Histopathological studies on hypoglycemic effect of aged garlic extract on experimentally induced diabetes mellitus in rat

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Abstract
The present study was conducted to evaluate the hypoglycemic effect of aged garlic extract in streptozotocin induced diabetes mellitus in rats and to compare its efficacy with standard hypoglycemic drug glibenclamide. Fifty rats weighing 200-250 grams were divided into five groups with ten rats in each group. The aged garlic extract was administered at the dose rate of 200 mg/kg body weight & 400 mg/kg body weight by oral gavaging in the treatment groups for a period of 45 days. Two rats from each group were sacrificed on 15th and 30th day of experiment and the rest were sacrificed on 45th day. Histopathologically, aged garlic extract progressively ameliorated the effect of streptozotocin by showing formation of new pancreatic islets, ductular hyperplasia, improvement in the architecture of pancreas. The treatment showed a dose dependant response with aged garlic extract at the rate of 400 mg/kg body weight being more effective in in normalizing the pancreatic architecture, improving the β-cell population than compared to the dose 200 mg/kg body weight. From the present study it can be concluded that the ameliorating effect of aged garlic extract at the dose 400 mg/kg body weight was almost on par with standard hypoglycemic drug, glibenclamide.

Citation:

1. Introduction
Diabetes is a disorder of carbohydrate, fat and protein metabolism attributed to diminished production of insulin or mounting resistance to its action. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries. Oral hypoglycemic agents such as biguanides, sulphonylureas and thiozolidiones are available along with insulin for the treatment of diabetes. But they have side effects associated with their uses. Long term treatment with sulphonylurea may desensitize the β-cells of the pancreas and high concentrations of sulphonylurea may inhibit insulin biosynthesis in vitro and perhaps also in vivo(Anderson and Borg, 1980; Melander et al., 1987). There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experiences and relatively low costs.

Aged garlic extract (AGE) differs from dietary garlic and most garlic supplements because, as indicated by its name, it is naturally aged for 20 months. During this lengthy period, the garlic changes in a few important ways: 1) the more irritating and pungent properties of the bulb are rendered much milder; 2) a conversion takes place wherein certain phytochemicals that are typically found in raw garlic are transformed into other "sulfur-containing compounds".
Aged garlic extract (AGE) inhibits formation of AGEPs (advanced glycation end products) in vitro and formation of glycation-derived free radicals. S-Allylcysteine, a key component of aged garlic, is a potent antioxidant and can inhibit AGEP formation (Ahmad MS, 2006). A comparison was made between the action of garlic extract and glibenclamide (600 µg/kg), the known antidiabetic drug. The antidiabetic effect of the extract was more effective than that observed with glibenclamide (Eidi.A.et al, 2006).

2. Material and Methods

Adult male Wistar albino rats weighing 200-250 g were obtained from Animal House, IISc, and Bangalore. The rats were given standard commercial rat feed and ad lib water. The animals were maintained at standard laboratory conditions. The experiment was carried out for a period of 45 days.

2.1 Source of aged garlic extract:
Alcoholic extract of aged garlic used in the present study was procured from M/S Vet Care, Yelahanka, Bangalore.

2.2 Glibenclamide solution:
Glibenclamide (Daonil®, 5 mg) an oral hypoglycemic drug purchased from local chemist shop was dissolved in distilled water (82.33 ml) to give a concentration of 60µg/ml. This was used as a stock solution and administered orally at a dose of 600 µg/Kg body weight (B w) (Babu and Prince, 2004).

2.3 Preparation of STZ solution:
Fresh 0.1 M citrate buffer of pH 4.5 was prepared and maintained at 4-8 °C. The STZ of required quantity was dissolved in ice – cold citrate buffer to give a concentration of 45 mg/Kg b w and injected intraperitoneally to rats immediately to avoid degradation. To induce diabetes in rats, Streptozotocin (STZ) was used which was procured from Sigma Chemicals, St. Louis, USA.

2.4 Experimental protocol:
All the experimental animals will be divided into 5 groups with 10 animals in each as follows:
Group I- negative control; Group II- Diabetic control: Streptozotocin induced diabetic rats.;
Group III- Diabetic rats supplemented with aged garlic extract at the dose rate of 200 mg/kg body weight in distilled water.;
Group IV- Diabetic rats supplemented with aged garlic extract at the dose rate of 400 mg/kg body weight in distilled water.;
Group V- Diabetic rats supplemented with glibenclamide. The rats of group-I and II were gavaged only with saline and the rats of all other groups were gavaged with their respective treatments using clean gavaging needle attached to an appropriate disposable syringe for 45 days.

