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TIME DEPENDENT EFFECT OF THE AQUEOUS SEED EXTRACT OF *HELIANTHUS ANNUUS L.* ON SOME BIOCHEMICAL PARAMETERS IN ALLOXAN INDUCED - DIABETIC RATS.

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Abstract: The study was carried out to investigate the effect of the aqueous extract of helianthus annuus L seed and time of treatment on serum glucose, protein, urea, albumin, creatinine and some electrolytes using Wista albino rats as the animal model. Twenty (20) adult male wista albino rats were randomly assigned to four (4) groups of five (5) rats each. Diabetes was induced in the test and control rats by the intraperitoneal injection of alloxan monohydrate at a dose of 150mg/kg b. w. The experimental rats were administered 400mg/kg b. w single daily dose of the extract for fifteen (15) days. Diabetes was confirmed in the experimental rats by the significant increase ($p<0.05$) in serum glucose level. Treatment with helianthus annuus seed extract showed a significant time dependant decrease ($p<0.05$) in serum glucose level. The diabetic rats showed significant ($p<0.05$) increase in the serum levels of urea, creatinine. Treatment with the extract resulted in a time dependent significant ($p<0.05$) decrease in these parameters. There was a significant ($p<0.05$) decrease in the serum levels of total protein and albumin. Treatment with extract produced significant ($p<0.05$) increase in these parameters. This investigation also showed a significant ($p<0.05$) decrease in serum levels of Na^+, K^+, Ca^+, Mg^{2+} and Cl^- in the diabetic rats. Treatment with the extract produced significant ($p<0.05$) time dependent increase in the levels of these electrolytes.

Keywords: Helianthus Annuus L, Diabetes Mellitus, Glucose, Electrolytes

INTRODUCTION

For thousands of years, plants were a primary source of therapeutic medication for cultures all over the word. The use of medicinal plants for treating diseases is therefore probably the oldest existing method that humanity has used in coping with illness. For this reason, medicinal plants have been used therapeutically all round the world, being an important aspect of various traditional medicine systems [23, 24]. The World Health Organization (WHO) has estimated that up to 80% percent of people in the developing world are dependent upon traditional herbal medicines primarily because of their ease of accessibility, wide affordability and cultural familiarity [14, 23]. Among the common diseases and conditions that

benefit from phytotherapy is *diabetes mellitus*. *Diabetes mellitus* is one of the major diseases affecting millions of people worldwide and the number is growing rapidly. It is one of the most prevalent epidemics of the twenty first (21st) centuries [16]. It can be defined as a group of metabolic diseases characterized by chronic hyperglycaemia due to defective insulin secretion, insulin action, or both, resulting in impaired carbohydrate, lipid and protein metabolism [2]. Several plant species have been reported to possess anti-diabetic properties and have also been used for prevention or managing diabetes in the traditional system of medicine in the different regions of the world [5, 8, 11].Helianthus annuus L is reported to have excellent nutritive and medicinal properties, it is an annual plant which originated in south America and can be grown successfully in the tropics and sub tropics. Its seeds are used by some communities in Nigeria as a complimentary treatment for diabetes mellitus, this study sought to ascribe validity or not to these claims. The aim of this study was to investigate the effect of the aqueous seed extract of helianthus annuus on some diabetes mellitus.

MATERIALS AND METHODS

Plant material

Helianthus annuus L (sunflower) seeds were obtained from the Institute of Agricultural Research (IAR), Faculty of Agriculture, Ahmadu Bello University Zaria, Nigeria. The variety was funtua oil rich type.

Preparation of Plant Extract

The *helianthus annuus L* seeds were sundried, dehulled and pounded into a fine powder using mortar and pestle. The powdery seed was poured into a beaker mixed properly with water and then placed on an electric hot plate to boil. The mixture was stirred continuously until it started boiling. It was then filtered. The filtrate was evaporated to dryness at 60^{0}c, and the extract was stored in a clean air-tight container in the refrigerator.

Experimental Rats

Adult male wista albino rats (Rattus norvegicus) ranging from 150 to 250g weight were used in the study. The animals were housed in cages, allowed two (2) weeks to acclimatize and maintained on standard animals feed and drinking water *ad libitum*.

