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# Biological Activities of Sunflower Protein Hydrolysate

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**Abstract:** There is a growing trend and interest in the use of food protein – derived peptides and hydrolysates as intervention against chronic human diseases and for maintenance of general well-being. Sunflower protein hydrolysate (SPH) was prepared by the hydrolysis of sunflower protein isolate, first with alcalase for 1h then with flavourzyme for 2h. The biological activity of the hydrolysate including its effect on serum lipids, effect on body weight loss, antihyperglycemic effect, hepatoprotective effect and antihypertensive activity were examined. Examination of rats after 24 hours of injection of high dose of hyrolysate showed no lethality or any manifestations of acute toxicity, which indicates the safety of SPH. The hepatoprotective effect in this study is displayed by 22.60% decline of serum GPT. This data points to the supportive role of the utilized hydrolysate. The results also demonstrated a positive antihyperglycemic effect of the high dose (200 mg/dl) of SPH which equals 30.42% decline in the high blood glucose levels. In the present work, administration of SPH resulted in significant decrease in both serum total cholesterol (18.55%) and triglyceride (29.70%) levels in induced hyperlipidemic rats ($p<0.01$). Unfortunately, SPH revealed no lowering effect on hypertensive rats. Also, the SPH did not exhibit any weight losing effect on obese rats.

**Key words:** Antihypertensive activity · Antihyper-glycemic · Body weight loss · Hepatoprotective effect · Serum lipids · Sunflower protein hydrolysate

## INTRODUCTION

Protein hydrolysates possess a number of functional properties, which make them attractive as a protein source in human nutrition, both in products for special medical use and in consumer products for more general use, e.g. diets for the elderly [1], sports nutrition [2], because of their higher solubility and because proteolysis is considered a predigestion of proteins. Hydrolysates are also used in weight-control diets [3]. In the course of preparing hydrolysates for human consumption it is preferable to use enzymatic hydrolysis than acid or alkali hydrolysis, because the conditions of hydrolysis are much milder and no amino acids are destroyed. Acid and alkali hydrolysis can destroy l-form amino acids and produce d-form amino acids and can form toxic substances such as lysino-alanine [4]. Hydrolysis is a chemical reaction in which water is used to break the bonds of certain substances. In biotechnology and living organisms, these substances are often polymers. In a hydrolysis reaction involving an ester link, such as that found between two amino acids in a protein, the products that result include one that receives the hydroxyl (OH) group from the water molecule and another that becomes a carboxylic acid with the addition of the remaining proton ($H^+$). Lately it has been reported that besides the dietary value of the protein hydrolysates, the hydrolysates and their peptides exhibit several biological activities. They act as antioxidants [5, 6, 7] have antimicrobial activity [8, 9] and are considered as immunomodulatory [10], antihypertensive [11,12], anticoagulant [13], anticarcinogenic agents [11,14] and have several other physiological functions. The aim of this work is to assess the biologic activity of Sunflower protein hydrolysate (SPH) regarding acute toxicity, antihyperglycemic, Anti hypertensive, serum lipid reducing effects and hepatoprotective activity.

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## MATERIALS AND METHODS

**Enzymes:** Alcalase (A) and Flavourzyme (F) are products of Sigma-Aldrich.

**Preparation of Hydrolyzate:** In a previous paper [15] we have examined several enzymes for the preparation of sunflower protein hydrolysates and peptides and their effect on the antioxidant activity (AOA) and antimicrobial activity (AMA) of the protein products. In this paper we have chosen the following hydrolysate from [15] to be subjected to further examination of several biological activities. The sunflower protein isolate (which is free of CGA) was enzymatically hydrolyzed using a mixture of alcalase (A) & flavourzyme (F) at a ratio of 1:1. Alcalase is an endopeptidases which breaks peptide bonds of nonterminal amino acids (i.e. within the molecule) and flavourzyme which is an exopeptidases, which break peptide bonds from their end pieces. Endopeptidases cannot break down peptides into monomers, while exopeptidases can break down proteins into monomers. The hydrolyzate was prepared by dissolving sunflower isolate at a meal: water ratio of 1: 10 (w/v) by continuous stirring, while adjusting the temperature to 50°C and the pH to 2.0 for Alcalase with continuous stirring for 1h, then the pH was raised to 7.0 for Flavourzyme and stirring continued for two more hours. The enzyme was added (2% enzyme concentration) after adjustment of temperature. During hydrolysis both the temperature and pH were kept constant. The enzyme mixture was deactivated at the end of the 3h. With the hydrolysis a control experiment was carried under same conditions but without the addition of the enzyme mixture. The hydrolyzate was taken for the determination of its biological activity.

