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Synergistic of Solenostemonmonostachyus and Ocimumgratissium for the Management of type -2 -diabetes

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The efficacy of synergistics of Solenostemonmonstachyus and Ocimumgratissimumfor the management of type -2 –diabetes using Zebra fishwas investigated. The plants were dried at room temperature, grounded and the powder pooled together, homogenized and extracted for 7 hours using the Soxhlet apparatus. A total of 75 zebra fish were used and were divided into five groups of five juveniles per test concentration in three replicates. Group A was given de-chlorinated water; B was treated with 0.25mg/L diazinon only while other groups were exposed to the same concentration as in group B, but with different concentrations of the plant's extract. Group C, D and E received 250, 500, and 750mg/L respectively. Water quality parameters and blood chemistry were estimated in the experimental and control fish on day 7, 14, 21 and 28th. All the blood parameters were significantly ($P< 0.05$) affected by diazinon when compared with the control. But were reversible in the plants extract mixture treated fish and were dose and time dependent reduction, even less than the control group. This findinghad shown the efficacy of these plants' extract and the use of zebra fish as toxicological tool. Thus,thoroughstudy of these plants mixture is indispensable, so as to find a lasting solution to the ailment that has been given people a nightmare.
Keywords: Solenostemon monstachyus, Ocimum gratissimu, Mixture, Diazinon, Zebra fish and Human.

Introduction

Diabetes is one of the most noxious and precarious ailment that has been a menace to the life of people. The condition is mainly defined by the level of hyperglycemia which gives rise to the risk of micro-vascular damage [1]. Diabetes mellitus is of two types; type I (insulin-dependent) , this is when the insulin producing β-cells are not functioningwell therefore no enough insulin production, and type 2 (noninsulin dependent) caused by relative insulin insufficiency due to insulin resistance-the inability of the insulin to tell the cells to use glucose in addition to insufficient insulin to overcome this resistance . Of a truth diabetes has robbed many people in the past years in living a healthy and normal life [2]. The controls of diabetes depend on the traid of food, activity and insulin or oral agents. All these must be balanced properly by the diabetic patient.

It has been estimates by the World Health Organization (WHO) that over 366 Million people worldwide are affected by diabetes mellitus, and many without efficacious diabetes care [3]. A recent revelation by the WHO indicates that diabetes has tripled in the last two decade globally with the highest prevalence rates found in developing countries [4]. The WHO report indicates that in extreme cases, up to 30 - 50% of the adult population in some developing countries have been afflicted with diabetes [5]. Chinenye (2015) reported that in Nigeria, with the population of over 180 million people [6], an estimated six million people have full blown diabetes mellitus. The 1992 National prevalence study on Non-communicable diseases conducted by the Federal Ministry of Health in 13 states of the Federation indicated a prevalence rate of 2.7% with a prevalence of 2.6% in adult males and 2.8% in adult females. Reports from subsequent studies as hospital records indicated an alarming increase in both prevalence and incidence of diabetes among all ethnic groups and social classes in the world [7].

Medicinal plants have been used in many years back to treat diseases all over the world. Some medicinal plants has been proven to be anti-diabetic plants used to treat diabetes mellitus by the local folks. Some of such plants are*Solenostemon monostachyus* (SM) and *Ocimmum gratissimum* (OG), but have not been examined medically. Therefore, the study was designed to investigate the acclaimed antidiabetic abilities of the extract mixture of *S. monostachyus* and *O gratissimum*extract macerated in acetone.

MATERIALS AND METHODS

Chemical / Equipment Analysis: Diazinon (98.5%purity) that was used for fish bioassay was obtained from chemical Service, WestChester, U.K., Aquaria (locally built), Aerators from Agro chemicals, Canada, ethyl 3-aminobenzoate methanesulfonate salt, Sigma

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Collection and Handling of Experimental fish

The zebra fish used for the test were raised in the laboratory in a well-aerated tarpaulin tank for three months. From the tank, 15 post juveniles fish weighing 23.20 ± 0.12 gm and 17.00 ± 0.13 cm in length were taken into well-aerated containers, to avoid hyperactivity, injuries and shock. The fish were examined for any pathological sign and washed with1% $KMnO_4$ solution, and transferred to glass aquaria (50 x 25 x 25 cm) containing dechlorinated tap water. They were allowed to acclimatize to their new environment (glass aquaria) conditions for 14 days prior to experiment and were fed with 30% protein pellets. Water was replaced regularly and limnological parameters of the water were kept within the recommended range with electrical aerators. At the end of the acclimatization, all the fish were in good health and no mortality observed.

