Efficacy of N. sativa oil and Glimepiride on the Histopathological Changes of Streptozotocin -Induced Diabetic Rats

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Abstract: Forty eight adult male Wistar rats were used to study the effect of N. sativa oil and glimepiride (Amaryl®) and its combination on the treatment of lesions induced by STZ- induced diabetic rats. The rats were randomly distributed into 8 groups (Gps) of 6 rats each. Gp. 1 was left without treatment, gp. 2 was orally given 2 ml physiological saline solution, gp. 3 was normal rats, intraperitoneally injected with 0.20 ml of N. sativa oil /kg B.wt, gp. 4 was normal rats, orally administrated with 0.08 mg Amaryl® /kg B.wt. and gp. 5 was intraperitoneally injected with a single dose of STZ (65 mg/kg B.wt.). While gp.6 was diabetic rats, intraperitoneally injected with 0.20 ml of N. sativa oil /kg B.wt., gp.7 was diabetic rats, orally administrated once daily with 0.08 mg Amaryl® /kg B.wt. and finally gp.8 was diabetic rats co-treated with N. sativa oil and Amaryl®. Diabetic rats (gp.5) revealed severe pathological changes in liver, kidneys, lung, brain and pancreas. Congested, thickened and hyalinized hepatic and renal arteries were seen. Hemorrhage and focal interstitial aggregations of lymphocytes besides massive degenerative changes and necrosis were observed in the liver and kidneys. The kidneys revealed also hyaline thickening and calcification of glomerular tufts. The lung showed focal thickening in the interalveolar septa. The bronchioles showed hyperplasia and desquamation of its lining epithelia. The pulmonary arteries showed narrowing of its lumina by thickened and hyalinized tunica media. The brain revealed congestion of the meningeal and cerebral blood vessels, encephalomalacia, satellitosis, neuronophagia and gliosis. The pancreas revealed congested, thickened and hyalinized blood vessels with perivascular edema and lymphocytic infiltration. Atrophied islets with massive degeneration and severe necrosis of β-cells were seen. Some pancreatic acinar cell showed vacuolation. The pancreatic ducts and intercalated ducts revealed hyperplasia. The electron microscopic examination of pancreas showed markedly decreased number and degranulated β-cells, with dilated rough endoplasmic reticulum, crytalysis in mitochondria and nuclear morphological changes. However, diabetic rats treated with Amaryl® (gp.7) ameliorated the histological alterations better than those treated with N. sativa oil (gp.6) but did not reach the normal structural pattern. On the other hand, liver, kidneys, lung, brain and pancreas of control group (gp.1), experimental groups (gps.2, 3, 4) and those co-treated by N. sativa oil and Amaryl® (gp.8) exhibited normal histological and fine structural picture. Collectively, it could be concluded that the N. sativa oil and Amaryl® are effective in amelioration the lesions of STZ induced diabetic rats. Co-treatment with Amaryl and N. sativa was more potent than each one alone where the lesions were completely absent.

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1. Introduction

Diabetes mellitus is the most common lifestyle disease characterized by hyperglycaemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action or, both. It affects 5% of the world population and becomes the third human killer following cancer and cardiovascular disease (Taylor, 1999). Type 1 diabetes mellitus is caused by cellular-mediated autoimmune destruction of pancreatic islet beta cells, leading to loss of insulin production. It usually starts during childhood, but can occur at all ages. Type 2 diabetes mellitus accounts for 90% - 95% of all diabetes and had global prevalence estimate of 2.8% in the year 2000 and is projected to be 4.4% in 2030 (Wild et al., 2004). Both Type 1 and Type 2 diabetes mellitus have complex pathophysologies, including insulin resistance syndrome and hyperglycaemia, which are associated with abnormalities in reactive nitrogen species and fat species (Brownlee, 2001 and Green, 2004).

