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Immuno-Modulatory Effects of *Solanium Nigrum* (UNH 333a) Leaves in Diabetic Geriatrics on Metformin Monotherapy

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Abstract: Solanium nigrum is a medicinal plant whose leaves and fruits are known to confer health benefits to humans. Its adoption as a dietary supplement for the regulation of blood sugar levels, especially for diabetic patients is now being practiced. This study aimed at monitoring the immuno-modulatory effects of S. nigrum supplementation in diets of diabetic geriatric patients on metformin monotherapy. A total of twelve patients were used for the study. Six of them served as control group while the other half were used as test patients. A 10 g portion of fresh leaves were administered to the patients twice daily through a three-month period. Packed cell volume, lipid profile, liver function and kidney function parameters were monitored over the period of study. There was significant difference ($p \leq 0.05$) between the initial and final packed cell volume values of the test patients; while no significant difference ($p \geq 0.05$) existed amongst the patients in their sodium, chloride, potassium, bicarbonate, urea and creatinine values. There was also no significant difference ($p \geq 0.05$) in the lipid profile and liver function parameters save for total protein and albumin values which had significant differences ($p \leq 0.05$); showing decrease from 73.67±1.97 to 11.78±2.74 and an increase from 44.00±1.55 to 47.00±2.37 respectively. This study has been able to show that S. nigrum has immuno-modulatory impact to diabetic patients who use them as dietary supplement for blood sugar regulation.

Keywords: Solanium Nigrum, Plant, Diabetic, and Patients.

INTRODUCTION

The use of foods as medicines or medicinal alternatives is a practice that has been around for some decades. Medicinal plants are known to contain certain phyto-constituents that aid in proper metabolic functioning of the body. *Solanium nigrum* is a tropical plant used for different medicinal purposes ranging from antibacterial, anti-helminthic, anti-inflammatory and likewise, anti-diabetic functions. Concomitant with these stated roles is its impact on the immune system of humans who take them. The pharmacological properties of *Solanium nigrum* have been attributed to the presence of certain chemical substances in the plants, such as fiber, ascorbic acid, phenols, anthocyanin, glycoalkaloids and α-chaconine (Gaikwad *et al*., 2014).

Diabetes is a carbohydrate, protein and fat metabolism disorder which affects persons of different age brackets (Aguwa & Omole 2012). It is not to affect millions of people round the world (Okpara & Odili, 2010). Metformin is a known antidiabetic medication used for the control of type II diabetes (Inzuchi, 2002). This study aimed at evaluating the immune-modulatory roles of S. nigrum on geriatric patients on metformin monotherapy, who use the leaves as dietary supplements.

METHODS

Study Design

The research made use of Interventional study design. A total number of twelve geriatric diabetic patients (six for control and other six patients were used as test patients) that are poorly managed were used for the study over a period of three months.

Ethical Consideration

Ethical approval was granted by the ethics board and research committee of Asokoro District hospital, Abuja. Patients consents were duly obtained after one on one discussion and consent forms filled, signed and documented.

Sampling Method

Convenience sampling method was adopted for this research.

Sampling Setting

Poorly managed Geriatric diabetic patients (≥65years) that attend clinic at Asokoro District Hospital Abuja.

Eligibility Criteria

- Geriatrics on metformin monotherapy only.

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- Geriatrics ≥65 years.
- Geriatrics on poorly controlled blood glucose.
- Patients willing to comply with the life style modifications and hospital visits.

Research Design for the Administration of *S. nigrum* Leaves

Baseline tests were first performed on the patients, after which they were placed on 10 g twice daily oral consumption of fresh, saline washed garden egg leaves (first thing in the morning and last thing at night). Participants were asked to maintain a routine of 30 minutes morning aerobics, healthy dietary habits and to take their last meals at most, 7 pm daily. Daily glucose parameters monitoring (HBA_{1C}, FBS and 2-HPP) of the test patients and control patients were performed for a 3-month period. Control patients were on metformin therapy only.

Fasting Lipid Profile Determination

Patients were asked to fast from food and drinks for 10 to 12 hours prior to test, and come fasting in the morning of the day of testing. A 4 ml aliquot of venous blood samples were obtained from the patients and transferred into Vacutainer Bottle containing littium Heparin anticoagulant. Blood samples were gently mixed and vortexed in a centrifuge to obtain plasma samples. Automated chemistry analyzer (Selectra Pro's) was prepared by re-filling reagents and switching on to boot. Thereafter the test plasma samples were transferred into the sample space of the machine and Cholesterol, Triglycride, HDL, LDL were analyzed.

