

(RESEARCH ARTICLE)



## Effects of *Helianthus annuus* seeds on antioxidants, lipid profile and serum urea and creatinine in obesity induced rats using high fat diet

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### Abstract

High-fat diets have been strongly associated with common metabolic diseases. Clinical practices often link these diseases, such as hyperlipidemia and oxidative stress resulting from the accumulation of reactive oxygen species, to severe conditions like cancer, diabetes, atherosclerosis, and cardiovascular diseases. Managing these conditions is often financially demanding. *Helianthus annuus* seeds, known to be non-toxic even at doses greater than 5000mg/kg in rats, with an LD<sub>50</sub> greater than 5000mg/kg, has demonstrated efficacy in reducing elevated blood sugar levels and BMI in both animal and human studies. Therefore, further research is needed to explore the potential effects of sunflower seeds on antioxidants, lipid profiles, and serum urea and creatinine levels in rats induced with obesity using a high-fat diet.

In this study, overnight fasting blood samples were collected to conduct lipid profile tests (HDL, LDL, VLDL, and triglycerides). These tests utilized a photometric system with procedures derived from the Diasys-G Emmany kit and the measurement and precipitation method. The modified Jaffe technique for in-vitro measurement of creatinine in serum was employed to measure urea and creatinine levels. The experiment involved 27 Wistar rats weighing between 350 and 400 grams, randomly divided into 9 groups, with 3 rats in each group. Group 1 served as the control, while groups 2 to 9 were subjected to various conditions: high-fat diets alone for 6 weeks (group 2), high-fat diets with high (5000mg/kg body weight), medium (3000 mg/kg body weight), or low (2000 mg / kg body weight) doses of *Helianthus annuus* seeds powder for 6 weeks (groups 3, 4, and 5, respectively). Additionally, there were variations in duration and doses in groups 6 to 9, where rats were fed high-fat diets 20 g/ kg / day /rats for 5 weeks and then supplemented with *Helianthus annuus* seeds powder (5000 mg / kg, 3000 mg / kg and 2000 mg / kg per rat for group 6,7,8 or Metformin 70 mg / kg per rat for group 9) for 1 week. The results revealed a significant ( $P < 0.05$ ) increase in serum cholesterol, triglyceride, and low-density lipoprotein (LDL) levels in rats across all groups compared to the control. However, all groups supplemented with *Helianthus annuus* seeds alongside high-fat diets displayed a significantly increased HDL level ( $P < 0.05$ ) compared to rats fed only high-fat diet alone. Creatinine levels were significantly increased in all *Helianthus annuus* seeds -supplemented groups compared to the high-fat diet alone, while urea levels showed no significant differences among the groups. Furthermore, rats in all *Helianthus annuus* seeds -supplemented groups alongside high-fat diets demonstrated a significantly increased glutathione reductase activity ( $P < 0.05$ ) compared to the control and high-fat diet alone. The study indicated that sunflower seeds significantly bolster antioxidant protection, elevate HDL levels, and increase creatinine levels in rats fed high-fat diets, suggesting potential beneficial effects in mitigating the adverse impacts of high-fat diets on health parameters.

**Keywords:** *Helianthus annuus*; High-fat diets; Rats; Glutathione; Serum cholesterol

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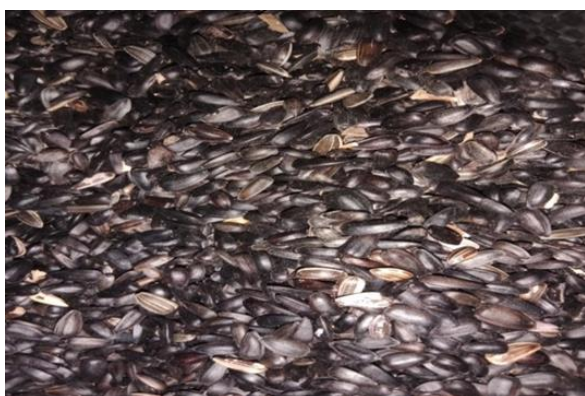
## 1. Introduction

Consumption of high-fat diets has been linked to an increased risk of metabolic disorders, leading to severe health complications that significantly impact human well-being, productivity, and overall quality of life (Kamlesh et al., 2015). These metabolic disorders, such as diabetes, are often associated with grave complications, with over 50% of individuals with diabetes succumbing to cardiovascular diseases (CVD), primarily heart diseases and strokes. Moreover, diabetes stands as a primary cause of end-stage renal diseases necessitating treatments like dialysis or kidney transplantation, and it is a leading cause of diabetic retinopathy, resulting in blindness (Mahammadi et al., 2015; Abi et al., 2018).

Research indicates that high-fat diets elevate serum cholesterol, triglyceride, and low-density lipoprotein levels, while concurrently reducing high-density lipoprotein (HDL) levels (Shuangshaug et al., 2017). Additionally, studies by Natalia et al. (2014) showed that high-fat diets hinder cell signaling transduction, impair effective insulin production compared to glucose, leading to fat accumulation and hyperglycemia which are —key contributors to persistent obesity in individuals consuming high-fat diets (Klein et al., 2022).

*Helianthus annuus*, originating herbaceous plants in the *asteraceae* family, are primarily native to African countries, North, and South America (Shuangshuang et al., 2017; Aishatu et al., 2023). *Helianthus annuus* is called Ododo sonofilawa and Yunyun in Yoruba, Orangila in Igbo, tozalin in Hausa, Edemedong in Efik, *sunflower* in Tiv, Igede and Idoma language of Benue State Nigeria.