Streptozotocin (STZ) is a is a naturally broad spectrum antibiotic and cytotoxic chemical that is particularly toxic to the pancreatic, insulin producing beta cells in mammals (Szkudelski, 2001, Hayashi et al, 2006 and Takeshita et al, 2006) It has been widely used to induce diabetes in animal models especially rats and mice (Brentjens and Saltz, 2001 and Hayashi et al., 2006).The increasing incidence of secondary complications associated with diabetes highlights the need to improve existing treatment regimens and preventive measures. Despite numerous preventive strategies and medical therapies, 300 million people worldwide are expected to developed diabetes mellitus by 2025 (Seidell, 2000). Amaryle® (glimepiride tablets) is an oral blood-glucose -lowering drug of the sulfonylurea class (El-Enany et al., 2012). It
effectively inhibits the hyperglycemia and development of oxidative stress in diabetes by its potent extrapancreatic effect on glucose metabolism and stimulating the release of insulin from functioning pancreatic β-cells (Groop, 1992 and Krauss et al., 2003). Herbal medicine is another therapeutic strategy in the treatment of diabetes mellitus. It considered to be less toxic and have fewer side effects than synthetic ones (Ozsosan et al., 2004). The seeds of Nigella sativa (N. sativa) plant have been used to promote health and fight disease for centuries especially in the Middle East and Southeast Asia. It was found that N. sativa oil and derived thymoquinone have anti-inflammatory (Houghton et al., 1995), anti diabetic (Kanter et al., 2003, 2004 and Fararh et al., 2005) anti-tumor (Bamerje et al., 2010), antioxidant, antihypertensive and anti hyperlipidemic properties (Bamosa et al., 1997). Also its oil is used as a natural remedy for a wide range of diseases, including various allergies (Kalus et al., 2003), asthma and diarrhea (Ali and Blunden, 2003) and nephrotoxicity and hepatotoxicity induced by either disease or chemicals (Kapoor, 2009).

To date there has very little information exists concerning the effects of N. sativa oil and its combination with Amaryl on lesions induced by STZ-induced diabetic rats. Therefore, this investigation was conducted to evaluate the effect of N. sativa oil and Amaryl and its combination on the treatment of lesions induced by STZ-induced diabetic rats.

2. Material and Methods

Experimental Animals:

Forty eight adult male Wistar rats (Unit of Laboratory Animal, Faculty of Veterinary Medicine, Zagazig University, Egypt) weighing 200–250 g were used in this study. The rats were housed in polycarbonate cages with stainless-steel wire tops and maintained at 24 to 26°C with 55 to 75% humidity and a 12-h light/dark cycle, and fed a commercial rodent diet and given water ad libitum.

Induction of Diabetes

The experimental animals (Gps. 5, 6, 7 and 8) were fasted for 12 hrs and then diabetes was induced by a single intraperitoneal injection of streptozotocin (Sigma Chemical Co., St. Louis, MO, USA), dissolved in a freshly prepared physiological saline solution (0.9% NaCl) at a dose of 65 mg/kg body weight (Kanter et al., 2003 and Dai et al., 2005). After injection, all animals were returned to their cages and given free access to food and water. After 3 days, the fasting blood glucose levels were measured from tail blood samples by using an One Touch Ultra® glucometer (Lifescan; Johnson & Johnson, Milpitas, CA, USA). Animals with blood glucose levels more than 277 mg/dL were considered diabetic and used for the experiment.

Experimental Design

After 1 week in quarantine, rats were distributed into 8 groups (Gps) of 6 rats each. Gp. (1) was the control. Gps. (2, 3, 4, 5) were the experimental control groups, while gps. (6, 7, 8) were the experimental groups.

Group 1: Was left without treatment.

Group 2: Normal rats, orally given 2 ml physiological saline solution by esophageal tube.

Group 3: Normal rats, intraperitoneally injected with 0.20 ml of N. sativa oil/kg B.wt. (Kanter et al., 2003)

Group 4: Normal rats were given 0.08 mg Amaryl® /kg B.wt., dissolved in physiological saline solution and orally administrated by esophageal tube (Das et al., 2008).

Group 5: Was given 65 mg STZ (/kg body weight (diabetic control rats).

Group 6: Diabetic rats, intraperitoneally injected with 0.20 ml of N. sativa oil/kg B.wt.

Group 7: Diabetic rats, orally administrated once daily with 0.08 mg Amaryl/kg B.wt.

Group 8: Diabetic rats, co-treated with N. sativa oil (0.2 ml/kg b.wt.) and Amaryl (0.08 mg/kg b.wt.).