Induction of Experimental Diabetes Mellitus

The animals were fasted overnight with free access to water prior to the induction of diabetes. Induction of diabetes was carried out by single intraperitoneal injection of alloxan-monohydrate (Sigman St. Louis, M.O; USA) dissolved in

normal saline solution at a dose of 150mg /kg body weight. Diabetes was confirmed in alloxan induced rats by determining the blood glucose level 48hrs after injection of alloxan using one touch (R) Glucometer (Life scan Inc, 1995; Milpitas Califonia 95395, USA).

Design of the Experiment

After the induction of diabetes mellitus in the rats, the animals were randomly divided into experimental and control groups of five rats each as follows;

Group A: Normal control (positive control)
Group B: Diabetic control (negative control)
Group C: Normal treated rats
Group D: Diabetic treated rats

Treatment of Experimental Animals and Collection of Samples

Groups A and B animals were given 0.2ml of distilled water daily, while groups C and D received 400mg/kg b.w single daily dose of the dried aqueous extract for fifteen (15) days by oral intubation. Blood glucose levels were taken at five (5) days interval using one touch ® glucometer. At the end of the experiments, the rats were starved overnight before they were anesthetized and sacrificed. Blood samples were collected in clean dry centrifuge tubes, allowed to clot for 40 minutes and then spun at 5.000 rpm for 10 minutes. The sera were collected, transferred to sterile bottles and kept for analysis.

Assay of Biochemical Parameters

Parameters assayed include serum glucose by the method of Trinder, 1969 [22], total Protein by the method of Gornall *et al*, 1949 [12], Urea by the method of Tobacco *et al*, 1979 [20], Uric Acid by the method of Fossati *et al*, 1980 [10], while Creatinine concentration was assayed by the method of Henry, 1974 [13], Albumin concentration by the method of Doumas, *et al*, 1971 [9]. Sodium ion (Na^+), and Potassium ion (K^+) concentrations were determined by flame photometry while Calcium ion (Ca^{2+}) and Chloride ion (Cl^-) concentrations were determined by the methods of Barnett *et al*, 1973[7] and Schales and Schales, 1941 respectively [18].

Statistical Analysis

Data were presented as Mean ± SD of 5 replicates and analyzed by one-way analysis of variance (ANOVA) using SPSS version 18 computer software package (SPSS INC, Chicago, USA). Differences at $P < 0.05$ were considered significant.

RESULTS

Table 1: Effect of *Helianthus Annuus* (Sunflower) Seed Extract on Serum Glucose Level (mmol/L) in Normal and Diabetic Rats

Group	Initial	48hrs	Day 5	Day 10	Day 15
A	4.65±0.22	11.80±0.07[a]	12.05±0.05[a]	13.64±0.08[a]	14.24±0.15[a]
B	4.70±0.32	4.54±0.22	4.60±0.31	4.69±0.31	4.65±0.05
C	4.63±0.35	4.50±0.24[b]	4.71±0.14[b]	4.45±0.05[b]	4.45±0.07[b]
D	4.72±0.07	11.86±0.07[c]	11.78±0.05[c]	9.14±0.01[c]	7.67±0.08[c]

Values are mean ± S.D for five determinations (n=5).

KEY: A = Diabetic Control; **B** = Normal Control; **C** = Normal + Aqueous extract; **D** = Diabetic + Aqueous extract;

a = statistically significant ($p<0.05$) when compared to the normal control (NC),

b = statistically insignificant ($p<0.05$) when compared to the normal control (NC).

c = statistically significant ($p<0.05$) when compared to the diabetic control (DC).

Table 1 shows the effect of *Helianthus annuus* (sunflower) seed extracts on serum glucose level (mmol/L) in the diabetic and normal rats . Induction of diabetes in the experimental rats was confirmed by the significant increase ($p<0.05$) in serum glucose level of the diabetic control rats group when compared with the normal control rats group. The diabetic treated group showed a significant decrease ($p<0.05$) in serum glucose level compared with the diabetic control rats group. There was no significant ($p<0.05$) difference between the normal control and the normal treated groups. For the diabetic treated group, day 15 showed a significant decrease ($p<0.05$) in serum glucose level when compared to day5 and day 10. The effect of treatment was therefore time dependent.

