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Effects of *Nigella sativa* Oil on Some Blood Parameters in Type 2 Diabetes Mellitus Patients

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Forty one type 2 diabetes mellitus (DM2) patients, selected and registered in accordance with especially laid down criteria were studied for the effect of *Nigella sativa* oil. All patients consumed *Nigella sativa* oil for 40 d followed by a placebo for another 40 d. Fasting blood sample of each subject was collected on 0, 40th and 80th day of the study. Glucose, insulin, platelet count, total leukocyte count, ALT, AST and blood urea were analyzed using standard methods. SPSS version-12 was used for statistical analysis and all the phases were compared by paired sample test. A significant fall in fasting blood glucose and rise in insulin and AST levels was observed after treatment with *Nigella sativa* oil as compared to concurrent control levels. Glucose and insulin levels significantly reversed after the placebo administration. Platelet count, total leukocyte count, ALT and blood urea remained statistically unchanged after *Nigella sativa* oil treatment or placebo as compared to the baseline levels. It is concluded that *Nigella sativa* oil may play a role in management of type 2 diabetes with reasonable renal and hepatic safety.

Key Words: *Nigella sativa*, Insulin, Hyperglycemia, Diabetes mellitus.

INTRODUCTION

Nigella sativa commonly known as black seed is an herbaceous annual plant belongs to family Ranunculaceae[1]. *Nigella sativa* plant is half meter tall, with blue flowers and triangular black seeds smelling pungent[2]. Seeds contain a considerable amount of fixed and volatile oils[3], proteins, alkaloids and saponins[4-8]. In Islamic medicine, the use of the seeds is recommended in daily use because it has prophesied cure for all known diseases[9]. Its high LD50 (28.8 mL/kg body weight of oil given orally)[10] is key to its lower toxicity. Its ability to stabilize hepatic enzymes, organ integrity and antioxidant activity adds on its safety, but the fall in leukocyte and platelet count must be taken into consideration[10].

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Increasing prevalence of diabetes mellitus and high cost of treatment in the world is a cause of concern[11], which aroused the need for cost effective and safe alternatives. Effect of *Nigella sativa* oil has previously been studied in diabetic type 2 like animal models at large with reasonable safety and effectiveness but its effect on human DM2 patients has not yet been reported. This study has been designed to see its effect on glucose and insulin profile in DM2 patients and also it's effect on parameters like platelet count, total leukocyte count, ALT, AST and blood urea in order to confirm its safety in humans.

EXPERIMENTAL

The study was initiated after the official approval from Directorate of Advanced Studies and Research Board, Arid Agriculture University, Rawalpindi, Pakistan.

All the patients signed a consent form was study. Initially, 75 type 2 diabetes mellitus (DM2) patients from twin cities of Rawalpindi/Islamabad, Pakistan, fulfilling a specified criteria were registered for the study. However, only 41 subjects completed the study. The patients were explained that they would receive two treatments one for first 40 days and second for another 40 days. They will keep taking their usual diabetes control medicine at constant dose throughout the study period but if that dose is increased or decreased by their consultant/physician at any stage, they informed us. They also keep taking their usual diet and continue their usual activity/exercise pattern. Their fasting blood samples was collected on 0, 40th and 80th day of the study. The levels of 0 day *i.e.* baseline were taken as concurrent control.

Selection criteria: The following criterion was used for the selection of patients suffering from type 2 diabetes mellitus: (1) Patients with known type 2 diabetic history of either sex. (2) Fasting blood glucose more than normal values, even with their usual diabetes control medicine. (3) Age between 30-60 years. (4) Patients not on insulin therapy. (5) Patients not suffering from any other chronic disease. (6) Should not be taking any medication other than that for diabetes. (7) Should not be taking black seed in any form or any other herbal treatment. (8) Pregnant women were not included in this study.

Collection of blood samples: Fasting blood samples (about 7 mL) were collected from the patients using disposable syringes by trained nurses. Each blood sample was collected in three different tubes *i.e.* (i) plain tube (4 mL), (ii) glucose tube (2 mL), (iii) CP tube (1 mL). (Plane tubes are without any additive, glucose tubes contain potassium fluoride and CP tubes contain K 3 EDTA). All the tubes were properly labeled to afford an identity of the patient.

