 mg/kg with Metformin and Glibenclamide increase serum HDL cholesterol by 108.35 and 99.03% respectively. Results are shown in table-2.

Serum protein

Significantly increase of serum protein was -18.71 and 1.13% after daily treatment with HAECC-285.71, 666.66 mg/kg respectively. Combination of HAECC-285.71, 666.66 mg/kg with Glibenclamide increase serum protein by 15.87 and 24.19% respectively. Combination of HAECC-285.71, 666.66 mg/kg with Metformin increase serum protein by -12.28 and 13.79% respectively. Combination of HAECC-285.71, 666.66 mg/kg with Glibenclamide and Metformin increase serum protein by 30.43 and 26.65 % respectively. Results are shown in figure-2.

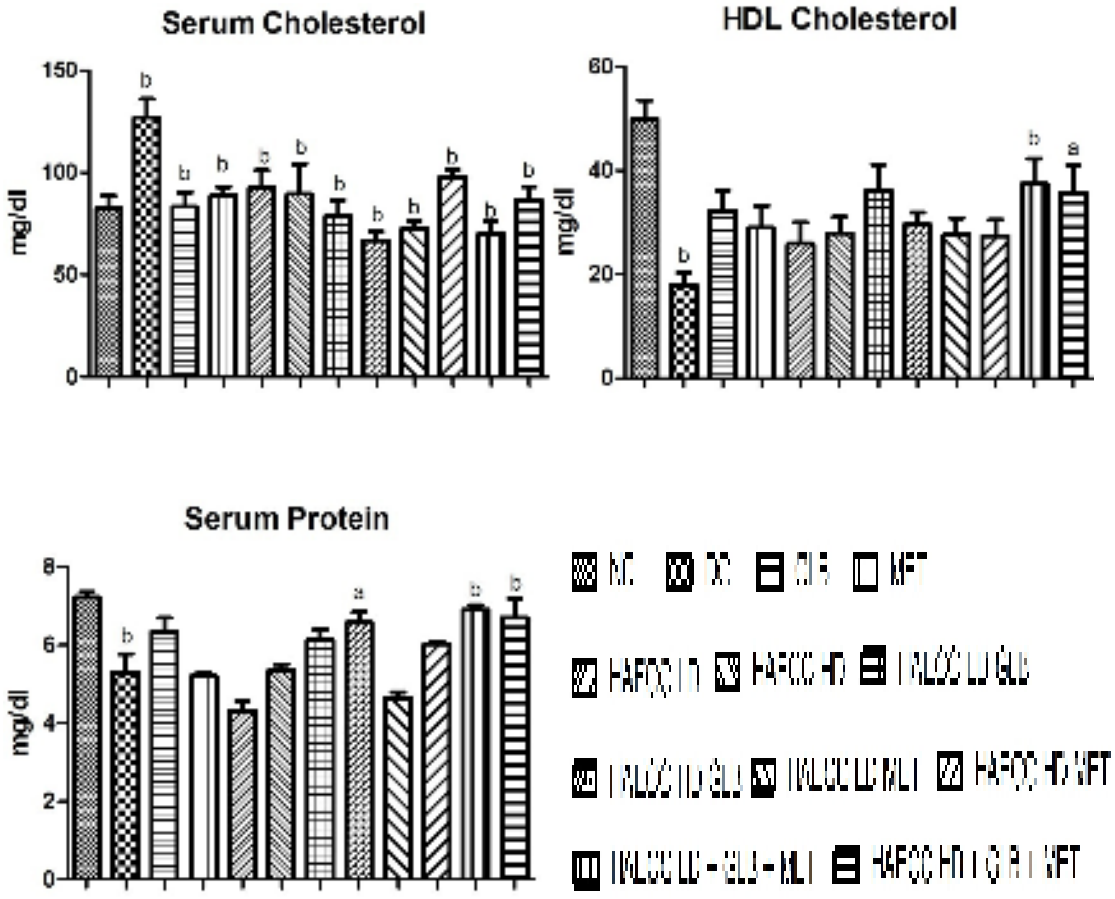

Figure 2: Effect of HAECC, Glibenclamide, Metformin and their combination on serum protein, cholesterol and HDL cholesterol levels in diabetic rats. Each bar represents the mean ± SEM (n=6), *P < 0.05, **P < 0.01, All treatment groups are compared against diabetic Control and diabetic control compared against normal control (HAECC- Hydroalcholic extract of *Cinnamomum cassia*, GLB-Glibenclamide, MET-Metformin, LD-lowdose(285.71mg/kg), HD-highdose (666.66mg/kg).