The phytochemicals in the *Helianthus annuus seeds* includes phenolic acid and chlorogenic acids, alkaloids, flavonoids, saponins, terpenes, cardiac glycosides polysaccharides, stilbenes, tannin, and tocopherols (Erbaş et al., 2016). The seeds' have diverse cultural significance in Nigerian communities despite their rich nutritional content and significant biological activities, particularly related to phytochemical constituents like phenolic acids, flavonoids, and tocopherols, their potential benefits remain inadequately researched (Ming-Chih et al., 2011; Hansa et al., 2020). These findings suggest promising avenues for further exploration into the health-promoting properties of *Helianthus annuus seeds* and their derivatives.



A



B

**Figure 1** Sunflower seeds and Sunflower Leaves

This study aimed to investigate the effects of sunflower seed powder on antioxidants, lipid profiles, and serum urea and creatinine in rats with induced obesity due to a high-fat diet. The research explores the potential health benefits of incorporating sunflower seeds into the diet to mitigate the adverse effects caused by obesity induced by high-fat intake.

## 2. Materials and Method

### 2.1. Sample

*Helianthus Anuus* seeds was obtained from Modern Market Makurdi Nigeria. *Helianthus Anuus* leaves and seeds were identify by a Botanist (Dr. Mrs, Dooshima Shirki) of Botany Department Benue State University for identification and sample was placed at the herbarium, with voucher number, HBI - 001- BSU23.

The seeds were carefully handpicked, cleaned to remove impurities using distilled water, and left to dry for ten days. Subsequently, they were finely ground into powder using an LG electric grinding machine 2023 model, following the method described by El-Kholy et al. (2018). Approximately 10kg of the finely ground sunflower seed powder was stored in a plastic container, which was later measured and mixed appropriately with high-fat diets tailored for the animals in various experimental groups. To ensure preservation, the sunflower powder was stored in a freezer set at either 4°C as outlined by Abalaka et al. (2012).

## 2.2. Compounded High Fat Diet Meal

High fat diet (HFD) was constituted locally by an animal nutritionist at the Faculty of Veterinary Medicine, Federal University of Agriculture Makurdi, Benue State Nigeria. The formula was designed using the method of Julia et al., (2012) with slight modification. The diet was made from chow, tallow and soy oil at an inclusion rate of 60%, 25% and 15% respectively. The total caloric value of the diet was about 5340kcal/kg of which energy contribution of fat was about 70%. The fat component comprises of 60% saturated fat and 40% unsaturated fat (Abi et al., 2018).



**Figure 2** High Fat Diet

### 2.2.1. Addition of sunflower seed powder to feed

From pilot study it was estimated that 1 rat consumed less than 20g feed per day, therefore with this the mixture of sunflower seed with HFD was calculated as follow:

- For high dose (5000 mg / kg) *Helianthus annuus seeds* powder in HFD
  - If 5000 mg is to be given to 1000 g rat, then 400 g rat will take 200 mg *Helianthus annuus seeds* powder
  - If rat consumed 20 g feed per day, then 200 mg sunflower seed was added to 20 g feed
  - By extension, 10 g *Helianthus annuus seeds* powder was mixed with 1 kg HFD
  - For medium dose (300 mg / kg) *Helianthus annuus seeds* powder in HFD
    - If 200 mg is to be given to 1000 g rat, then 400 g rat will take 120 mg *Helianthus annuus seeds* powder
    - If 1 rat consumed 29 g feed per day, then 120mg *Helianthus annuus seeds* was added to 20 g feed
    - By extension, 6 g *Helianthus annuus seeds* powder was mixed with 1 kg HFD
    - For low dose (200 mg / kg) *Helianthus annuus seeds* powder in HFD
      - If 200 mg is to be given to 1000 g rat, then 400 g rat will take 80 mg *Helianthus annuus seeds* powder
      - If 1 rat consumed 20 g feed per day, then 80mg *Helianthus annuus seeds* was added to 20 g feed
      - By extension, 4 g of *Helianthus annuus seeds* powder was mixed with 1 kg HFD

**2.3. Animals;** A total of 27 male Wistar rats, 14- 16 weeks old, weighing 350 -400 g was obtained from the Animals House Benue State University Makurdi, animals were kept in plastic cages and were allowed to acclimatize for 2 weeks before commencement of the experiment. Ethical clearance for the uses of animals for experiment was obtained from the ethical committee in the College of Health Sciences, Benue State University, Makurdi, CHS REC No: CREC/THS/002, the rats were maintained in a standard condition at room temperature ( $27 \pm 2^{\circ}\text{C}$ ) and relative humidity ( $50 \pm 5\%$ ), with 12 hours Light / dark cycle.

Weighing balance (Ohaus Navigator NVA422) was used to monitor the rats weight weekly.

Syringes and needles were used to collect blood sample during the experiments. Cotton wool and methylated spirit were used to clean the tables during the experiments. Test tubes were used for sample collection and were all obtained from Vincal pharmacy Wadata Makurdi, Benue State.