Preparation and storage of serum and plasma: Blood samples in plain tubes were hold at room temperature for *ca.* 0.5 h to clot and then centrifuged at 5000 rpm (85 G) for nine minutes to separate serum from the blood. Similarly glucose tubes were also centrifuged to separate plasma from the blood. Separated serum and plasma was transferred to new plain tubes for storage at -20 ºC for assay at proper time. All tubes were labeled accordingly.

Tests for total leukocyte count (TLC) and platelet count: Blood samples in CP tubes were processed on the day of collection for TLC and platelet count using auto analyzer (Sysmex KX-21, Made in Japan).

Analysis of plasma and serum: Analysis of plasma for glucose and serum for ALT, AST and blood urea were determined on auto analyzer (VITALAB Selectra E, Made in Netherlands). Kits used for this purpose was (Linear chemicals, Spain). Serum insulin levels on (IMMULITE-1000, Hormone Analyzer, Made in USA). Kits used for this purpose was (Diagnostic Products Corporation, PILINC-23, USA).

***Nigella sativa* seeds:** *Nigella sativa* seeds were purchased from a herborist from the local market (Rawalpindi, Pakistan) after careful selection. The seeds were identified at "Herbarium Medicinal Botanic Centre; Pakistan Council of Scientific and Industrial Research (P.C.S.I.R.) Laboratories Complex, Peshawar, Pakistan". A voucher specimen No. (PES): 9747 was also deposited there for future reference.

Preparation of Ist treatment (*Nigella sativa* oil): *Nigella sativa* seeds were cleaned manually and then after washing with distilled water, were oven dried at a temperature of 40 °C. Oil of *Nigella sativa* seeds (7 Kg) was extracted by an electrically driven mechanical extractor, without the use of any solvent. Oil was extracted in small amounts avoiding heating. Oil obtained was 1855 mL. Extracted oil was filled into small size (30 mL) washed and sterilized brown glass bottles having a dropper and cap at their mouth. The dose per day for the patients was adjusted in such a way that it was equivalent to oil obtained from 0.7 g seeds of *Nigella sativa*.

Preparation of 2nd treatment (Placebo): The second treatment was actually a placebo. Ready to use wheat bran was made available from the local market and dispensed in capsules.

RESULTS AND DISCUSSION

Forty one patients completed the study. Their mean age was 47.80 ± 1.10 years falling in forties, 41.47 % of the patients were males and 58.53 % females.

Glucose profile: The mean glucose level ± SEM at the base line (concurrent control) was 190.780 ± 8.042 mg/dL, which fell to 168.317 ± 7.150 mg/dL after treatment with *Nigella sativa* oil for 40 d and again rose to 186.487±7.491 mg/dL after placebo administration for a similar period. The fall in glucose level after treatment was highly significant ($p = 0.000$), which increased significantly ($p = 0.000$) after the placebo. Fall in fasting blood glucose level was observed in 80.48 % cases ($n = 41$) while increase was seen in 19.52 % cases after the treatment with *Nigella sativa* oil. This fall was reversed in 75.60 % of the cases after the placebo, while glucose level in 4.878 % cases remained unchanged. The individual glucose levels of the patients at base line, after *Nigella sativa* oil and after the placebo are articulated in Fig. 1.

Insulin level: The mean insulin level ± SEM at base line was 8.013 ± 0.758 ulU/mL, which increased to 13.194 ±1 .404 ulU/mL after treatment with *Nigella sativa* oil and again decreased to 8.850 ± 0.694 ulU/mL after the placebo. The rise