2.5 Experimental induction of diabetes:
The animals were fasted overnight and diabetes was induced in group II to V by a single intraperitoneal injection of a freshly prepared solution of steptozotocin(45 mg/kg b w) in 0.1 M cold citrate buffer of pH 4.5. Control (Group I) animals received citrate buffer alone.

2.6 Confirmation of diabetes:
The blood glucose levels were estimated 72 hours post STZ injection using Span diagnostic kit with semi-automatic biochemical analyzer in order to confirm the diabetic state in animals. The animals with blood glucose levels above 200 mg/dL were considered as diabetic. After confirmation of diabetic state, all the groups received their respective treatments daily for 45 days.

2.7 Pathology:
Two animals from each group were sacrificed humanely on 15th, 30th day and rest at the end of study. The sacrificed animals were subjected to detailed post-mortem examination. Gross lesions, if any, in various organs were recorded. The representative tissue samples of 3-5 mm thickness were collected in 10 percent neutral buffered formalin for histopathological examination. The tissues were processed by routine paraffin embedding technique and 5µ sections were cut and subjected for H & E staining.

3. Results and Discussion

3.1 Gross pathology:
3.1.1 Diabetic group:
Grossly, pancreas appeared slightly atrophied and showed progressive decrease in size from 15th day onwards which appeared as a thin gelatinous strip on 45th day in the present study (Figure 1). The progressive decrease in the size of pancreas may be attributable to the cytotoxic effect of streptozotocin on β-cells of islets as well as damage to exocrine portion (Jelodaret al., 2005; Mir et al., 2008; Dhanush, 2009; Mallikarjuna, 2009; Atangwho et al.,
2010). Grossly, liver appeared pale, soft and friable from 30th day of the treatment which could be attributed to fatty liver due to hyperlipidemia in streptozotocin induced diabetic rats (Ohno et al., 2000; Dhanush, 2009; Mallikarjun, 2009; Zafar et al., 2009). The other organs such as kidney, heart, lungs and intestine did not show any gross lesions throughout the study period.

3.1.2 Group III & IV:
Gross pathological changes observed in various organs in both the groups reduced progressively from Day 15 to Day 45 of the study.

3.1.3 Glibenclamide group:
Gross pathological changes observed in the glibenclamide treated rats reduced progressively from Day 15 to 45 post treatment. The results were in agreement with the findings of Ananthan et al., 2003 where they attributed the improvement to glibenclamide effect.

3.2 Histopathology:
3.2.1 Diabetic group:
Microscopically, in diabetic rats pancreas revealed lesions both in endocrine as well as exocrine portion. In the endocrine portion there was a reduction in the number of islets which were irregular with loss of demarcation with the adjacent exocrine portion. The islets revealed reduced number of β-cells that were either highly swollen with vaculated cytoplasm or elongated and fusiform with condensed nucleus. Some islets revealed total necrosis of the β-cells while in others there was presence of apoptotic cells. Most of the islets revealed hypocellularity with altered distribution of α and β-cells. There was reduced cytoplasmic granularity of β-cells which varied between cells with complete loss in some cells. In some islets hypercellularity was observed with increase in the number of α-cells and infiltration of inflammatory cells and fibroblasts resulting at later period of experimentation (Figure 3).

In exocrine portion there was a loss of normal lobular architecture characterized by reduction in the lobular size. In the affected acini the lining cells showed vacuolar degeneration and necrosis with loss of zymogen granules into the surrounding. Exocrine portion also revealed increase in the number of apoptotic cells. The change in the shape of β-cells and increase in apoptotic bodies could be attributed to partial damage caused by streptozotocin. The fibrotic change in the affected islets late in the study could be due to replacement of necrosed β-cells by substitution with fibrous connective tissue (Figure 3).

The exocrine damage in the present study could be secondary to the β-cells damage due to the release of free radicals from the STZ damaged β-cells into the surrounding exocrine portion exaggerated by reduced antioxidant activity in STZ cytotoxicity. In addition, enzymes of exocrine portion such as trypsin and lipase may also be contributory. Liver parenchyma showed swelling of hepatocytes with highly vacuolated and granular cytoplasm, obliteration of the sinusoidal space and moderate to severe congestion. The vacuolar appearance of the hepatocytes indicated fatty change and could be due to the increased influx of fatty acids into the liver induced by hypoinsulinemia and low capacity of lipoprotein secretion from liver due to deficiency of apolipoprotein B synthesis (Ohno et al., 2000).