The physiological saline solution, Amaryl and N. sativa oil were given once daily for 30 days. Amaryl was produced by Sanofi-aventis Egypt S.A.E under license of Sanofi-aventis/ Germany. This study was performed following an institutionally approved protocol in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Necropsy was performed at the end of the experiment. Specimens from the liver, kidneys, lungs, brain and pancreas were collected from all the necropsied animals and fixed in 10% neutral buffered formalin solution. Five micron thick paraffin sections were prepared for light microscopic examination (Bancroft and Gamble, 2002). For Transmission electron microscopic investigation, 1 mm³ tissue specimens were obtained from the tail of pancreas from both control and experimental groups. The specimens were immediately fixed in 2.5% glutaraldehyde (pH: 7.3) in 0.1M phosphate buffered saline at 4°C. The specimens were then fixed in 2% osmium tetroxide (0.1 M), dehydrated through a graded series of ethanol, and embedded in araldite. The araldite blocks were sectioned using a Leica ultract R microtome. Seventynanometre thick ultrathin sections were stained with uranyl acetate and subsequently with lead citrate (Bancroft and Gamble, 2002). Finally, they were
examine and photographed using a JEOL, 100 CX transmission electron microscope.

3. Results

Diabetic rats (gp.5) revealed severe pathological changes. The liver showed congestion of the hepatic blood vessels and sinusoids. Portal areas showed hyperplasia in the biliary epithelium with newly formed bile ductules besides hyalinization and thickening of the wall of hepatic arteries (Fig.1). Focal interstitial aggregations of lymphocytes were noticed. The hepatic cells revealed degenerative and necrotic changes(Fig.2). Diffuse vacular and hydropic degeneration and fatty changes were seen (Fig.3). Coagulative necrosis and individualization of hepatocytes were recorded(Fig.4). Some hepatocytes were destroyed and replaced by eosinophilic material and extravasated blood (Fig.5). Hypertrophied kupffer cells was detected. The kidneys revealed thickening of renal glomerular basement membrane and hyaline thickening of glomerular tufts (Fig.6). The latter showed calcification represented by basophilic substance(Fig.7). Thickening and vacuolation of the wall of renal blood vessels with perivascular edema were seen(Fig.8). The renal tubules revealed cloudy swelling, hydropic degeneration and coagulative necrosis. The latter represented by pyknosis and karyolysis. Other tubules showed cystic dilation or contained hyaline and cellular casts. Lymphocytes infiltration were showed among the degenerative and necrotic renal tubules (Fig.9). The lung showed focal thickening in the interalveolar septa by leukocytic infiltration and extravasated blood (Fig.10). Some alveoli are occluded by blood and lymphocytes alternative with others of compensatory alveolar emphysema. The bronchioles showed hyperplasia and desquamation of its lining epithelia in the lumina. The pulmonary arteries and arterioles showed thickening and hyalinization of tunica media. The brain revealed congestion of the meningeal and cerebral blood vessels (Fig.11). Encephalomalacia, satellitosis, neuronophagia and gliosis were seen (Fig.12). Demyelination was also noticed in the white matter. The pancreas revealed congested blood vessels with lymphocytosis (Fig.13) besides hemorrhage and lymphocytic infiltration among pancreatic acini. Some pancreatic blood vessels showed hyalinization of its walls besides few perivascular lymphocytic infiltration (Fig.14). Others showed thickening and vacuolation of its walls and perivascular edema (Fig.15). Reduction in number and size (atrophy) of islets of Langerhans were showed. Atrophied islets revealed massive degeneration and severe necrosis of β-cells (Fig.16). Loss of islet architecture and severe islet destruction were recorded. Some pancreatic acinar cell showed vacuolation (Fig.17). Hyperplasia of epithelial lining of intercalated and pancreatic ducts with newly formed pancreatic ductules was seen (Figs.18&19). The electron microscopic examination of diabetic pancreas showed markedly decreased number and degranulated pancreatic islet β-cells (cell- producing insulin) and nuclear shrinkage with inactive heterochromatin (Fig.20). Pancreatic islet β-cells revealed crystallysis in swollen mitochondria and separation between inner and outer membrane of nucleus. The acinar cells exhibited reduction in the general size of the zymogen granules and crystallysis in mitochondria (Fig.21).