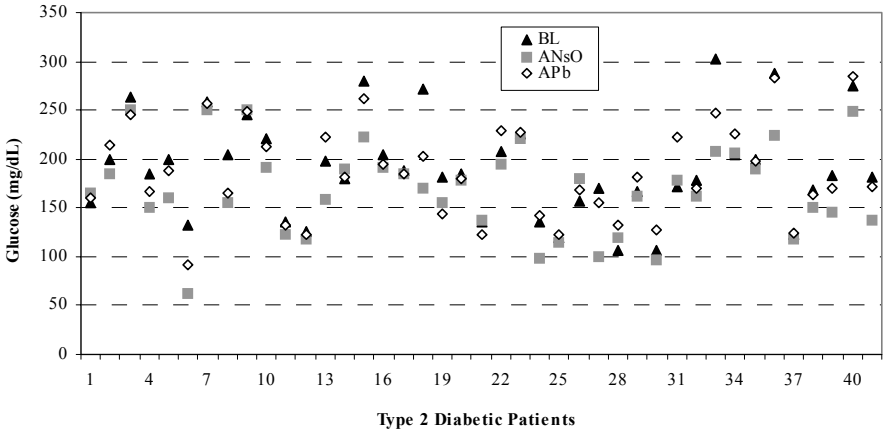

Fig. 1. Individual levels of glucose in DM2 patients at base line (BL), after *Nigella sativa* oil (ANsO) and after placebo (APb)

in insulin level was highly significant (p = 0.000) after *Nigella sativa* oil treatment and the fall after the placebo was also highly significant (p = 0.000). Rise in fasting blood insulin level was seen in 80.48 % cases (n = 41) after treatment with *Nigella sativa* oil and a fall was observed in 85.36 % of the cases after the placebo. Detailed pattern of each DM 2 patient is articulated in Fig. 2.

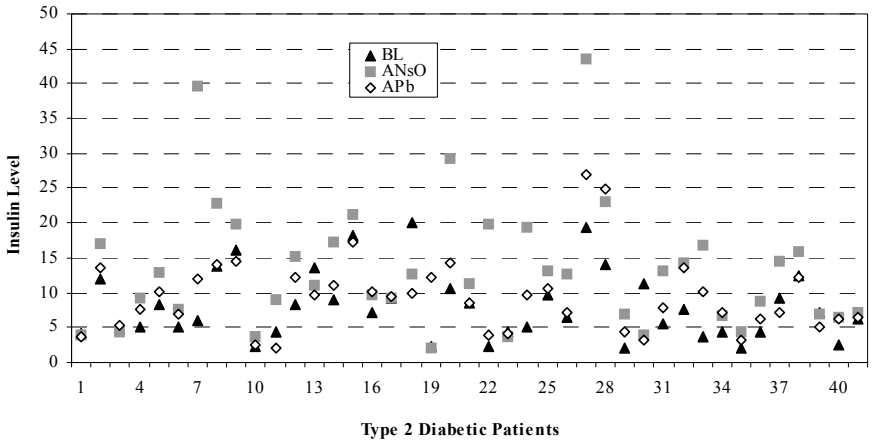

Fig. 2. Individual levels of insulin in DM2 patients at base line (BL), after *Nigella sativa* oil (ANsO) and after placebo (APb)

Platelet count: The mean platelet count ± SEM at base line was 229.463 ± 9.659 × 10^9/L, which changed to 227.365 ± 9.171 × 10^9/L after treatment with *Nigella sativa* oil and changed to 233.024 ± 9.282 × 10^9/L after administration with placebo. The change after *Nigella sativa* oil treatment was non significant (p = 0.550) and also non-significant after the placebo (p = 0.063). The individual levels of platelet count of subjects are articulated in Fig. 3.

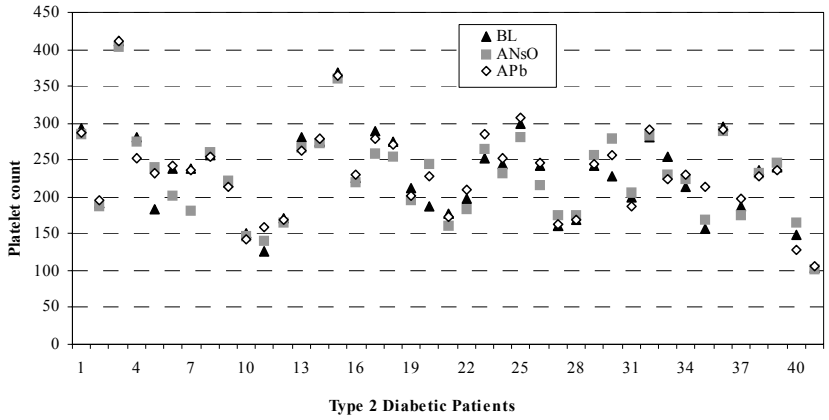

Fig. 3. Individual levels of platelet count in DM2 patients at base line (BL), after *Nigella sativa* oil (ANsO) and after placebo (APb)

Total leucocytes count (TLC): The mean TLC level ± SEM at base line was $7.524 \pm 0.266 \times 10^9$/L, which changed to $7.604 \pm 0.259 \times 10^9$/L after treatment with *Nigella sativa* oil and changed to $7.573 \pm 0.254 \times 10^9$/L after the placebo. The change after *Nigella sativa* oil treatment was non significant ($p = 0.511$) and changed after the placebo was also non-significant ($p = 0.674$). The individual levels of platelet count in all the patients are articulated in Fig. 4.