Table 2: Effect of *Helianthus annuus* (Sunflower) Seed Extract on Serum Total Protein Level (g/L).

Group	48hrs	Day 5	Day 10	Day 15
A	62.34±0.24[a]	64.25± 0.15[a]	63.44±0.20[a]	65.35± 0.30[a]
B	73.67±0.75	72.57± 0.25	71.30± 0.50	73.47± 0.70
C	72.00± 0.90[b]	72.23±0.18[b]	72.18±1.42[b]	74.93±1.42[b]
D	63.56± 0.20	66.88±0.77[c]	70.74±0.52[c]	73.61±0.72[c]

Values are mean ± S.D for five determinations (n=5).

KEY: A = Diabetic Control; **B** = Normal Control; **C** = Normal + Aqueous extract; **D** = Diabetic + Aqueous extract.

a = statistically significant ($p<0.05$) when compared to the normal control (NC),

b = statistically insignificant ($p<0.05$) when compared to the normal control (NC).

c = statistically significant ($p<0.05$) when compared to the diabetic control (DC),

Table 2 shows the effect of *Helianthus annuus* (sunflower) seed extract on serum total protein level. The diabetic control group rats showed significant ($p<0.05$)

decrease when compared to the normal. There was however no significant ($p<0.05$) difference between the normal control and normal treated group rats. On days 5, 10 and 15 the diabetic treated group rats showed a significant ($p<0.05$) increase in total protein level when compared to the diabetic control rats. For the diabetic treated group day 15 showed a significant ($p<0.05$) increase in protein level when compared to day 5 and Day 10. Similarly day 10 showed significant ($p<0.05$) increase compared to day 5.This is evidence of time dependence.

Table 3: Effect of *Helianthus annuus* (Sunflower) Seed Extract on Serum Albumin Level (g/L)

Group	48hrs	Day 5	Day 10	Day 15
A	27.08±0.79[a]	27.10± 0.60[a]	25.20± 0.59[a]	24.05± 0.45[a]
B	36.87±0.72	37.57± 0.60	35.00± 0.55	34.24± 0.09
C	36.90± 0.60[b]	37.00±0.38[b]	36.19±1.42[b]	35.29±0.07[b]
D	27.02± 0.80	29.74±0.21[c]	31.81±0.29[c]	34.83±0.59[c]

Values are mean ± S.D for five determination (n=5).

KEY: A = Diabetic Control; **B** = Normal Control; **C** = Normal + Aqueous extract; **D** = Diabetic + Aqueous extract.
a = statistically significant ($p<0.05$) when compared to the normal control (NC),
b = statistically insignificant ($p<0.05$) when compared to the normal control (NC).
c = statistically significant ($p<0.05$) when compared to the diabetic control (DC).

Table 3 shows the effect of *Helianthus annuus* (sunflower) seed extracts on serum albumin level. The diabetic control group rats showed significant ($p<0.05$) decrease compared to the normal control group rats while there was no significant ($p<0.05$) difference when the normal treated group rats are compared to the normal control group rats. On days 5, 10 and 15 the diabetic treated group rats showed a significant ($p<0.05$) increase in albumin level when compared to the diabetic control group rats. For the diabetic treated group day 10 and day 15 showed significant increases compared to day 5. The effect of treatment was time dependent.

Table 4: Effect of *Helianthus annuus* (Sunflower) Seed Extract on Serum Creatinine Level (µmol/L).

Group	48hrs	Day5	Day10	Day15
A	85.21±0.72[a]	86.20± 0.32[a]	87.25± 0.62[a]	87.10± 0.50[a]
B	81.20±0.61	81.50± 0.70	80.51± 0.65	80.40± 0.50
C	81.00± 0.50[b]	80.81±0.71[b]	81.59±0.79[b]	79.81±0.72[b]
D	90.25± 0.69	82.50±0.79[c]	79.48±0.70[c]	76.69±0.71[c]

Values are mean ± S.D for five determinations (n=5).