Cultivation of the plants (*S. monostachyus*and *O. gratissimum*)

A well-drained, sunny location within Federal University Otuoke Nigeria premises earmarked for botanical gardens was used for the cultivation of the herbs. The soil was tested to determine the soil pH (6.0 - 6.8) and nutrient levels. Geophysics Department of the University assist in ascertained the physicochemical parameters of the soil. The seeds of *S. monostachyus*and *O. gratissimum* were purchased from the market and cultivated within 2 hours. The plants were monitored and grew into maturity within two months.

Crude Extraction: The fresh leaves of SM and OG were collected from the garden and dried separately at room temperature (22 + 0.15)ºC for 21 days in the laboratory . They were grounded separately using Nakai blender and filtered through a 40-mesh screen.

They were grounded separately using Nakai blender and filtered through a 40-mesh screen Equal volume (1.5kg) each of the two plants powders and was homogenized. The dry powder was extracted by maceration at 35–37°C; five times for 18 to 20 hrs with 70% ethanol (Jones and Kinghorn, 2012). Filtered under vacuum and dried using rotary evaporator (Heidolph, Schwabach, Germany) at 35–40°C. The extract was weighed and preserved aseptically at 4oC.

Phytochemical Determination: The phytochemical compositions of the plants were analyzed using the methods described by Trease and Evans (1989). The following chemical composition of the plants leaves were determined quantitatively;Alkaloid, Terpene, Flavonoids, Saponins, Anthraquinones ,Tanin, Phlobatanins, Phenol and Cardiac glycoside

Bioassay Test

The fish were divided into five groups comprising five fish per test concentration. They were exposed to the concentrations of organophosphate that has been experimentally proven to induce hyperglycemia by obstructing pancreatic islets (Ikpesuet al., 2014), and other treatments as stated below.

Group A was given de-chlorinated tap water; B was treated with 0.25mg/L diazinon only while other groups were treated to the same concentration as in group B, but with different concentrations of the plants extracts mixture. Group C, D and E received 250, 500, and 750mg/L respectively. Water quality parameters were kept within the normal range with the help of aerator. The blood sugar was estimated in the experimental and control fish on day 7, 14, 21 and 28th

Blood Sugar Determination

At the end of each experimental period (day 7, 14, 21 and 28th) , a fish was removed from each aquarium and immediately anesthetized with MS222 (ethyl 3-aminobenzoate methanesulfonate salt, Sigma). Blood sample was taken by puncturing the caudal vessels with a 20-gauge needle and aspirating 0.2–0.4mL sample of mixed arterial and venous blood into a heparinized syringe, a technique shown to minimize dilution by tissue fluids (Congleton and LaVoie,2001). The blood sample was stored in heparinized blood collecting duct for the estimation of blood sugar level. The blood was allowed to clot for 30min, centrifuged at 2000 g for 15 min for clear separation of the serum, and stored at −80oC until the analysis.

Glucose Analysis

Folin-Wu protein free filtrate was prepared by adding nine volumes of the mixture containing eight parts N/12 H_2SO_4 and one part of sodium tungstate directly to the sera separated from the whole blood [8]. The medium was filtered and the filtrate was taken in a tube and two ml of alkaline copper sulphate solution was added. The optical density was measured at 420 nm in a spectrophotometer. Simultaneously, standard glucose solution containing 1 mg·ml and a blank containing 2 ml distilled water was taken for the observations.