**Analysis of Sunflower Protein Hydrolyzate:** Protein, ash and crude fibre and were determined according to A.O.A.C. [16]. Amino acid composition of samples was measured at the Central Laboratory for Food & Feed, Agricultural Research Centre, Cairo, Egypt, using an amino acid analyser (Model 7300) as described by Moore & Stein [17]. The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentages of the total protein.

**Animals and Treatments:** Adult albino rats of both sexes, weighing 120-140g were used. Animals were obtained from the animal house (National Research Centre Cairo). All animals were housed under conventional laboratory conditions through the period of experiment. Rats were fed standard lab pellets (20% proteins, 5% fats, 1% multivitamins, Malacado, Obour city). They were allowed free access to tap water. Animals were allowed at least one week acclimatization before using them. Experiments were performed under standard conditions at Pharmacology Research Unit of National Research Centre. The rats were kept in separated animal rooms with a 12h light–dark cycle and the temperature and humidity were kept at 23 ± 2°C and 55–65%, respectively. This study was approved by the Ethical Committee of National Research Centre. All animals received humane care according to the guidelines in the Guideline for the Care and Use of Laboratory Animals. All the animals were acclimatized for at least one week prior to experiment and then randomly divided into groups (8 rats per group) for testing acute toxicity, anti diabetic activity and hepatoprotective, serum lipids, antihypertensive and weight loosing effects.

**Test for Acute Toxicity:** Group 1 served as normal control and group 2 was tested for acute toxicity. The hydrolysate was provided orally in rising doses of 50, 100 and 200 mg/kg. Twenty four hours after the hydrolyate was taken, the rats were examined for lethality and acute toxicity manifestations.

**Evaluation of Antihyperglycemic Effect:** Rats were rendered hyperglycaemic by a single intraperitoneal injection of alloxan monohydrate (Sigma-Aldrich, Switzerland) in a dose of 150 mg/kg as recommended by Aruna *et al.* [18]. Alloxan was first weighed individually for each animal according to the body weight and then dissolved in 0.2 ml saline just prior to injection. Rats with plasma glucose levels of 140 mg/dl or higher levels were included in the study. Five groups (8 males in each group) of rats were prepared: Group 1: is the normal control group. Group 2: Rat rendered hyperglycemic with alloxan with no treatment. Group 3: Rat rendered hyperglycaemic with alloxan with oral hypoglycemic treatment (Glicazide). Groups 4 and 5: Rat rendered hyperglycaemic with alloxan treated with hydrolysate in two doses (100 and 200 mg/kg). Treatment was started 24 hours after alloxan injection in hyperglycaemic rats. Blood glucose was estimated before and after treatment, serum glucose levels were calculated in mg/dl.

**Detection of Hepatoprotective Activity:** Three groups of rats were prepared: Group 1 and 2, rats were allowed to ingest hydrolysate in two dose levels (100 and 200 mg/Kg) for 7 days in addition to regular diet. Group 3: rats ingesting regular diets. Induction of acute liver injury was

done for the 3 groups by injection of thioacetamide (Sigma-Aldrich, Switzerland) in a dose of 200 mg/kg body weight as recommended by Aydin *et al.* [19]. The chemical was dissolved in sterile distilled water and injected intraperitoneally. Serum levels of liver enzymes (GOT, GPT) were assessed before and after acute liver injury induction. Hepatic enzymes, asparate aminotransferase (AST) and alanine aminotransferase (ALT), were used as the biochemical markers of the hepatic damage. The serum activities of AST and ALT were assayed using commercial kits. The enzyme activities of AST and ALT were expressed as U/L.