KEY: A = Diabetic Control; **B** = Normal Control; **C** = Normal + Aqueous extract; **D** = Diabetic + Aqueous extract.
a = statistically significant ($p<0.05$) when compared to the normal control (NC),
b = statistically insignificant ($p<0.05$) when compared to the normal control (NC).
c = statistically significant ($p<0.05$) when compared to the diabetic control (DC).

Table 4 shows the effect of *Helianthus annuus* (sunflower) seed extracts on serum creatinine level. The diabetic control group rats showed significant ($p<0.05$) increase compared to the normal control group rats. The difference between the normal treated group rats and the normal control group rats was statistically not significant ($p<0.05$). On days 5, 10 and 15 the diabetic treated group rats showed a significant ($p<0.05$) decrease in serum creatinine level when compared to the diabetic control group rats. Day 15 shows a significant ($p<0.05$) decrease in serum creatinine level when compared to day 5 and day 10. Similarly day 10 shows a significant ($p<0.05$) decrease compared to day 5 for the diabetic treated groups rat. The effect of treatment was therefore time dependent.

Table 5: The effect of *Helianthus annuus* (Sunflower) Seed Extract on Serum Urea Level (µmol/L)

Group	48hrs	Day 5	Day 10	Day 15
A	20.41±0.76[a]	21.30± 0.41[a]	23.37± 0.30[a]	22.50± 0.41[a]
B	15.32±0.75	16.33± 0.50	14.30± 0.45	15.45± 0.30
C	15.20± 0.61[b]	15.68±0.71[b]	15.76±0.71[b]	13.97±0.75[b]
D	21.14± 0.67	19.69±0.71[c]	16.32±0.23[c]	15.89±0.76[c]

Values are mean ± S.D for five Determinations (n=5).
KEY: A = Diabetic Control; **B** = Normal Control; **C** = Normal + Aqueous extract; **D** = Diabetic + Aqueous extract.
a = statistically significant ($p<0.05$) when compared to the normal control (NC),
b = statistically insignificant ($p<0.05$) when compared to the normal control (NC).
c = statistically significant ($p<0.05$) when compared to the diabetic control (DC).
Table 5 shows the effect of *Helianthus annuus* (sunflower) seed extracts on serum urea level. The result shows a statistically significant ($p<0.05$) increase in the diabetic control group rats compared to the normal control and a statistically no significant ($p<0.05$) difference in serum urea level in the normal treated group rats when compared to the normal control. On days 5, 10 and 15 the diabetic treated group rats showed a significant ($p<0.05$) decrease when compared to the diabetic control group rats.

Table 6: Effect of *Helianthus annuus* (Sunflower) Seed Extract on Serum Sodium ion (Na^+) Level (mmol/L).

Group	48hrs	Day 5	Day 10	Day 15
A	125.00±2.55[a]	124.00± 3.50[a]	121.00± 2.15[a]	123.25± 0.35[a]
B	139.00±3.60	139.42± 3.50	1.44.05± 2.50	137.00± 0.60
C	138.00± 2.60[b]	140.00±0.79[b]	138.00±1.12[b]	136.00±0.79[b]
D	130.0±0 2.05	139.00±3.54[c]	140.00±1.46[c]	145.00±2.15[c]

Values are mean ± S.D for five determinations (n=5).

KEY: A = Diabetic Control; **B** = Normal Control; **C** = Normal + Aqueous extract; **D** = Diabetic + Aqueous extract.

a = statistically significant ($p<0.05$) when compared to the normal control (NC),
b = statistically insignificant ($p<0.05$) when compared to the normal control (NC).
c = statistically significant ($p<0.05$) when compared to the diabetic control (DC).

Table 6 shows the effect of *Helianthus annuus* (sunflower) seed extracts on serum sodium ion (Na^+) level. The result shows a statistically significant ($p<0.05$) decrease in the diabetic control group rats compared to the normal control and a statistically no significant ($p<0.05$) difference in serum sodium ion (Na^+) level in the normal treated group rats when compared to the normal control. On days 5, 10 and 15 the diabetic treated group rats showed a significant ($p<0.05$) increase when compared to the diabetic control group rats. Day 15 showed a significant ($p<0.05$) increase in serum sodium ion level compared to day 5 and day10 for the diabetic treated group rats. Day 10 also showed a significant ($p<0.05$) increase compared to day 5.