Streptozotocin, in addition to pancreatic β-cells also damages the hepatocytes through GLUT2 transporter expression and with generation of free radicals resulting in the increased number of apoptotic cells. The increased formation of hydroxyl radicals in the liver of STZ-induced diabetic rats may causelipid peroxidation and cell damage. Microscopically, spleen revealed hypocellularity of the white pulp. There was depletion of lymphocytic mass from follicles as well as periarteriolar sheath which was very prominent on 15th day of the study. One of the effects of STZ is defective host defense system which includes failure of lymphocytes to release mediators involved in the recruitment of target cells (Kazura et al., 1979), depressed T-cell function and reduced phagocytic activity of macrophages (Saiki et al., 1980) could be probably due to secondary damage by free radicals released during STZ toxicity.

3.2.2 Group III & IV:
Histopathologically, there was a progressive improvement in the architecture of pancreas, liver, kidney and spleen from Day 15 to 45 of the study. In Group-III (AGE 200 mg/kg b w) the microscopic changes in the pancreas on 15th day included appearance of a few small sized islets with moderate cellularity. The islets were irregular and showed STZ effect in the form of cell swelling, cytoplasmic vacuolation and sparse cellularity. Gradual improvement was observed in the form of increase in size and architecture of islets and decrease in
cellular vacuolation. By the 45th day, islets revealed hypercellularity with increase in the number of α-cells although, cells with granular cytoplasm were also seen (Figure 4).

The microscopic changes observed in Group-IV animals (AGE 400 mg/kg b w) were almost similar to those of Group-III animals. However, a dose dependent improvement was observed which included formation of more number of compact islets with high cellularity, consisting of moderate number of β-cells. The improvement in architecture of pancreatic islets could be attributed to the regeneration and repair of damaged β-cells by the stimulating effect of AGE (Sheela and Augusti, 1992). The improvement in insulin levels could be attributed to SACS (S-allyl cysteine sulfoxide), which has been found to significantly stimulate insulin secretion from β-cells (Augusti and Sheela, 1996). The neogenesis of β-cells from ductal epithelial cells could be the possible reason for improvement in number of islets and β-cells (Paris et al., 2004) (Figure 5, 6).

Microscopically, in the present study liver and kidney revealed progressive improvement in the architecture from STZ-induced damage and attained almost normal structure by 45th day post treatment with few apoptotic cells persisting in liver till the end. The protective effect of AGE has been attributed to its antioxidant activity. In spleen there was an increase in the lymphoid components of white pulp in spleen in both the groups although they were more predominant in rats treated with 400mg/kg b w of AGE. Other organs were normal throughout the study in AGE 200 and 400 mg/kg b w (Group III and IV) treated rats.

**Figure 1:** Comparison between pancreas of normal, diabetic and treated groups. Note the improvement in the treatment group
3.3.3 Glibenclamide group:
There was a progressive improvement in the microscopic pathology of pancreas of glibenclamide treated rats from 15th to 45th day post treatment. The improvement could be attributed to increased proliferation as well as recruitment of subpopulation of β-cells and thereby increase in the β cell mass upon treatment with glibenclamide.

Liver section revealed progressive improvement in the architecture from 15th day onwards in the glibenclamide treated rats. Studies using [3H]-glibenclamide boluses have suggested that hepatocytes possess specific binding sites that may be relevant in mediating the action of the drug on the liver. Additional studies have shown that the drug has a positive action on glycogen deposition with direct action on the synthesis of GLUT-2 rather than GLUT-4 proteins and at the glycogen phosphorylase level. The effect of glibenclamide on the insulin levels and altered metabolism of various macromolecules could be the reason for improvement in the microscopic architecture of liver (Luzi and Pozza, 1997). The spleen section revealed persistence of lymphocytic depletion on 15th day with improvement in cellular mass on 30th and 45th day post treatment compared to diabetic group. This indicated that with improvement in hyperglycaemic state and insulin level, the free radical injury reduced contributing to improvement in the lymphoid component of spleen.

Conclusion

From this we can conclude that Aged garlic extract has dose dependant ameliorating effect on the STZ induced diabetes. Aqueous aged garlic extract at the dose rate of 400 mg/kg b w was on par with glibenclamide in ameliorating the STZ induced diabetes. Future studies on aged garlic extract with high dose rate could be taken up. Studies on effect of other garlic preparations on amelioration of diabetes could be thought off in future.

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