Accu-check Active glucometer & LG electric grinding machine were used to check blood sugar & grind the sunflower seeds to powder respectively. Drug: Mixture of isoflurane 30% (inhalational anesthesia) by API Manufacturer with FDA, UK. Marketed by Macfes medical high level markurdi. Soaked in a cotton wool and dropped in clean and covered plastic container, was used to anesthetized the rats. The hand gloves, hand sanitizer, scissors obtained from Macfes medical shop Makurdi, Benue State.

#### Animal Grouping

Preventive groups (3,4,5) and Curative groups(6,7,8) studies in animals were done concurrently for 6 weeks. Rats, were randomly assigned to 9 groups consisting of 3 each as follows:

- Group 1 (Control): Received normal rat chow 20 g / kg body weight, per animal / day and rats were allowed to feed at *libitum*) plus water for six weeks.
- Group 2 (Pre-diabetic model): Received HFD alone 20 g / kg body weight, per rat / day and rats were allowed to feed at *libitum*) for six weeks
- Group 3: Received HFD 20 g per / kg body weight, per rat plus *Helianthus annuus seeds* powder 5000 mg / kg body weight, per rat / day, concurrently, mixed completely together and rats were allowed to feed at *libitum*).
- Group 4: Received HFD 20 g per / kg body weight per rat plus *Helianthus annuus seeds* powder 3000 mg / kg body weight, per rat / day, concurrently, mixed completely together and rats were allowed to feed at *libitum*).
- Group 5: Received HFD 20 g per / kg body weight per rat plus *Helianthus annuus seeds* powder 2000 mg / kg body weight, per rat / day, concurrently, mixed completely together and rats were allowed to feed at *libitum*).
- Group 6: Received HFD 20 g per / kg body weight / rat, alone for 5 weeks. Then after 5 weeks rats received *Helianthus annuus seeds* powder 5000 mg /kg body weight/ day +normal rat chow 20 g / kg body weight per rat / day, measured and mixed completely together and rats were allowed to feed at *libitum* for one week.
- Group 7: Received HFD 20 g per / kg body weight / rat, alone for 5 weeks. Then after 5 weeks, rats received *Helianthus annuus seeds* powder 3000 mg / kg body weight, per rat / day + normal rat chow 20 g / kg body weight per rat / day, measured and mixed completely together and rats were allowed to feed at *libitum* for one week.
- Group 8: Received HFD 20 g per / kg body weight / rat, alone for 5 weeks. Then after 5 weeks, rats received *Helianthus annuus seeds* powder 2000 mg /kg body weight, per rat / day + normal rat chow 20 g / kg body weight, per rat / day, measured and mixed completely together and rats were allowed to feed at *libitum* for one week.
- Group 9: Received HFD 20 g per / kg body weight per rat, alone for 5 weeks. After 5 weeks rats received metformin 70 mg / kg body weight per rat / day + normal rat chow 20 g / kg body weight, per rat / day, measured and mixed completely together and rats were allowed to feed at *libitum* for one week.

## 2.4. Biochemical analysis of antioxidants (Superoxide Dismutase, Catalase and Glutathione)

### 2.4.1. Measurement of superoxide Dismutase (SOD)

Superoxide dismutase activity was assessed using the Winter bourn et al (1975) technique. The capacity of SOD to prevent nitro-blue tetrazolium (NBT) from being reduced served as the foundation for the assay's basic premise. The reaction mixture included 0.1 ml of enzyme samples, 2.7 ml of 0.067 M phosphate buffer, pH 7.8, 0.12 M riboflavin, 1.5 MNBT, and 0.01M methionine, putting the tubes in a box with a 15-fluorescent light for 10 minutes, air aluminum foil was used to provide uniform illumination of the tubes. There was also a control group without the enzyme source. At 560nm, the absorbance was measured; the quantity of enzyme necessary to prevent NBT from being reduced by 50% under the given circumstances was determined to be one unit of SOD. Enzyme activity was measured in units per milligram of protein

### 2.4.2. Measurement of catalase (CAT) activity

The 1983 Aebi technique was used to measure this specific one. A cuvette containing 1.9 mL of 50mM phosphate buffer, pH 7.0, and 0.1 mL of tissue were pipetted together. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), newly manufactured at 30% (v/v), was added to begin their action. Utilizing spectrophotometry, therate of H<sub>2</sub>O<sub>2</sub> oxidation was calculated from variations in absorbance at 240nm. Enzyme activity was measured in units per milligram of protein. (Alpco Diagnostics, Salem, USA) ELISA kit.

#### 2.4.3. Measurement of Glutathione (GSH) level

By titrating with 0.1mmol/L of 5, 5' Dithibios (nitrobenzoic acid) in a 0.1mol/L disodiumphosphate buffer solution with a pH of 8, it was possible to measure the reduced glutathione content. At 412nm, the reduced product thio-nitrobenzene's production was quantified spectrophotometrically. The GSH concentration was given as mol/g of moist tissue.

#### 2.4.4. Urea Level Estimation

The Quimica Clinica Applicada (QCA) Urea test kit was used to determine the urea concentration utilizing the modified Jaffe technique for the in-vitro measurement of creatinine in serum. Picrate interacts with urea in an alkaline solution to produce a colored complex. The amount of urea in the sample determines how quickly the absorbance at 546 nm increases as a result of the development of the urea–picrate complex. It was expressed as mg / dl.