Statistical analysis

The data of the efficacy of synergistic fof different concentrations of *S.monstachyus* and *O.gratissimum*for the management of type -2 –diabetes were presented as mean ± SD, from 5 independent experiments with triplicates. The difference between the control and the various treatments and within the treatments were analyses using the student's t- test at 95% confidence level (Steel and Torrie, 1980) and one-way analysis of variance SPSS (14.0 version), SPSS Inc, Chicago, USA. P≤0.05 was considered to be significant.

Results

Hydo-chemical Properties of the Test Media

The water quality parameters (pH, temperature, dissolved oxygen, alkalinity and hardness) monitored during the investigation was not significantly different between various concentrations of the pesticide and the control and within concentrations ($p > 0.05$) (Table 1)

Phytochemical Screening: The results of the phytochemical composition of the leaves extracts of the investigated plants showed the presents of Alkaloid, Terpene, Flavonoids, Saponins, Deoxy-Sugar, Anthraquinones , Tanin, Phlobatanins and Cardiac Glycoside. The magnitude of concentrations of the chemicals in these plant leaves extract differs as represented with depiction (Table 2).

Blood glucose: The responses of the zebra fish to diazinon and to the different concentrations of the synergistic of SM and OG is shown in figure 1. The glucose level in aqueous treated fish (group A) shows no significant difference between the days of exposure ($p > 0.05$). However, in the diazinon alone treatment, the fish were hyperglycemic, which

Table 1: The physiochemical parameters of the test media

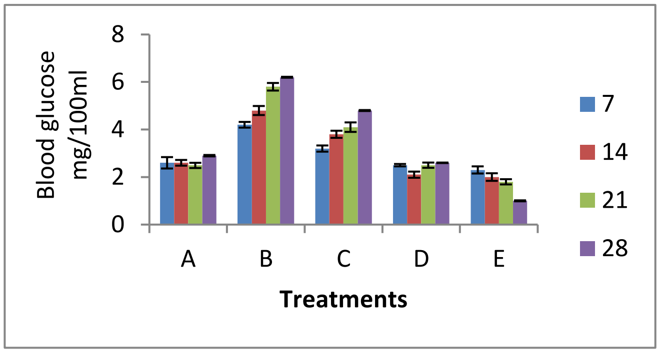
Parameters	pH	Temp (^{O}C)	DO (mg/l)	Turbidity (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)
Group (μg/L)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A	7.22 ±0.02	25.67 ±0.16	8.10 ±0.22	0.23± 0.04	17.40 ±0.72	31.23 ±1.15
B	7.36 ±0.16	25.00 ±0.30	8.12 ±0.19	0.23±0.06	17.63 ±0.42	31.33 ±1.15
C	7.25 ±0.10	27.33 ± 0.68	8.30 ±0.31	0.24 ±0.02	17.40 ±0.36	31.20 ±1.02
D	7.12 ±0.25	27.00 ±1.20	8.03 ±0.22	0.25 ±0.04	17.13 ±1.20	30.60 ±0.50
E	7.12 ±0.20	26.33 ±0.48	8.16 ±0.02	0.26±0.03	17.23 ±0.12	30.60 ±0.16

A: Distilled water; **B**: 0.25 mg/L diazinon treatment; **C**: 0.25 mg/L diazinon and 250mg/L equal mixture of crude extract of *S.monostachyus* and *O. gratissimum*; **D**; 0.25 mg/L diazinon and 500mg/L equal mixture of crude extract of *S.monostachyus* and *O. gratissimum*; **E;** with 0.25 mg/L diazinon and 750mg/L equal mixture of crude extract of *S.monostachyus* and *O. gratissimum*

Table 2: Phytochemical compositions of the leaves extracts of S.monostachyus and O. gratissimum

Phytochemicals	*S.monostachyus*	*O. gratissimum*
Anthraquinones	**	*
Phenol	**	*
Flavonoids	****	**
Saponins	*	**
Alkaloid	*	*
Phlobatanins	*	**
Terpenes	**	#
Tannin	**	*
Cardiac Glycoside	*	*

Keys: *Present in moderately concentration; **Present in high concentrations; ≠ Not detected