Determination of Serum Urea

Three specimen tubes were labelled A-C, 10µl serum sample was added into test tube A (sample), 10µl of standard (CAL) was added into test tube B (standard), 10µl of distilled water was added into test tube labeled C; while 100 µl of reagent R1 (EDTA) was added into tubes A, B and C mixed and incubated for 10 minutes at the temperature of 37^0C. The absorbance was spectrophotometrically read at 546nm and calculation was made thus;

Urea concentration (mg/dl) = A_{sample} / $A_{standard}$ x Standard concentration (Reddy *et al*., 2011).

Determination of Serum Creatinine

This was determined with alkaline picrate method using the creatinine kit according to the method of Reddy *et al*. (2011). A 2 ml aliquot of picric acid reagent in a test tube was added to 0.2 ml of serum (for deproteinization of serum), mixed well and centrifuged at 3000 rpm to obtain a clear supernatant. A 100 µl aliquot of buffer reagent was added to 1.1 ml of supernatant, 0.1 ml of standard creatinine and 0.1 ml of distilled water to prepare the test solution, standard and blank respectively. A 0.1 ml of picric reagent was added to blank and standard. The test tubes were mixed well and kept at room temperature for 20 minutes. The alkaline picrate reacts with the creatinine to form an orange coloured complex, which was read at 520 nm with a spectrophotometer. The serum creatinine concentration was calculated thus:

Serum creatinine concentration (mg/dl) = absorbance of test/ absorbance of standard x 2.

Determination of Sodium (Na^+)

Freshly collected blood was centrifuged and the serum was collected. Into labeled test tube A, B, C and D, 1.0 ml of filtrate reagent was added into each tube and then 50µl of serum sample was added to the test tube B,Cand D while distilled water was added to the blank test tube (A). The tubes were shaked vigorously and mixed continuously for 3 minutes. The tubes were centrifuged at 1,500 RPM for 10 minutes and the supernatant was tested ensuring that the protein precipitate was not disturbed. A 1.0ml aliquot of acid reagent followed by 50µL of supernatant and 50µL of color reagent were added and mixed. The absorbance of the labelled tubes was spectrophotometrically read at 550 nm and calculation was made thus:

Abs. of Sample-Abs. of Standard/Abs. of blank-Abs. of STD x Conc of STD (mEq/L) = Conc of Sodium (mEq/L).

Determination of Chlorides (Cl^-)

Test tube A, B, C and D were labelled and 1.5ml chloride reagent was added into each labelled tube and 0.01ml (10µl) of calibrator was added in tube A, while serum samples were added in other tubes and were all mixed. The tubes were incubated at room temperature for 5 minutes. The absorbances of all the tubes were spectrophotometrically read at a wave length of 480 nm-520 nm**.** Calculation was made thus:

Abs. of unknown/ Abs. of calibrator x concentration of calibrator = concentration of chloride (mEq/L)

Determination of Potassium (K^+)

Into labeled test tubes (A-D), 1.0 ml of potassium reagent was added. Then 0.01 ml (10µl) of serum samples were also added into the labeled tubes mixed and was allowed to stand for 3 minutes at room temperature. After 3 minutes the absorbance was spectophotometrically read at the wavelength of 500 nm. Calculation was made thus:

Abs. of unknown/Abs. of STD x Conc of STD (mEq/L) = Potassium conc (mEq/L).

Liver Function Test (LFT)

A 4 ml aliquot of venous blood samples were obtained from the patients and transferred into Vacutainer Bottle containing littium Heparin anticoagulant. Blood samples were gently mixed and vortexed in a centrifuge to obtain plasma samples. Automated chemistry analyzer (Selectra Pro's) was prepared by re-filling reagents and switching on to boot. Thereafter the test plasma samples were transferred into the sample space of the machine. LFT parameters assessed include; SGOT, SGPT, Alkaline Phosphates,

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Total Bilirubin, direct Bilirubin, Total Protein and Albumin.

RESULTS

Phytoconstituents of *Solanium aethiopicum*

Solanium nigrum had ascorbic acid as the highest phytoconstituent (0.786 %), followed by Alkaloid (0.590 %), while flavonoid had the least content of 0.090 % as shown on Table 1.