#### 2.4.5. Creatinine Level Estimation

The Quimica Clinica Applicada (QCA) creatinine test kit was used to determine the creatinine concentration utilizing the modified Jaffe technique for the in vitro measurement of creatinine in serum. Picrate interacts with creatinine in an alkaline solution to produce a colored complex. The amount of creatinine in the sample determines how quickly the absorbance at 546 nm increases as a result of the development of the creatinine-picrate complex. It was expressed as mg/dl.

### 2.5. Lipid Profile Determination (Cholesterol, Triglyceride, Low Density and High Density Lipoprotein)

Measuring of total cholesterol, LDL-C Level and triglyceride was done by photometric systems with procedure from DiaSys®, Germany kit (Diagnostic System International) cat no. 1 4121 99 10 021. Blood serum 3Pl was added 280 Pl of reagent 1, then was mixed and incubation for 5 min at 37 °C. Absorbance is read at wave length ( $\lambda$ ) 600/700 nm (bichromatic measurement) as A1. After that, it was added 70 Pl of reagent 2, then was mixed and incubation for 5 min at 37 °C. Absorbance is read at wave length ( $\lambda$ ) 600/700 nm as A2.

$$\Delta A = [(A2 - A1) \text{ sample or calibrator}] - [(A2 - A1) \text{ blank}]$$

The LDL-cholesterol level was measured with formula :

$$LDL - C [mg/dL] = \frac{\Delta A \text{ sample}}{\Delta A \text{ Calibrator}} \times \text{Conc. Calibrator} [mg/dL]$$

Measuring of HDL-C was done by the LDL, VLDL and chylomicrons precipitation method with procedure from DiaSys®, Germany kit (Diagnostic System International) cat no. 10 350 022. Blood serum 500 Pl was added 1000 Pl of HDL-reagent, then was mixed and incubation for 10 min at room temperature. After that, it was centrifuged for 2min at the speed of 10000 m/sec. Supernatant was separated from precipitant. Supernatant of 100  $\mu$ l was added 1000 Pl of cholesterol reagent, then it was mixed and incubation for 10 min at 20-25 °C or 5 min at 37 °C. Absorbance was measured at wave length ( $\lambda$ ) 500 nm. The HDL cholesterol level was measured with formula:

$$HDL - C [mg/dL] = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{st}} [mg/dL]$$

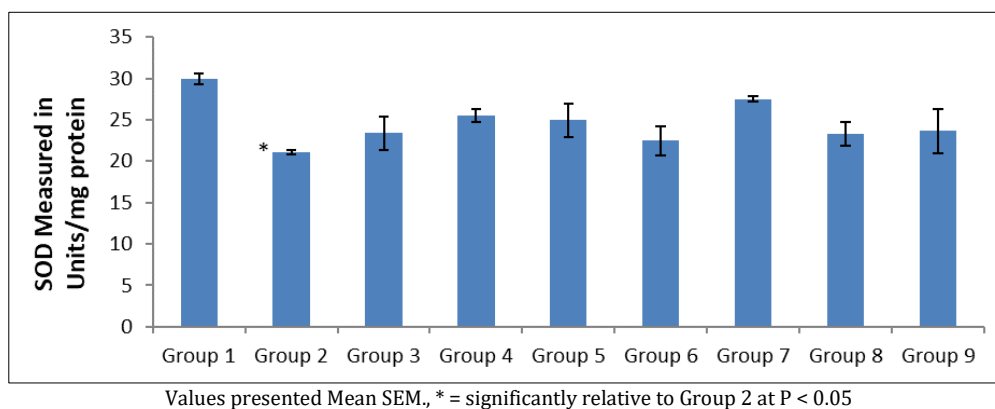
### 2.6. Statistical analysis

Results were presented as mean  $\pm$  SEM. Differences between two groups was determined using independent t test, while differences between more than two groups was determined using One-Way ANOVA with Tukey post hoc test. Differences were considered significant when  $P < 0.05$ . Data were analyzed using SPSS version 20.0 software (International Business Machines Corporation).

## 3. Results and Discussion

**3.1. Superoxide Dismutase (SOD) Activity** was notably affected in the experimental groups. Rats that consumed only the high fat diet (HFD) (group 2) exhibited a significant ( $P < 0.05$ ) reduction in SOD levels compared to the control. Conversely, rats that received a combination of *Helianthus annuus seeds* alongside the HFD for six weeks showed an increase in SOD levels ( $P < 0.05$ ). Similarly, administering *Helianthus annuus seeds* powder to rats after five weeks on the HFD resulted in a significant elevation of SOD levels compared to rats exclusively fed the HFD. These

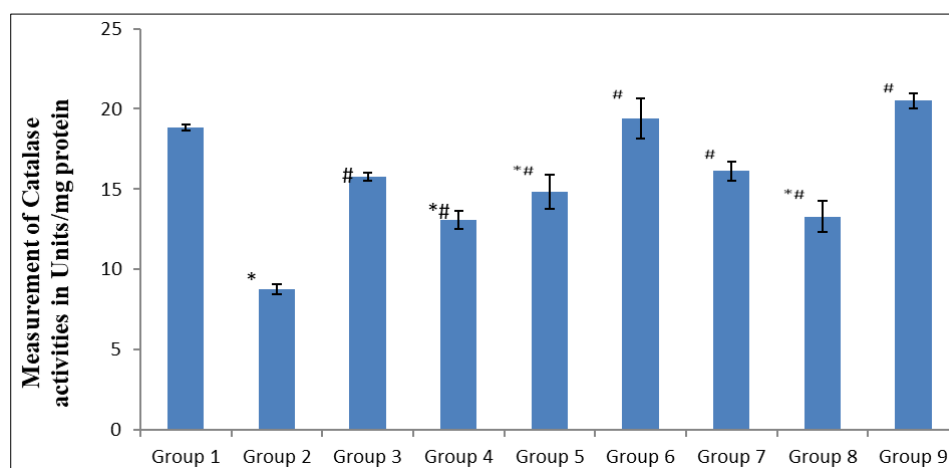
findings confirm the significant impact of *Helianthus annuus seeds* intake on SOD levels in rats subjected to a high-fat diet regimen. This outcome is illustrated in Figure 3 below.