However, diabetic rats treated with Amaryl (gp.7) ameliorated the histological alterations better than those treated with N. sativa oil (gp.6) but did not reach the normal structural pattern . Group (6) showed mild amelioration of the encountered lesions in group (5), where the liver and kidneys showed congestion and hemorrhage (Fig. 22), besides hydropic degeneration of hepatic and renal cells. Few interstitial aggregations of round cells were observed among the hepatic cells and renal tubules. The lung showed congested blood vessels and few peribronchial aggregations of round cells. The brain showed degenerated neurons and mild hemorrhage among meningeal blood vessels. The pancreas revealed congested blood vessels with mild hemorrhage among pancreatic acini. Islets with mild degeneration and necrosis of β-cells were also seen (Fig.23). The electron microscopic examination of pancreas showed mild decreased number of β-cells and minimal crystallysis in mitochondria (Fig.24).

Group (7) showed moderate congestion in hepatic, renal, pulmonary, meningeal and pancreatic blood vessels. The pancreas revealed moderate amelioration of cytological structure of islet represented by intact islets with slight necrosis of β-cells (Fig.25). The electron microscopic examination of pancreas revealed slight decrease in the number of β-secretory granules (Fig.26). On the other hand, liver, kidneys, lungs ,brain and pancreas of control group(gp.1), experimental groups(gps.2,3,4) and those co-treated by N. sativa oil and Amaryl (gp.8) exhibited normal histological and fine structural picture. The pancreas revealed intact islet of Langerhans surrounded by normal acinar cells (Fig. 27). Some islets of Langerhans of group (8) showed hyperplasia of β-cells (Fig.28). The electron microscopic examination of pancreas revealed numerous β-cells with typical secretory granules consisting of electron dense core with broad, clear halo surrounded by a membrane and normal nucleus with active eucromatin (Fig. 29).
Figs.(1-5): liver of gp. 5 (Diabetic rats), showing:

(1) Portal area showed hyperplasia in the biliary epithelium with newly formed bile ductules (arrow) besides hyalinization and thickening of the wall of hepatic arteries (arrowhead), HE x1200.

(2) The hepatic cells revealed hydropic degeneration (arrow) and coagulative necrosis of hepatocytes represented by pyknosis (arrowhead), HE x1200.

(3) Fatty changes of hepatocytes represented by signet ring appearance (arrowhead), HE x1200.

(4) Individualization of hepatocytes (arrowhead), HE x1200.

(5) Some hepatocytes were destroyed and replaced by eosinophilic material and extravasated blood (arrow), HE x1200.

Fig(6)-Kidney of gp.5 showing: Hyaline thickening of glomerular tufts (arrow), HE x1200.
Figs.(7-9): Kidneys of gp. 5, showing:

(7)- Calcification of the glomerular tufts and some renal tubules represented by basophilic substance (arrowhead), HE x1200.

(8)- Thickening and vacuolation of the wall of the interlobular artery (arrowhead) with perivascular edema (arrow), HE x1200.

(9)- Lymphocytes infiltration among the degenerative and necrotic renal tubules (arrow), HE x1200.

(10)- Lung of gp.5 showing: Focal thickening of interalveolar septa by leukocytic infiltration (arrowhead), besides thickening and hyalinization of peribronchial blood vessel (arrow), HE x300.

(11)- Brain of gp.5 showing: Congestion of the meningeal blood vessels (arrowhead) and encephalomalacia (arrow), HE x1200.

(12)- Brain of gp.5 showing: Satellitosis (arrowhead) and neuronophagia (arrow), HE x1200.
Figs.(13-18): Pancreas of Gp. 5 (Diabetic rats) ,HE x1200, showing:
(13)- Congested blood vessels with leukocytosis (arrowhead).
(14)- Hyalinization of pancreatic blood vessels(arrow) with few perivascular lymphocytic infiltration (arrowhead).
(15)- Thickening and vacuolation of pancreatic artery (arrowhead) with perivascular edema (arrow).
(16)- Atrophied islets with massive degeneration and necrosis of β-cells, (arrowhead).
(17)- Vacuolation of some pancreatic acinar cell, (arrow).
(18)- Hyperplasia of epithelial lining of intercalated duct, (arrow).
Fig. (19)- Pancreas of gp. 5 showing: Hyperplasia of epithelial lining of pancreatic duct with newly formed pancreatic ductules (arrow), HE x150.