Table 7: The effect of *Helianthus annuus* (Sunflower) Seed Extract on Serum Potassium ion (K^+) Level (mmol/L).

Group	48hrs	Day 5	Day 10	Day 15
A	3.58±0.71[a]	3.25± 0.38[a]	3.15± 0.30[a]	2.58 ±0.61[a]
B	4.77±0.57	4.92± 0.72	4.6±1 0.65	5.01± 0.40
C	4.95± 1.15[b]	5.00±1.12[b]	4.60±0.74[b]	5.00±0.35[b]
D	4.00± 0.65	5.50±0.79[c]	5.50±0.38[c]	5.60±0.47[c]

Values are mean ± S.D for five determination (n=5).

KEY: A = Diabetic Control; **B** = Normal Control; **C** = Normal + Aqueous extract; **D** = Diabetic + Aqueous extract.

a = statistically significant ($p<0.05$) when compared to the normal control (NC),
b = statistically insignificant ($p<0.05$) when compared to the normal control (NC).
c = statistically significant ($p<0.05$) when compared to the diabetic control (DC).

Table 7 shows effect of *Helianthus annuus* (sunflower) seed extracts on serum potassium ion (K^+) level. The diabetic control group rats showed significant ($p<0.05$) decrease compared to the normal control group rats. The difference

between the normal treated group rats and the normal control group rats was statistically not significant ($p<0.05$). On days 5, 10 and 15 the diabetic treated group rats showed a significant ($p<0.05$) increase in serum potassium ion (K^+) level when compared to the diabetic control group rats. For the diabetic treated with aqueous extract, day 15 showed a significant ($p<0.05$) increase in serum potassium ion level compared to day 5 and day 10 while there is no significant ($p<0.05$) difference between days 10

Table 8: Effect of *Helianthus annuus* (Sunflower) Seed Extract on Serum Chloride ion (Cl^-) Level (mmol/L).

Group	48hrs	Day 5	Day 10	Day 15
A	85.00±1.58[a]	85.25± 0.76[a]	83.2±0 1.40[a]	82.00± 0.65[a]
B	107.60±0.76	106.50± 1.50	108.40± 2.54	110.45± 1.25
C	107.00±0.80[b]	105.00±1.46[b]	109.80±1.79[b]	111.00±1.46[b]
D	87.00 1.25	107.20±3.51[c]	110.00±3.54[c]	116.00±1.58[c]

Values are mean ± S.D for five determinations (n=5).

KEY: A = Diabetic Control; **B** = Normal Control; **C** = Normal + Aqueous extract; **D** = Diabetic + Aqueous extract.

a = statistically significant ($p<0.05$) when compared to the normal control (NC),

b = statistically insignificant ($p<0.05$) when compared to the normal control (NC).

c = statistically significant ($p<0.05$) when compared to the diabetic control (DC).

Table 8 shows the effect of *Helianthus annuus* (sunflower) seed extracts on serum chloride ion (Cl^-) level. The result showed a statistically significant ($p<0.05$) decrease in the diabetic control group rats compared to the normal control and a statistically non significant ($p<0.05$) difference in serum chloride ion (Cl^-) level in the normal treated group rats when compared to the normal control.

On days 5, 10 and 15 the diabetic treated group rats showed a significant ($p<0.05$) increase when compared to the diabetic control group rats. Day 5 showed a significant ($p<0.05$) increase in serum chloride ion level compared to day 10 and day 15.There was also a significant increase ($P< 0.05$) on day 10 compared to day 5.

Table 9: Effect of *Helianthus annuus* (Sunflower) Seed Extract on Serum Calcium ion (Ca^{2+}) Level (mmol/L).

Group	48hrs	Day 5	Day 10	Day 15
A	1.83±0.02[a]	1.82± 0.15[a]	1.80± 0.05[a]	1.80± 0.06[a]
B	2.28±0.08	2.10 ±0.07	2.25± 0.05	2.30 ±0.08
C	2.15± 0.05[b]	2.08±0.06[b]	2.27±0.09[b]	2.29±0.10[b]
D	1.80 ±0.04	1.99±0.06[c]	2.20±0.36[c]	2.39±0.15[c]

Values are mean ± S.D for five determination (n=5).