Table 1: Phytoconstituents of *Solanium nigrum*

Phytoconstituents	Actual quantity in%
Flavonoid	0.090
Saponin	0.180
Ascorbic acid	0.786
Alkaloid	0.590
Tannin	0.180

Effect of Oral Administration of *Solanium nigrum* and Metformin on Blood Chemistry Profiles

There was significant difference ($p \leq 0.05$) between the initial and final packed cell volume values of the test patients; while no significant difference ($p \geq 0.05$) existed amongst the patients in their sodium, chloride, potassium, bicarbonate, urea and creatinine values. There was also no significant difference ($p \geq 0.05$) in the lipid profile and liver function parameters save for total protein and albumin values which had significant differences ($p \leq 0.05$); showing decrease from 73.67±1.97 to 11.78±2.74 and an increase from 44.00±1.55 to 47.00±2.37 respectively.

Table 2: Effect of Oral Administration of *Solanium nigrum* on Packed Cell Volume

Packed Cell Volume	Mean±S.D
Initial	40.50±5.84
Final	43.00±7.07
P value	0.027

Table 3: Effect of Oral Administration of *Solanium nigrum* on Lipid Profile

Parameters	Initial (Mean±S.D)	Final (Mean±S.D)	P value
Total Cholesterol	5.60±0.38	5.47±0.38	0.563
Total Glyceride	1.20±0.53	1.35±0.56	0.258
HDL	1.37±0.29	1.25±0.19	0.158
LDL	3.70±0.25	3.58±0.57	0.537
VLDL	0.53±0.23	0.62±0.26	0.224

Table 4: Effect of Oral Administration of *Solanium nigrum* on Kidney Function Profile

Parameters	Initial (Mean±S.D)	Final (Mean±S.D)	P value
Urea	3.85±1.98	3.15±0.44	0.497
Creatinine	96.50±5.09	105.33±11.04	0.817
Sodium	144.33±4.37	140.83±2.93	0.090
Potassium	4.25±0.59	4.17±0.53	0.683
Chloride	100.83±2.32	100.17±4.31	0.800
Bicarbonate	28.00±2.03	28.00±4.24	1.000

Table 5: Effect of Oral Administration of *Solanium aethiopicum* on Liver Function Profile

Parameters	Initial (Mean±S.D)	Final (Mean±S.D)	P value
Total Bilirubin	13.15±5.13	11.78±2.74	0.495
Direct Bilirubin	5.80±2.47	5.42±1.62	0.496
GOT	17.17±7.08	21.17±15.60	0.327
GPT	12.17±5.88	12.67±14.50	0.912
ACP	191.50±66.55	183.67±51.00	0.563
TP	73.67±1.97	79.10±2.74	0.001
ALB	44.00±1.55	47.00±2.37	0.007

DISCUSSION

Solanium nigrum is a medicinal plant with varying antidiabetic properties as well as antioxidant activities. Its phytoconstituents such as alkaloid and ascorbic acid concentrations as shown on Table 1, reflect its ability to perform the aforementioned roles. Ascorbic acid had the highest phytoconstituent, which is suggestive of the nutritional value of the leaf as rich in antioxidant and as such an immune booster (Chinedu *et al*., 2011). Furthermore, other phytochemicals present such as the flavonoids,tannins equally suggests the ability of the leaves to boost immunity (Rao &Gurfinkel, 2000). Pan *et al.* (2003) explains that plants rich in alkaloids readily exert a wide range of antidiabetic activities through different mechanisms of metabolism.

The evaluation of immune-modulatory effects of *S. nigrum* leaves oral administration was conducted by monitoring packed cell volume and blood chemistry parameters such as lipid profile, liver function and renal function parameters (Tables 2-4). Packed cell volume of the patients was significantly ($p \leq 0.05$) increased with the administration of *S. nigrum* and this finding is similar to that of Duru *et al*. (2013) that reported significant increase ($p \leq 0.05$) in the packed cell volume of Wister rats fed with *S. macrocarpon*. There was also no significant difference ($p \geq 0.05$) in the lipid profile of the patients which partly agrees with the reports of Sohrabipour *et al.* (2013); & Azarkish *et al*. (2017) that did their investigation using albino rats. Same was the observed effect on liver function tests, save for total protein and albumin values that significantly ($p \leq 0.05$) increased in the patients. This development could be fairly explained from the works of Ezeugwu *et al*. (2004) who reported that *S. nigrum* and *S. aethopicum* fruits contains a high amount of protein in proximate analysis, which is the third highest nutrient after moisture content and carbohydrate content; however, nutritional composition of fruits does not automatically infer nutritional composition of leaves. No significant

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difference (p ≥0.05) was observed in the kidney function tests, which varies with the reports of Nandita *et al*. (2016). However, there was increase in creatinine value which signifies muscle wasting and thus, is indicative of the exercise that was incorporated into the diabetes control regimen of the patients.

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