Figs.(20&21)- Electron micrography of the pancreas of gp. 5 showing:
(20)- β-cells with markedly decreased number and degranulation of β-granules and nuclear shrinkage, (arrow) uranyl acetate Lead citrate stain x5000.
(21): Acinar cells exhibited reduction in the general size of the zymogen granules (arrow), and crystalysis in mitochondria, (arrowhead) uranyl acetate Lead citrate stain x8000.
Figs.(22-24): Gp. 6 (Diabetic rats treated with N. sativa oil) showing,
(22)- Liver: Congestion of central vein and hepatic sinusoids, (arrowhead) HE x1200.
(23)- Pancreas: Islet with mild degeneration and necrosis of β-cells, (arrowhead) HE x1200.
(24)- Electron micrography of the β-cells showed mild decreased number of β-granules (arrow) and minimal crystallization in mitochondria, (arrowhead) uranyl acetate Lead citrate stain x 6000.

Fig.(25): Pancreas of gp. 7 (Diabetic rats treated with Amaryl) showing: Intact islet of Langehans with slight necrosis of β-cells, (arrowhead) HE x1200.
Fig.(26): Gp. 7 (Diabetic rats treated with Amaryl ) showing: Electron micrography of the islets with slight decrease in the number of β-secretory granules of β-cells , (arrow) uranyl acetate Lead citrate stain x 4000.

Figs.(27-29): Pancreas of gp. 8 (Diabetic rats co-treated with N. sativa oil and Amaryl) showing,
(27)- Intact islet of Langehans surrounded by normal acinar cells, (arrowhead) HE x1200.
(28)-Hyperplasia of β-cells of islet of Langerhans, (arrow) HE x1200.
(29)- Electron micrography of the β-cells with typical secretory granules consisting of electron dense core with broad, clear halo surrounded by a membrane(arrow) and normal nucleus with active euochromatin, uranyl acetate Lead citrate stain x 6000.