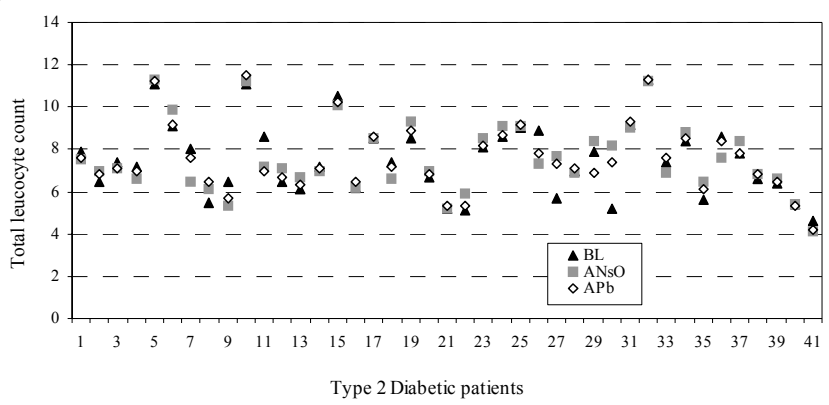

Fig. 4. Individual levels of total leukocyte count in DM2 patients at base line (BL), after *Nigella sativa* oil (ANsO) and after placebo (APb)

ALT Level: The mean ALT level ± SEM was 20.585 ± 1.240 at base line, 23.902 ± 1.820 after treatment with *Nigella sativa* oil and 23.048 ± 1.327 after the placebo. The change after treatment with *Nigella sativa* oil was non-significant ($p = 0.078$) and after placebo was also non-significant ($p = 0.465$). The individual levels of ALT are expressed in Fig. 5.

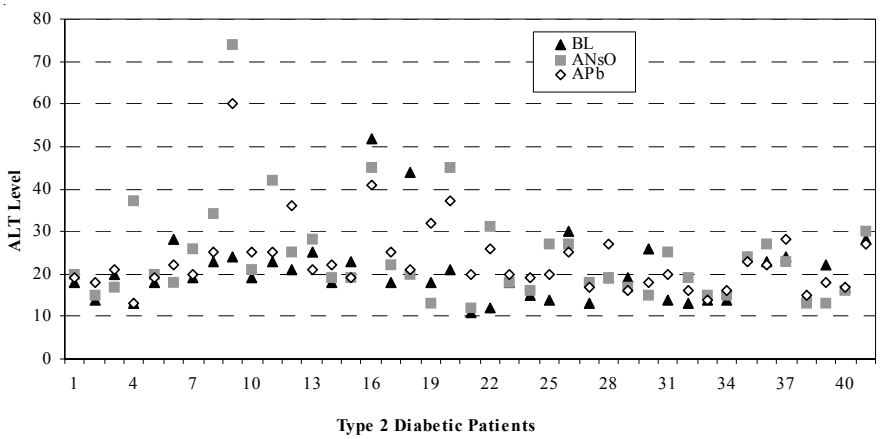

Fig. 5. Individual levels of ALT in DM2 patients at base line (BL), after *Nigella sativa* oil (ANsO) and after placebo (APb)

AST Level: The mean AST level ± SEM at base line was 23.926 ± 1.728 which increased to 28.390 ± 2.234 after treatment with *Nigella sativa* oil and slightly dropped to 27.390 ± 1.535 after the placebo. The increase in AST after treatment with *Nigella sativa* oil was significant (p = 0.045) and the change after placebo was non significant (p = 0.472). The individual levels of AST are articulated in Fig. 6.