**Figure 3** Effect of *Helianthus annuus seeds* powder consumption on superoxide dismutase (SOD) activity in HFD fed rats. n = 3,

### 3.2. Catalase (CAT) Activity

The catalase (CAT) activity measurements revealed distinct patterns among the groups. Rats that consumed the high fat diet (HFD) alone (group 2) exhibited a significant ( $P < 0.05$ ) reduction in catalase levels compared to the control group. Interestingly, the consumption of *Helianthus annuus seeds* alongside the HFD for six weeks also led to a significant ( $P < 0.05$ ) decrease in catalase activity. However, there was no significant ( $P > 0.05$ ) difference observed between the rats given *Helianthus annuus seeds* powder after five weeks on the HFD when compared to the control group. These findings suggest a varied impact of *Helianthus annuus seeds* intake on catalase levels in rats subjected to different dietary conditions. This outcome is depicted in Figure 4 below.

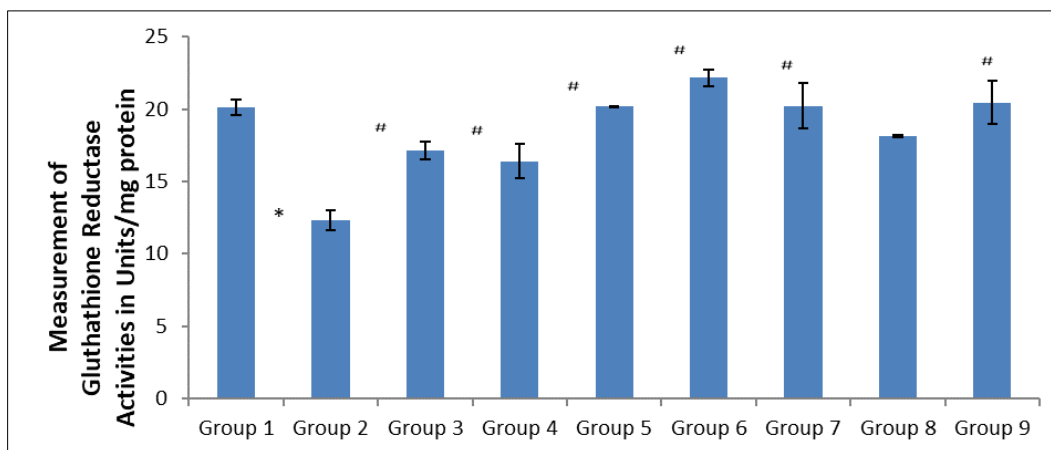


Values presented Mean SEM, \* = significantly relative to Group 1 at P < 0.05, # = significantly relative to Group 2 at P < 0.05

**Figure 4** Catalase (CAT) activity Effect of *Helianthus annuus seeds* powder consumption on Catalase (Cat) Activity in HFD fed rats. n = 3

### 3.3. Effects Of *Helianthus annuus seeds* Consumption On Glutathione Reductase (GSH) Level In High Fat Diet Fed Rats.

Rats that consumed HFD alone (group 2) showed a significant ( $P < 0.05$ ), decrease in glutathione reductase activity compared to control, and rats fed with *Helianthus annuus seeds* along with HFD for six weeks significantly ( $P < 0.05$ ) increased glutathione reductase activity compared to HFD alone group. Likewise the glutathione reductase activity was significantly ( $P < 0.05$ ) increased in rats that were given *Helianthus annuus seeds* powder or metformin after 5 weeks of HFD when compared with the HFD alone group

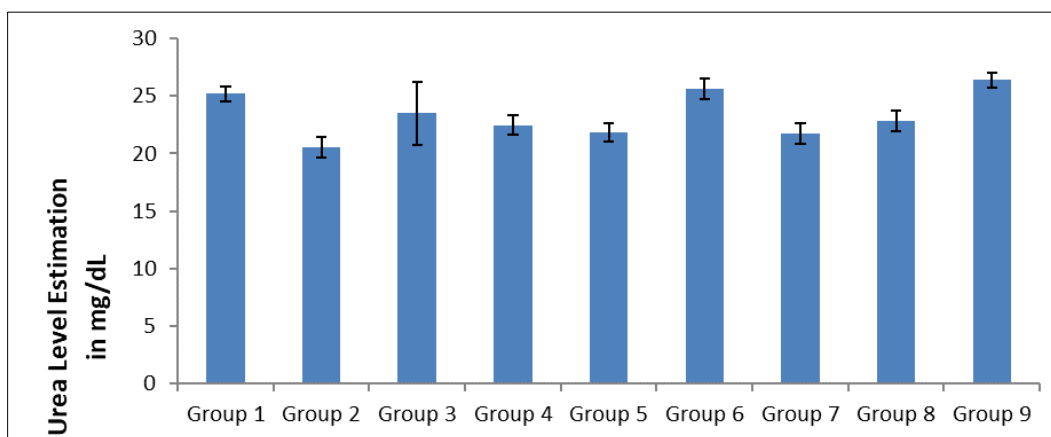


Values presented Mean SEM, \* = significantly relative to Group 1 at  $P < 0.05$ , # = significantly relative to Group 2 at  $P < 0.05$