A: Distilled water; **B**: 0.25 mg/L diazinon treatment; **C**: 0.25 mg/L diazinon and 250mg/L equal mixture of crude extract of *S.monostachyus* and *O. gratissimum*; **D**; 0.25 mg/L diazinon and 500mg/L equal mixture of crude extract of *S.monostachyus* and *O. gratissimum*; **E;** with 0.25 mg/L diazinon and 750mg/L equal mixture of crude extract of *S.monostachyus* and *O. gratissimum*

increases with exposure duration and varies significantly ($p < 0.05$). In the treatment C, the fish responses is similar to B treated fish but was not significant ($p > 0.05$), though the effect of the plant extract was slightly obvious. There was fluctuation in the D treatment, although not significant ($p > 0.05$), but the effects was quite obvious and comparable to the control. E treated fish shows hypoglycemic as the day's progresses. On day 7 and 14th, the sugar level is similar to that of the control, while day 21 and 28th, the level was lower than the control, the fish were hypoglycemic and the variation was highly significant ($p < 0.01$)

Discussion

Herbal medicines have played an important role in treating diabetes in various parts of the world for centuries, and their synergistic properties due to the presence of variety of components within herbals extract are beneficial to multifactorial diseases like diabetes [9].

In this investigation, the responses of the hyperglycemic induced zebra fish to the synergistic of *S.monostachyus*and *O. gratissimum* revealed doses and time dependent recovery, with the concentration of 500mg/L plants extracts comparable with the control. When the concentrations of the extracts was increased to 750mg/L there was spontaneous decrease in the sugar level, far below the reference value (Fig. 1) and was highly significant ($p < 0.1$) on day 28th.

This antidiabetic's property SM and OG may be attributed to the synergistic and the potency of their phytochemical compositions. Alkaloid is known to exhibits hypoglycemic activity and antioxidant properties [10, 11]. Terpene often refers to as terpenoids has a broad range of the biological properties, including cancer chemo preventive effects, antimicrobial, antifungal, antiviral, ant hyperglycemic, anti-inflammatory, and ant parasitic activities [12]. Saponin is essentially good in glycemic control as well as its principal roles in preventing different metabolic disorders and liver damage caused by hyperglycemia. Its antidiabetic activity is attributed to its components that plays vital activity in the releasing of insulin from the pancreas that is, it exerts a direct insulinotropic effect [13].

2-hydroxy-3-methyl-anthrquinone is another phytochemical component of the investigated plants and was also identified as the hypoglycemic active components of binate [14,15]. Although anthraquinone glycosides have been known to cause diarrhea, aglycone has several other biological activities such as antibacterial and anti-inflammatory effects [16]. Similarly Ye et al. (2010) reported that early intervention with anthraquinone glycosides significantly improved glucose tolerance and restored early-phase insulin secretion in db/db mice. Equally, the tannin phytochemical observed in SM and OG was also reported in extract from the amaranth grain, finger millet, field bean, sunflower seeds, drumstick, and amaranth leaves, and it exerted significant higher antioxidant and antidiabetic activities [17]. Phenols are known for their anti-hyperglycemic activity (Nkirote, et al., 2011), was present in high concentrations in *S.monostachyus*and moderately concentrated in*O. gratissimum*.

This agrees with the local uses of the leaves of the plant for the treatment of diabetes by traditional medicine practitioners [18, 19].

Conclusion

Diabetes mellitus is becoming a threat to world, with the significant achievements in treatment modalities and preventive measures; its prevalence has risen exponentially. Because of these limitations, there is continuous need for new and more effective therapies to combat the surge.

The synergistic of S.monostachyusand O. gratissimum had shown ameliorating effects in the control of hyperglycemia and justify the traditional uses of the plants for the treatment of diabetes.

Further study is necessary to investigate the mechanism behind the hypoglycemic effect of the synergistic of these two plants. We strongly believe that they are promising antidiabetic constituents that will be helpful for the management of type 2 diabetes, and stabilize blood sugar level for longer periods.

Conflict of Interest

There is no conflict of interest.

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Author's Contribution

Dr.Ikpesu,T. O.: Design the research and take part in the writing of the article

Dr. Ezenwaka, C. O.:Monitor the fish in the laboratory, collect the blood samples and did all the analysis. She also participated in the writing of the article.

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