SGOT

SGOT level was significantly reduced by 38.82 and 62.17% after daily treatment with HAECC-285.71, 666.66 mg/kg respectively and it is dose dependent. Effect observed by HAECC 666.66 mg/kg is almost similar to Glibenclamide. Combination of HAECC-285.71, 666.66 mg/kg with Glibenclamide reduced SGOT levels by 55.48 and 63.60%.

Combination of HAECC-285.71, 666.66 mg/kg with Metformin reduced SGOT by 54.77 and 53.86%. Combination of HAECC-285.71, 666.66 mg/kg with Metformin and Glibenclamide reduced SGOT by 56.09 and 55.83% respectively. Effects observed by the treatment with combinations are almost similar and not much difference was observed to their individual effects. Results are shown in table-3.

SGPT

A SGPT level was significantly reduction by 21.41 and 7.63% after daily treatment with HAECC-285.71, 666.66 mg/kg respectively. Daily treatment with Glibenclamide & Metformin reduced SGPT by 10.20 and 38.33%. Combination of HAECC-285.71, 666.66 mg/kg with Glibenclamide reduced SGPT by 25.12 and 23.61% respectively. Combination of HAECC-285.71, 666.66 mg/kg with Metformin reduced SGPT by -3.25 and 24.99% respectively. Combination of HAECC - 285.71, 666.66 mg/kg with Glibenclamide and Metformin reduced SGPT by 27.69 and 40.02% respectively. Results are shown in figure-3.