**Figure 5** Effect of *Helianthus annuus seed* powder consumption on Glutathione Reductase (GSH) in HFD fed rats.  $n = 3$ ,

### 3.4. 3 Urea Level Estimation

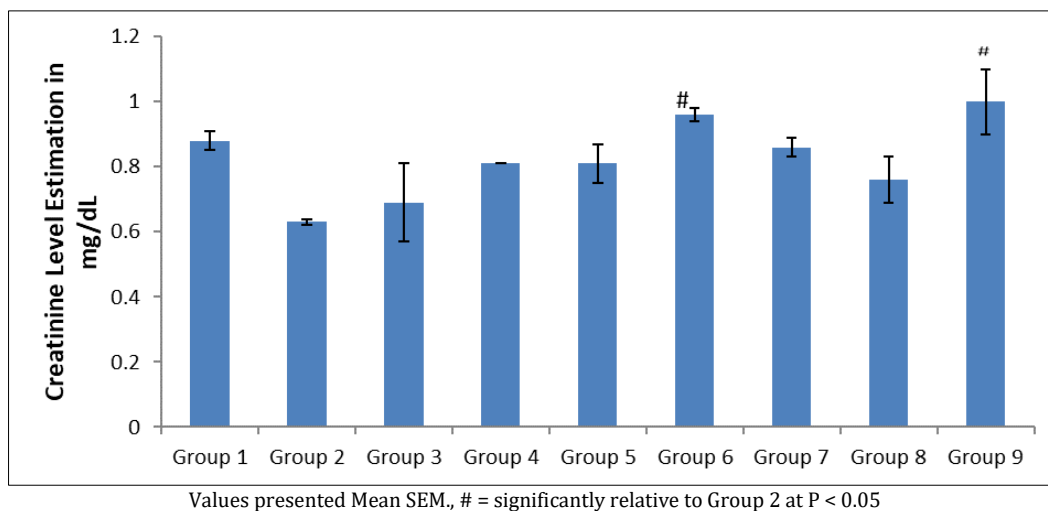
The assessment of urea levels among the experimental groups revealed no significant differences ( $P > 0.05$ ) between the rats consuming the High Fat Diet (HFD) alone (group 2) and the control group. Similarly, administering sunflower seeds alongside the HFD for six weeks or providing *Helianthus annuus seed* powder after five weeks on the HFD did not result in any significant ( $P > 0.05$ ) differences in urea levels compared to the control group. These observations suggest that both the HFD and the inclusion of sunflower seeds did not markedly alter urea levels in the studied rats, as depicted in figure 6.



**Figure 6** Effect of *Helianthus annuus seeds* powder consumption on Serum Urea in HFD fed rats.  $n = 3$ , Values presented Mean SEM

### 3.5. Creatinine Level Estimation

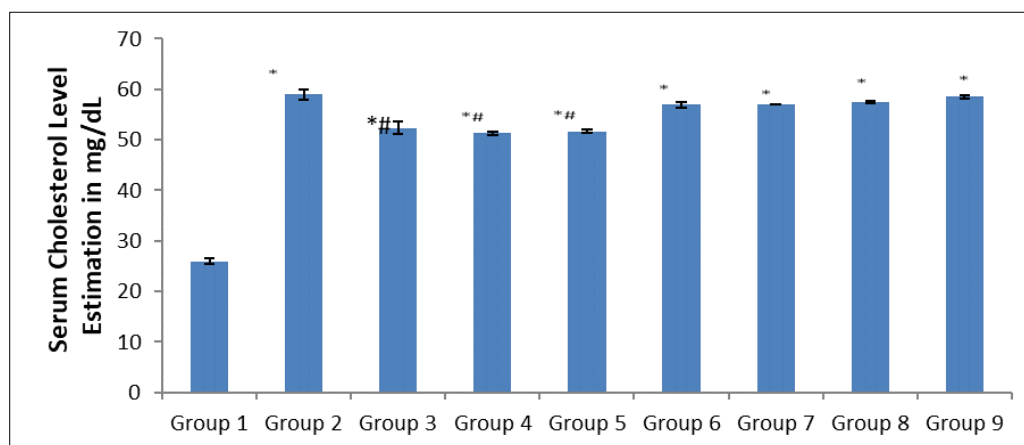
The data analysis on creatinine levels revealed distinct trends among the experimental groups. Rats consuming the High Fat Diet (HFD) alone showed a significant ( $P < 0.05$ ) decrease in creatinine levels compared to the control group. Interestingly, rats fed sunflower seeds alongside the HFD for six weeks or those provided with sunflower seed powder after five weeks on the HFD exhibited a notable and significant ( $P < 0.05$ ) increase in creatinine levels compared to the group consuming the HFD alone. This suggests that while the HFD led to reduced creatinine levels, the inclusion of sunflower seeds seemed to reverse this effect, resulting in elevated creatinine levels when compared to the group consuming the HFD alone. This distinct pattern is clearly illustrated in figure 7.