KEY: A = Diabetic Control; **B** = Normal Control; **C** = Normal + Aqueous extract; **D** = Diabetic + Aqueous extract.
a = statistically significant ($p<0.05$) when compared to the normal control (NC),
b = statistically insignificant ($p<0.05$) when compared to the normal control (NC).
c = statistically significant ($p<0.05$) when compared to the diabetic control (DC).

Table 9 shows the effect of *Helianthus annuus* (sunflower) seed extracts on serum calcium ion (Ca^{2+}) level. The result showed a statistically significant ($p<0.05$) decrease in the diabetic control group rats compared to the normal control and a statistically no significant ($p<0.05$) difference in serum calcium ion (Ca^{2+}) level in the normal treated group rats when compared to the normal control. On days 5, 10 and 15 the diabetic treated group rats showed a significant ($p<0.05$) increase when compared to the diabetic control group rats. The diabetic treated group rats, showed a significant ($p<0.05$) increase in calcium ion level on day 15 compared to day 5 and day 10. The same was the case between day 10 and day 5.

Table 10: Effect of *Helianthus annuus* (Sunflower) Seed Extract on Serum Magnesium ion (Mg^{2+}) Level (mmol/L).

Group	48hrs	Day 5	Day 10	Day 15
A	0.99 ± 0.29^{a}	1.01 ± 0.25^{a}	0.95 ± 0.08^{a}	0.92 ± 0.10^{a}
B	1.60 ± 0.02	$1.62\ 0\pm.05$	$1.6\pm0\ 0.07$	$1.59\pm\ 0.09$
C	$1.62\pm\ 0.05^{b}$	1.63 ± 0.08^{b}	1.59 ± 0.08^{b}	1.62 ± 0.06^{b}
D	$0.95\pm\ 0.15$	1.25 ± 0.08^{c}	1.41 ± 1.10^{c}	1.49 ± 0.06^{c}

Values are mean ± S.D for five determination (n=5).
KEY: A = Diabetic Control; **B** = Normal Control; **C** = Normal + Aqueous extract; **D** = Diabetic + Aqueous extract.
a = statistically significant ($p<0.05$) when compared to the normal control (NC),
b = statistically insignificant ($p<0.05$) when compared to the normal control (NC).
c = statistically significant ($p<0.05$) when compared to the diabetic control (DC).

Table 10 show the effect of *Helianthus annuus* (sunflower) seed extracts on serum magnesium ion (Mg^{2+}) Level. The result showed a statistically significant ($p<0.05$) decrease in the diabetic control group rats compared to the normal control and a statistically no significant ($p<0.05$) difference in serum magnesium ion (Mg^{2+}) level in the normal treated group rats when compared to the normal control. On days 5, 10 and 15 the diabetic treated group rats showed a significant ($p<0.05$) increase when compared to the diabetic control group rats. Day 15 shows a significant increase ($p<0.05$) in serum magnesium ion levels when compared to day 5 and day 10.

DISCUSSION

Table 1 shows the effect of *Helianthus annuus* (sunflower) seed extracts on serum glucose level in diabetic and diabetic treated rats. Diabetes was confirmed in the experimental rats after 48hours by the significant increase ($P<0.05$) in serum glucose level of the diabetic control group rats when compared to the normal control group rats. The diabetic rats treated with *Helianthus annuus* (sunflower) seed extract showed a significant decrease ($P<0.05$) in serum glucose level compared with the diabetic control group rats. The result obtained in this study indicates that the aqueous extract of *Helianthus annuus* (sunflower) seed produced significant hypoglycaemic effects in alloxan induced diabetic rats. This is consistent with the findings of Al-Attar, (2012) [3]. It is possible that the plant extracts may reverse the catabolic features of insulin deficiency, decrease the release of glucagon or increase that of insulin, stimulate directly glycolysis in peripheral tissues, increase glucose removal from the gastrointestinal tract [15]. Hypoglycaemic effect of Sunflower (*Helianthus annuus*) seed extract could also possibly, be due to increased peripheral glucose utilization. For all the treated groups day 15 showed a significant decrease ($P<0.05$) in serum glucose level when compared to days 5 and 10. This implies that the treatment was time dependent for serum glucose level.