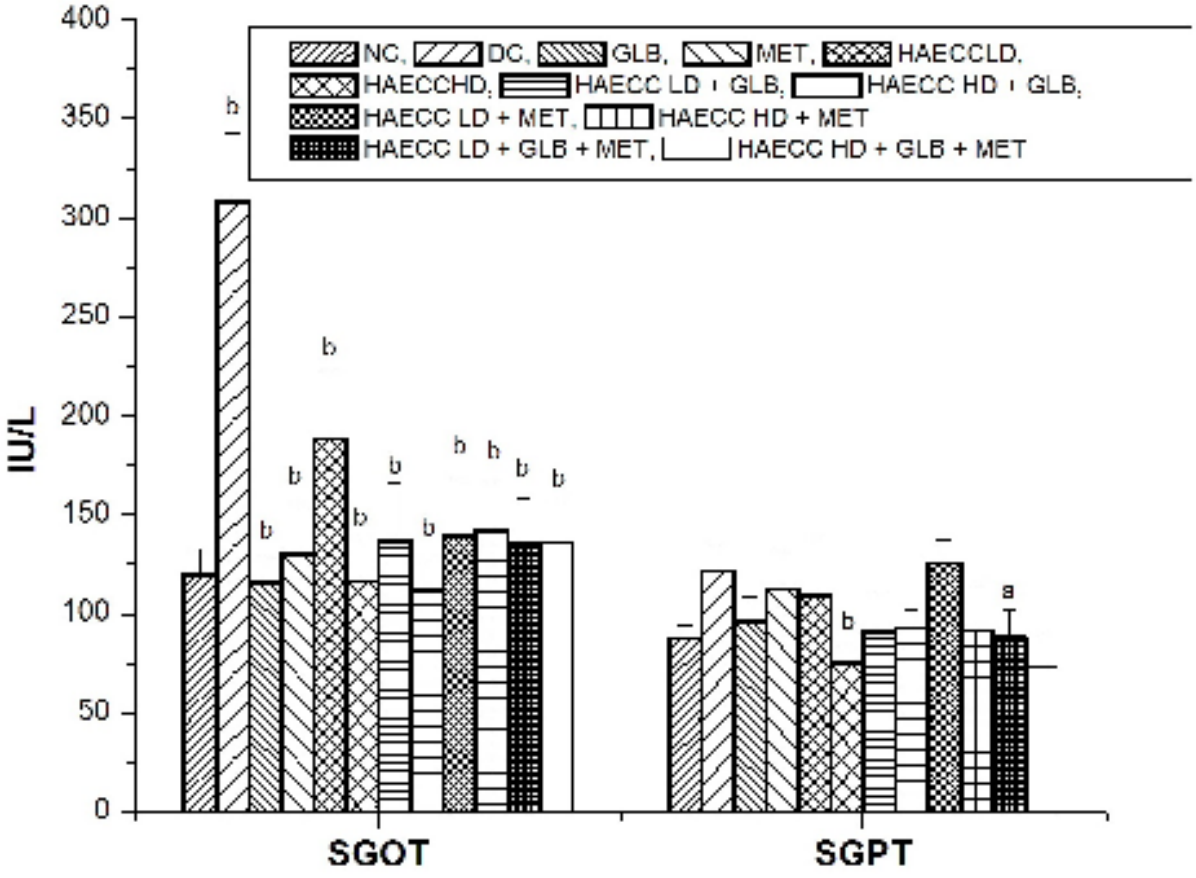

Figure 3: Effect of HAECC, Glibenclamide, Metformin and their combination on SGOT and SGPT levels in diabetic rats. Each bar represents the mean ± SEM (n=6), *P < 0.05, **P < 0.01, All treatment groups are compared against diabetic Control and diabetic control compared against normal control (HAECC-Hydroalcholic extract of *Cinnamomum cassia*, GLB-Glibenclamide, MET-Metformin, LD-lowdose(285.71mg/kg), HD-highdose (666.66mg/kg).

Discussion

Plants may act on blood glucose through different mechanisms some may inhibit hepatic gluconeogenesis[13], some may mimic or improve insulin action at the cellular level, and or that it may possess extra-pancreatic hypoglycaemic activity[14]. The literature survey revealed that Cinnamomum cassia has shown significant increase in the levels of serum insulin after administration of cinnamon extract either by increasing the pancreatic secretion of insulin from the existing beta cells or its release from the bound form[15]. Methylhydroxychalcone polymer (MHCP) of Cinnamomum cassia was found to be an effective mimetic of insulin. MHCP demonstrated invitro activation of glycogen synthesis and inhibiton of glycogen synthase kinase-3β as well as insulin receptor phosphorylation homologous to the effects of insulin in 3T3-LI adipocytes. Invivo studies showed an increase in insulin-stimulated IR-β and the IRS-1 tyrosin phosphorylation treated with cassia cinnamomum. Cinnamon acts as an agonist with insulin invivo to decrease blood glucose levels after a glucose tolerance test and in chronically high fructose diets[16]. Similarly, Kham *et al*., (2003) had reported that blood glucose level decreased after administration of cinnamon extract in people with type II diabetes[17]. There was also a significant increase in the levels of serum insulin after administration of C200 cinnamon extract to db/db.

Anti-hyperglycemic effect of HAECC 285.71 mg/kg was very significant from 7th day onwards till 21st day but fasting blood glucose levels did not come to normal levels, this is probably due to insufficient dose. The anti-hyperglycemic effect of HAECC 666.66 mg/kg is very significant and even blood glucose levels came to normal by 21st day of treatment. The effect of HAECC 666.66 mg/kg is almost equal to Glibenclamide and Metformin. It was reported that *cinnamomum cassia* has Anti-diabetic affect on blood glucose in db/db mice model[16] and also reported that treatment with combination of Glibenclamide and Metformin produce synergistic effect[18]. But no synergistic effect was observed when both anti-diabetic drugs given together with HAECC and further no hypoglycemic effect were observed.