**Figure 7** Effect of *Helianthus annuus seeds* powder consumption on Serum Creatinine in HFD fed rats. n = 6,

### 3.6. Effect Of Sunflower Seed Consumption On Lipid Profile In Rats Fed With High Fat Diet

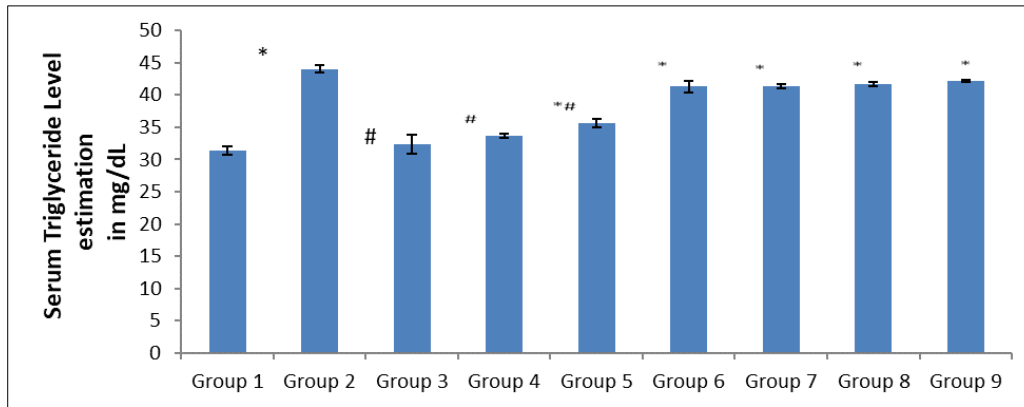
Rats that consumed HFD alone showed a significant ( $P < 0.05$ ) increase in serum cholesterol, triglyceride and low density lipoprotein level compared to control. So also, rats that consumed *Helianthus annuus seeds* along with HFD for six weeks significantly ( $P < 0.05$ ) have increased serum cholesterol, triglyceride and low density lipoprotein levels compared to control. Likewise, the serum cholesterol, triglyceride and low density lipoprotein levels were significantly ( $P < 0.05$ ) increased in rats that were given *Helianthus annuus seeds* powder after 5 weeks of HFD when compared with the control. However, rats that consumed HFD alone showed a significant ( $P < 0.05$ ) decrease in high density lipoprotein (HDL) level compared to control. The rats that consumed sunflower seed along with HFD for six weeks significantly ( $P < 0.05$ ) have increased HDL level compared with rats fed with HFD alone. Likewise, the HDL level was significantly ( $P < 0.05$ ) increased in rats that were given *Helianthus annuus seeds* powder after 5 weeks of HFD when compared with rats fed with HFD alone. These findings corroborate the research conducted by Nuru et al. (2022), highlighting the preventive effects of *Helianthus annuus seeds* against the adverse impact of high-fat diets on serum lipid levels. Specifically, *Helianthus annuus seeds* were found to mitigate the risks associated with high-fat diets, such as increased total cholesterol, triglycerides, LDL, and decreased levels of HDL. These results are visually represented in figures 8, 9, 10, and 11.



Values presented Mean SEM, \* = significantly relative to Group 1 at  $P < 0.05$ , # = significantly relative to Group 2 at  $P < 0.05$

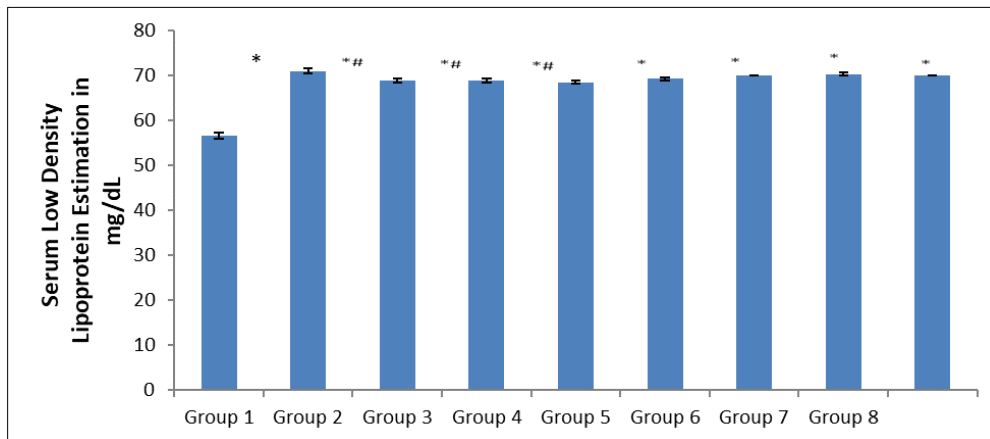
**Figure 8** Effect of *Helianthus annuus seeds* powder consumption on Serum Cholesterol in HFD fed rats. n = 3,