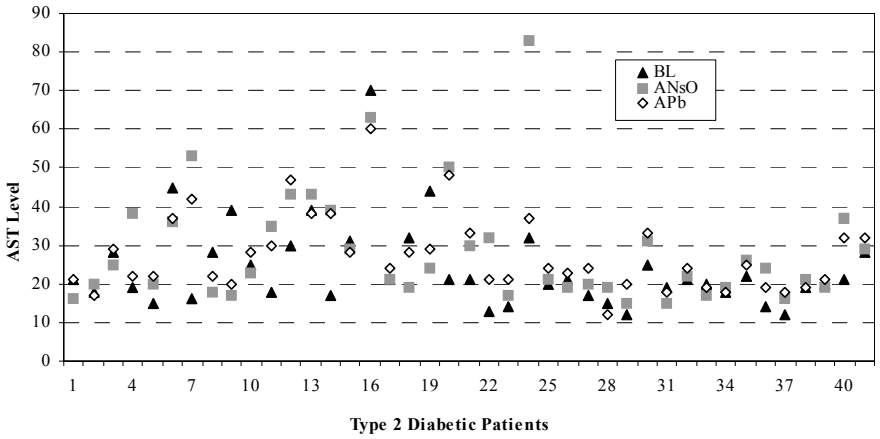

Fig. 6. Individual levels of AST in DM2 patients at base line (BL), after *Nigella sativa* oil (ANsO) and after placebo (APb)

Blood urea level: The mean blood urea level ± SEM at base line was 25.146 ± 1.092 which changed to 26.780 ± 1.117 after treatment with *Nigella sativa* oil and further changed to 25.707 ± 0.933 after the placebo. However, the change after treatment with *Nigella sativa* oil was non-significant (p = 0.089) and the change after placebo was also non-significant (p = 0.051). The individual levels of blood urea are shown in Fig. 7.

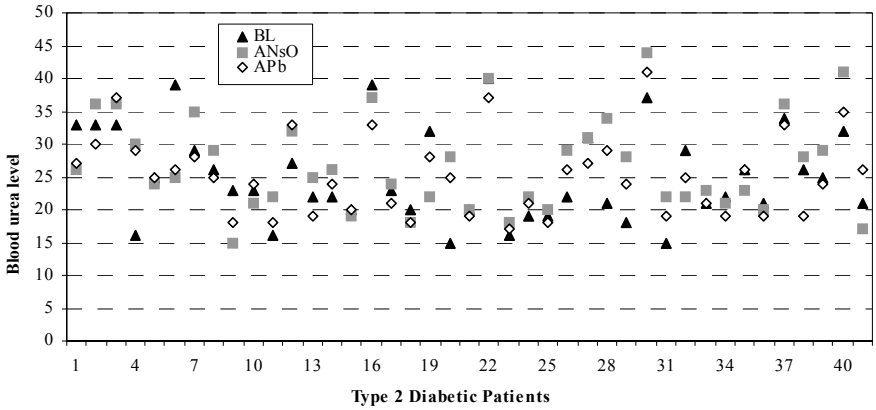

Fig. 7. Individual levels of blood urea in DM2 patients at base line (BL), after *Nigella sativa* oil (ANsO) and after placebo (APb)

Glucose and insulin: It is reasonably believed that the changes in glucose and insulin level were due to the treatment with *Nigella sativa* oil. This statement is strongly supported by the fact that insulin and glucose levels were significantly reversed after the consumption of placebo by the patients. An interesting point to note here is that after treatment with *Nigella sativa* oil a rise in insulin level was observed in 33 (80.48 %) patients (n = 41) while fall in glucose level was also seen in 33 (80.48 %) patients. It also emphasize that the increase in insulin secretion was actually responsible for the decrease in glucose level of the patients. Similarly after the placebo period, a fall in insulin level was observed in 85.36 % of the cases however a rise in glucose level was evident in 75.60 % of the patients.