4. Discussion
This study is an approach to find whether N. sativa oil alone or its combination with Amaryl can treat the lesions induced by STZ- induced diabetic rats. The current study revealed that STZ (gp.5) exerted severe pathological changes in liver, kidneys, lungs, brain and pancreas of the rats. Congested, thickened and hyalinized hepatic and renal arteries were seen. Hemorrhage and focal interstitial aggregations of lymphocytes besides massive degenerative changes and necrosis were observed in the liver and kidneys. The kidneys revealed hyaline thickening and calcification of glomerular tufts. The Lung showed focal thickening in the interalveolar septa by leukocytic infiltration and extravasated blood. Some alveoli are occluded by blood and lymphocytes alternative with others of compensatory alveolar emphysema. The bronchioles showed hyperplasia and desquamation of its lining epithelia in the lumina. The pulmonary blood vessels showed narrowing of its lumina by thickened and hyalinized tunica media. The aforementioned findings are in partial agreement with previous reports that showed that, streptozotocin injection leads to the degeneration and necrosis of many tissue including the
Langerhans islets beta cells, heart, eyes, skin, nerves and kidneys (Elsner et al., 2000 and Ikebukuro et al., 2002). The brain revealed congestion of the meningeal and cerebral blood vessels, encephalomalacia, satellitosis, neuronophagia and gliosis. Demyelination was noticed in the white matter. The previous results in brain were correlated with Baydas et al. (2003) and Navaratna et al. (2011) who demonstrated that STZ-induced diabetes causes reactive gliosis and neurodegeneration. The pancreas revealed congested, thickened and hyalinized blood vessels with perivascular edema and lymphocytic infiltration. Reduction in number and size of islets of Langrehans were showed. Atrophied islets revealed islets with mild degeneration and severe necrosis of β-cells. Some pancreatic acinar cell showed vacuolation. Hyperplasia of the intercalated and pancreatic ducts were noticed. The electron microscopic examination of diabetic pancreas showed markedly decreased number were noticed. The electron microscopic examination of diabetic pancreas showed markedly decreased number and degranulated β-cells, crytalysis in swollen mitochondria and nuclear morphological changes. The previous findings are in partial agreement with previous reports that clearly revealed that STZ -treated rats resulting in massive necrosis of β-cells (Desco et al., 2002 and Yavuz et al., 2003). Hawkins and Davies (2001), Baydas et al. (2002) and Kanter et al. (2004) attributed the STZ- induced the previous lesions to formation of oxygen free radicals production and reactive oxygen species (ROS) ,an increase in lipid peroxidation and serum nitric oxide (NO) concentrations, and decreases antioxidant enzyme activity in the tissues as a result of diabetes. These render the tissues more vulnerability to oxidative stress ,damaging DNA and inducing the lipoperoxidation of cellular membranes. Moreover, STZ has been shown to deplete the antioxidant pool in cells, making them more susceptible to oxidative damage (Low et al., 1997). Furthermore, it is suggested that diabetes mellitus modifies the angiogenic and synthetic properties of endothelial cells(Cines et al., 1998 and Georgescu, 2011). The thickened and hyalinized blood vessels causing not enough oxygen reaches the tissue and degenerative changes and necrosis resulted. The fatty changes of liver of diabetic rats in the current study are may be due to an increase in the mobilization of free fatty acids from the peripheral fat depots (Pushparaj et al., 2000). The hyaline thickening of glomerular tufts and necrosis of renal tubules were predisposing factor for dystrophic calcification. On the other hand, administration of the N. sativa oil for one month after inducing diabetes(gp.6) ameliorate the lesions of STZ- induced diabetes mellitus in male Wistar rats, where mild pathological changes were recorded. The liver , kidneys lungs ,brain and pancreas showed hemorrhage and congestion of blood vessels besides hydropic degeneration of hepatic and renal cells with few interstitial aggregations of round cell. The pancreas revealed islets with mild degeneration and necrosis of β-cells. The electron microscopic examination of pancreas showed mild decreased number and degranulated β-cells and minimal crytalysis in mitochondria. However, the slight improvement in the liver, kidney ,lungs ,brain and pancreas tissue may be due to the antioxidant properties of N. sativa and its ability to scavenge free radicals generated from STZ, in addition to its role in enhancing the functional capabilities of immune system (Ali and Blunden, 2003, Salem et al., 2005 and Hamdy and Taha , 2009). The previous results were correlated with Murli et al. (2011) who proved that N. sativa oil was useful for prevention and control of diabetes mellitus and at the same time have not exhibited adverse effects. Moreover, the previous pathological findings in pancreas were similar to those described by Kanter et al. (2003) who recorded that, intraperitoneal injection of 0.20 ml/kg volatile oil of N. sativa seeds for 30 days in STZ–diabetic rats, caused gradual partial regeneration/proliferation of pancreatic beta-cells, decrease in the elevated serum glucose and increase of the lowered serum insulin concentrations. It seems that N. sativa protect β-cells against oxidative stress. Furthermore, Salem et al. (2005) and Ali and Blunden (2003) recorded ameliorative effect of N. sativa oil upon the pathological alterations induced by the STZ could be explained by its role in regulating vital cellular functions, including cell proliferation and differentiation. The current study recorded few leukocytic infiltration. This may be attributed to the anti-inflammatory action of N. sativa oil as deduced by Houghton et al. (1995). Regarding group(7) ,the present study showed moderate lesions. Krauss et al. (2003) and Rabbani et al. (2009) attributed the previous results to the ability of Amaryl to enhance the antioxidant enzymes (CAT, SOD and GPX) and reduced the LPO, H2O2 and malondialdehyde and hyperglycemia. So, they suggested that Amaryl by increasing the level of antioxidant enzymes lead to decrease the ROS mediated damage in the host cells. Kecskemeti et al. (2002) and Yassin and Mwafy (2007) indicated that the main effect of the sulfonylureas (Amaryl) is enhancement of insulin secretion and improvement of metabolism both by pancreatic and extra-pancreatic mechanisms. In the present study, the different organs of the diabetic rats, which co-treated by N. sativa oil and Amaryl (gp.8) exhibited normal histological picture. Collectively, it could be concluded that the N. sativa oil and Amaryl are effective in amelioration the lesions of STZ- induced diabetic rats. However, the antidiabetic activity of Amaryl was relatively better than that of N. sativa. Co-treatment with Amaryl and N. sativa was more potent than each one alone where the lesions were completely absent.
was found to be an excellent adjuvant support in the therapy of diabetes and its complications.

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**Reference**