The diabetic control group rats showed a significant decrease ($P<0.05$) in serum total protein level when compared to the normal control group rats (Table 2). The significant decrease in serum total protein in the diabetic rats suggests a derangement in protein metabolism. Insulin stimulates protein synthesis as well as inhibits the key enzymes of gluconeogenesis. Since there is insulin deficiency in diabetes, the functions of insulin are prevented leading to low level of serum total protein in the diabetic control group rats. The increase in serum total protein observed in the diabetic treated group rats seem to suggest that the *Hellianthus annuus* (sunflower) seed extracts reverse the derangement in protein metabolism in the diabetic rats. Furthermore, the significant increase ($P<0.05$) in serum total protein level for the diabetic treated group rats on day 15 compared to days 5 and 10 showed that the effect of the treatment on serum total protein was also time dependant. Serum albumin is a protein made specifically by the liver and can be easily measured. It reflects the extent of functioning of liver cell mass. Results (Table 3) showed that the diabetic control group rats decreased significantly ($P<0.05$) in serum albumin level compared to the normal group rats. This is suggesting some damage to the liver as a result of the diabetic state. This is also indicative of inhibition of protein synthesis. Following treatment with *Helianthus annuus* (sunflower) seed extract the level of serum albumin increased compared to the diabetic control group rats. The effect of treatment was time dependent.

Table 4 shows a statistically significant (P<0.05) hypercreatinaemia in the diabetic control group rats compared to the normal control group rats suggestive of impaired renal function. The diabetic treated group rats showed increase in the serum levels of creatinine after treatment with *Helianthus annuus* (sunflower) seed extract meaning that it may provide some protection against renal dysfunction. The result (Table 5) of this study showed increase serum levels of urea in the diabetic control group rats compared to the normal control group rats. This is consistent with the findings of Akah, *et al.*, (2009) [2]. Increase in urea level could be due to both liver and kidney dysfunction. The elevated levels of serum urea observed was reduced significantly (P<0.05) by the *Helianthus annuus* (sunflower) seed extract and this effect was time dependant which suggests that *Helianthus annuus* (sunflower) seed may provide some protection against renal dysfunction. Results from this investigation show a statistically significant decrease (P<0.05) in the serum levels of sodium ion (Table 6), potassium ion (Table 7) chloride ion (Table 8), calcium ion (Table 9) and magnesium ion (Table 10). Akah, *et al.*, (2004) [1], also reported decrease in the levels of electrolytes in diabetic rats as a result of osmotic diuresis with subsequent loss of water and electrolytes induced by glycosuria.

Hypomagnesemia, was reported as a frequent condition in patients with diabetes [19, 21] and could be involved in the development of poor metabolic control and chronic complications [17]. A large body of evidence that shows a link between hypomagnesaemia and reduced tyrosine-kinase activity at the insulin receptor level, which may result in the impairment of insulin action and development of insulin resistance, has been progressively accumulated in previous year's Chemical evidence also shows the essential role of magnesium on insulin mediated glucose uptake. Hypomagnesaemia may worsen insulin resistance or may be the consequences of insulin resistance. The kidneys possibly lose their ability to retain magnesium during period of severe hyperglycemia. The increase loss of magnesium in urine then results in lower blood levels of magnesium. ATP requires magnesium to be stable and that may be part of the reason for very low energy level (weakness) in diabetic patients. The observed decrease in the serum levels of sodium ion and potassium ion of alloxan – induced diabetic rats may also be due to hyperosmolar non-ketosis. .Treatment with *Helianthus annuus* (sunflower) seed extracts increase significantly (P<0.05) the serum levels of these electrolytes. The observed significant increase (P<0.05) in the serum levels of these electrolytes in the diabetic treated suggest that the extracts enhance rehydration, transfer of intracellular electrolytes to the extracellular space thereby reducing or preventing against hyperosmolar non-ketotic state and metabolic acidosis. *Helianthus annuus* (sunflower) seeds are excellent sources of minerals

such as potassium, magnesium, iron, phosphorus, selenium, calcium and zinc [3]. The effect of the treatment was time dependant.

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