Present results showing the decreasing trend of glucose level after the use of *Nigella sativa* oil in type 2 diabetic patients are in line with those described by El-Dakhakhny *et al.*[12] in a study executed on diabetic rats. According to their results, *Nigella sativa* oil significantly lowered blood glucose concentrations in diabetic rats after 2, 4 and 6 weeks. Al-Hader *et al.*[13] reported that the intraperitoneal administration of volatile oil of *N. sativa* to fasting normal and alloxon-diabetic rabbits produced significant hypoglycemic effects. Similarly in another study by Fararh *et al.*[14] a significant decrease in blood glucose level together with significant increase in serum insulin level were observed in streptozotocin plus nicotinamide-induced diabetic hamsters after treatment with *Nigella sativa* oil for 4 weeks. Big areas with positive immuno-reactivity for the presence of insulin were observed in the pancreases from *Nigella sativa* oil-treated group compared to non-treated one using immunohistochemical staining. They concluded in their study that the hypoglycemic effect of *Nigella sativa* oil in streptozotocin plus nicotinamide diabetic hamsters resulted, at least partly, from a stimulatory effect on β-cell function with consequent increase in serum insulin level. Their results indicate that *Nigella sativa* oil has insulinotropic properties in type 2-like model. Another study executed on rats with steptozotocin indused diabetes mellitus by Kanter *et al.*[11] suggested that the hypoglycaemic action

of *Nigella sativa* oil could be partly due to amelioration in the β-cells of pancreatic islets causing an increase in insulin secretion.

Another possibility may be that diabetics exhibit high oxidative stress due to persistent and chronic hyperglycemia, which depletes the activity of the antioxidative defense system and thus promotes de novo generation of free radicals[15]. Numerous studies have found increased lipid peroxides or reactive oxygen species (ROS) and oxidative stress (or both) in different animal models of diabetes[16,17]. Oxidative stress refers to an imbalance between the intracellular production of free radicals and the cellular defense mechanisms. Proteins, lipids and DNA are sensitive targets of ROS. An excess availability of free radicals accompanied by a reduction in the capacity of the natural antioxidant systems leads to cellular dysfunction and death[18]. The results of the present study indicate that the preventive effects of *Nigella sativa* oil may also be due to inhibition of lipid peroxidation as a result of its antioxidant nature. Similar results and observation were made by Kanter *et al.*[19] who studied the effect of volatile oil of *Nigella sativa* in diabetic rats and concluded that *Nigella sativa* oil has a therapeutic protective effect against diabetes by decreasing oxidative stress and preserving pancreatic beta cell integrity. Consequently, *Nigella sativa* may be clinically useful for protecting β-cells against oxidative stress.

Platelet and leukocyte counts: The total leukocyte and platelet count were a part of present study, because a previous study by Zaoui *et al.*[10] showed that treatment with *Nigella sativa*, decreased leukocyte and platelet count. It is consoling the total leukocyte and platelet count did not change significantly neither after the treatment with *Nigella sativa* oil nor after the placebo as compared to the base line levels. It verifies the safe use of *Nigella sativa* oil by DM2 patients. However high doses might be harmful regarding the fall in total leukocyte and platelet count as stated by Zaoui *et al.*[10] who used comparatively a higher oral dose of *Nigella sativa* oil extract (0.6 mL/kg body weight/day) for the rats.

ALT, AST and Blood urea: Although the levels of ALT and AST were in normal ranges at base line of the study and remained in normal status after the treatment with *Nigella sativa* oil yet AST increased significantly after *Nigella sativa* oil treatment. This increase within the normal levels, might be benifical rather than harmful. Mahmoud *et al.*[20] in his study on *Schistosomiasis mansoni* infected mice stated that administration of *Nigella sativa* oil succeeded partially to correct the previous changes in ALT and AST. In another study taking place at USA, Daba and Rahman[21] observed that thymoquinone (the active component of *Nigella sativa*, which is present in its oil) protected the liver enzymes leakage in isolated rat hepatocytes while investigating the hepatoprotective activity of thymoquinone. The lack of hepatocellular toxicity of the *Nigella sativa* oil has previously been confirmed in rats[10]. Non-significant changes observed in the blood urea levels of the patients after *Nigella sativa* oil treatment or the placebo also suggest its renal safety. These results also confirm the findings of Ali and Blunden[4] who reported that administration of either the seed extract or oil of *Nigella sativa* has been shown not to induce significant adverse effects on liver or kidney functions.

Conclusion

A significant fall in fasting blood glucose and rise in insulin levels was observed after treatment with *Nigella sativa* oil as compared to concurrent control levels without affecting the platelet count, total leukocyte count with reasonable hepatic and renal safety in patients with type 2 diabetes.

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