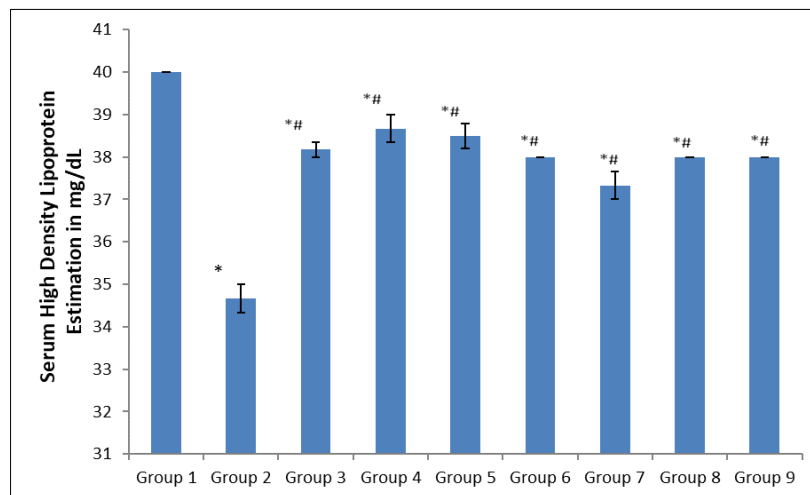
Values presented Mean SEM, \* = significantly relative to Group 1 at P < 0.05, # = significantly relative to Group 2 at P < 0.05

**Figure 9** Effect of sunflower seed powder consumption on Serum Triglyceride in HFD fed rats.n= 3,



Values presented Mean SEM, \* = significantly relative to Group 1 at P < 0.05, # = significantly relative to Group 2 at P < 0.05

**Figure 10** Effect of *Helianthus annuus seeds* powder consumption on Serum Low Density Lipoprotein in HFD fed rats. n= 3



Values presented Mean SEM, \* = significantly relative to Group 1 at P < 0.05, # = significantly relative to Group 2 at P < 0.05

**Figure 11** Effect of *Helianthus annuus seeds* powder consumption on Serum High Density Lipoprotein in HFD fed rats .n = 3,

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## 4. Conclusion

This study investigated, if chewing of sunflower seed can prevent the progression of obesity to prediabetes and diabetes. In this case, because animal subjects were used, the sunflower seed was grounded into powder and mixed with high fat diet meal. High fat diet was used to induce prediabetes. Because excessive consumption of fatty food especially saturated fatty acid has been associated with obesity, hyperglycemia, prediabetes and type 2 diabetes mellitus. The findings from this study shed light on the impact of *Helianthus annuus seeds* consumption in rats subjected to a High Fat Diet (HFD). Several key observations emerged, revealing significant alterations in various physiological parameters.

Firstly, the supplementation of *Helianthus annuus seeds* alongside the HFD resulted in notable changes in the rats' antioxidant enzyme activities. Superoxide Dismutase (SOD) levels exhibited a significant increase in rats consuming *Helianthus annuus seeds*, suggesting a potential role in enhancing antioxidant defenses. Similarly, Glutathione Reductase (GSH) activity levels showed variations, indicating the potential influence of *Helianthus annuus seed* intake on oxidative stress mechanisms. Moreover, the study revealed shifts in renal function markers. Creatinine levels, initially reduced in rats consuming the HFD alone, were significantly elevated upon supplementation with sunflower seeds, potentially indicating an impact on renal health and function. Furthermore, the lipid profile analysis demonstrated contrasting effects. While rats consuming the HFD alone experienced an unfavorable increase in serum cholesterol, triglycerides, and LDL, there was a concurrent decrease in HDL levels. However, supplementation with *Helianthus annuus seeds* alongside the HFD led to a further rise in cholesterol, triglycerides, and LDL but showed a positive influence by significantly elevating HDL levels. These outcomes highlight the complex interplay of *Helianthus annuus seeds* consumption within a high-fat dietary context. While *Helianthus annuus seeds* showed potential beneficial effects on antioxidant enzyme activity and HDL levels, they also appeared to exacerbate lipid-related parameters and creatinine levels.

Overall, these findings underscore the multifaceted impact of *Helianthus annuus seeds* intake on various physiological markers in the context of a high-fat diet. Further research exploring specific mechanisms underlying these observations is warranted to better understand the intricacies of *Helianthus annuus seeds* supplementation in dietary contexts, particularly in relation to oxidative stress, renal health, and lipid metabolism.

### Highlights

- *Helianthus annuus seeds* influenced serum lipid levels countering the detrimental impact of high-fat diets on cholesterol and triglycerides.
- *Helianthus annuus seeds* exhibited antioxidative properties
- Rats consuming *Helianthus annuus seeds* alongside a high-fat diet displayed elevated creatinine levels, indicating potential impacts on renal function.
- Supplementation of *Helianthus annuus seeds* in the diet showed a significant increase in High-Density Lipoprotein (HDL) levels
- findings indicated complex interactions between *Helianthus annuus seeds* consumption and various physiological markers

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## Compliance with ethical standards

### Disclosure of conflict of interest

No conflict of interest to be disclosed.

### Statement of ethical approval

Ethical clearance for the uses of animals for this experiment was obtained from the ethical committee in the College of Health Sciences, Benue State University, Makurdi, CHS REC No: CREC/THS/002.

### Authors contribution

- Conceptualization, data curation, was done by author Augustine Oko Adugba
- Resources was done by Author Sunday Adakole . Ogli, Christian Onahinon and Stephen Olasupo Adeniyi
- Supervision was done by Sunday Adakole . Ogli and Stephen Olasupo Adeniyi
- Original draft by Author Augustine Oko Adugba.
- Writing - review & editing by author Stephen Olasupo Adeniyi.

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