It is well reported that there is rise in serum lipid levels in diabetics, so significant control in the levels of lipids in serum of db/db mice treated with the cinnamon extract may be an encouraging result that improves the insulin level by cinnamon extract therapy[19]. The serum cholesterol level was reduced after treatment with HAECC-285.71, 666.66 mg/kg and the effect observed is almost similar to Metformin treated group. Glibenclamide effect was comparatively more than HAECC and Metformin. And further no hypolypidimic effect was observed by any of the combination therapy. Qin *et al*., (2003) have also reported that triglyceride and total cholesterol were decreased by administration of cinnamon extract in rats treated with streptozotocin for 3 weeks[8]. The mechanism is explained by the AMPK-enhanced triacylglycerol lipase activity that increases glycogen synthesis in the liver and enhances glucose uptake in skeletal muscle and adipocytes.

Hyperlipidemia is a recognized consequence of diabetes mellitus demonstrated by the elevated levels of tissue cholesterol, phospholipids and free fatty acids[20]. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand, glucagon, catecholamine and other hormones enhance lipolysis.

The increase in serum HDL cholesterol level was observed after treatment with HAECC-85.71, 666.66 mg/kg and the effect observed is almost similar to Glibenclamide and Metformin treated groups. The effect observed by the combinations i.e. HAECC with Glibenclamide and

HAECC with Metformin was almost similar. But very significant (P <0.01) reduction was observed by HAECC in combination with Glibenclamide and Metformin respectively.

The significant increase in serum protein level was observed after daily treatment with HAECC 666.66 mg/kg, Glibenclamide and Metformin. The effect of HAECC 666.66 mg/kg is almost similar to Metformin treated group. Glibenclamide effect was slightly more compared to HAECC and Metformin. Effect observed by the combination of HAECC-285.71, 666.66 mg/kg with Glibenclamide and HAECC 666.66 mg/kg with Metformin was almost similar and slightly more compared to combination of HAECC-285.71 mg/kg with Metformin treated group. Very significant (*P* <0.01) increase in serum protein level was observed by combination of HAECC with Glibenclamide and Metformin respectively. This indicates proteolysis is decreased after treatment of diabetic rats.

The dose dependent reduction of SGPT level was observed after treatment with HAECC. The effect observed by HAECC 666.66 mg/kg is slightly more than the Glibenclamide and Metformin. Significant effect was observed by the combination of HAECC with Glibenclamide and Metformin respectively. Even though there is reduction in SGPT levels in other treatment groups but which are not statistically significant.

Ghosh and Suryawanshi observed elevation in transaminase activity (GOT and GPT) in liver and kidney in diabetic rats. The increased gluconeogenesis and ketogenesis observed in diabetes may be due to high level in the activities of these transaminases. The elevation of the activities of SGOT and SGPT in plasma may be mainly due to the leakage of these enzymes from liver cytosol to blood stream which gives an indication on hepatotoxic effect of alloxan[9] (Ao Y *et al*., 2008). There is a significant decrease in SGOT levels to their normal levels after treatment with HAECC, Glibenclamide, Metformin alone and their combinations further strengthen the antidiabetogenic effect of this HAECC. Even though there is a reduction in SGPT levels compared to the diabetic controle after treatment but the results are statically not significant. This indicates that gluconeogenesis and ketogenesis was decreased.

To conclude hydroalcoholic stem bark extract of Cinnamomum cassia has potential anti-diabetic action in alloxan induced diabetic rats but combination of HAECC with antidiabetic drugs does not produce any synergistic/hypoglycaemic effects. Significantly reduction in serum cholesterol and transaminases levels and significant increase in serum protein and HDL cholesterol levels observed in diabetic treated rats than the diabetic control rats. This indicates treatment decreased the lipolysis, proteolysis, gluconeogenesis and glycogenolysis in diabetic treated rats. Further studies are required by reducing the doses of Glibenclamide and Metformin to know better antihyperglycemic activity of HAECC.

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