**Effect on Hyperlipidemia** Hyperlipidemia was induced by maintaining the rats on high fat diet for 30 days. The diet was prepared by adding 1% cholesterol powder, 0.2% cholic acid and 10% fat to the rat powdered standard laboratory diet [20]. Rats were fasted for 14 h before blood sampling, which was carried out under light ether inhalation anaesthesia. Blood samples were withdrawn from the retro-orbital venous plexus using a heparinised micropipette according to the method of Itiar *et al.* [21]. Each blood sample was collected in a test tube, then allowed to clot at room temperature and serum was separated by centrifugation at 4000 rpm for 15 minutes by cooling centrifuge (Laborzentrifugen, 2k15, Sigma, Germany).

**Determination of Total Cholesterol Level (TC):** The method described by Richmond [22] was utilized to estimate serum TC using a test reagent kit.

**Determination of Triglycerides (TG):** The method described by Fassati and Prencipe [23] was employed to estimate serum TG using a test reagent kit.

**Effect on Hypertension:** Systolic blood pressure and heart rate of animals were measured each week by non invasive blood pressure monitor (ML 125NIBP, AD Instruments, Australia). The animals were restrained in the tubes for 10-20 min/day for 5 days prior to recording blood pressure in the tail-cuff technique, animals were warmed for 30 min at 28°C in a thermostatically controlled heating cabinet (Ugo Basille, Italy) for better detection of tail artery pulse, the tail was passed through a cuff and a tail-cuff sensor that was connected to an amplifier (ML 125 NIBP, AD Instruments, Australia). The amplified pulse was recorded during automatic inflation and deflation of the cuff. Hypertension was induced according to Majithiya *et al.* [24]. Rats were weighed and given orally L-NAME (50 mg/kg) in distilled water daily for 4 successive weeks. The blood pressure was measured by a tail-cuff method then rats were divided into three groups, of 8 rats / each as follows:

*Group 1*: Normal rats were given distilled water (0.5ml/0.1kg) orally throughout the experiment and served as negative control group.

*Group 2*: Rats were given L- NAME (50 mg/kg/day) as single oral dose for 4 weeks and served as positive control group.

*Group 3*: Rats given orally the extract of (40 mg/kg) for 4 weeks simultaneously with L-NAME (50 mg/kg/day).

**Weight Losing Effect**

**Induction of Obesity:** Animals were fed standard diet in addition to Cafeteria diet 20 gm/ rat for three weeks [25]. Three groups of 8 rats /each as follows:

*Group 1*: Normal rats were fed standard diet in addition to Cafeteria diet 20 gm/ rat for three weeks.

*Group 2*: Rats were fed standard diet in addition to Cafeteria diet 20g/rat for three weeks. In addition, silbutramine was given in a dose of 3g/kg as a standard weight losing drug for 3 weeks.

*Group 3*: Rats were fed standard diet in addition to Cafeteria diet 20 gm/ rat for three weeks. Hydrolysate was added in a dose of 0.4ml/ rat daily for 3weeks.

## RESULTS AND DISCUSSION

As expected the protein content of the hydrolysates resulting from enzymatically hydrolysed using a mixture of alcalase (A) & flavourzyme (F) increased reaching almost 100% protein (Table 1).

**Amino Acids Analysis of Sunflower Protein Hydrolysate:** The amino acids lysine and sulphur amino acids (methionine + cysteine) increased due to hydrolysis (Table 2). The increase in total protein and the amino acids lysine and sulphur amino acids is certainly a nutritional privilege, especially with special concern to lysine which is originally limiting in sunflower protein isolate. Khalil *et al.* [26], Kain *et al.* [27] and

Table 1: Chemical composition of sunflower protein isolate and the protein hydrolysate prepared from there A & F hydrolyzate: alcalase and flavourzyme hydrolysate

| Composition (%) | Sunflower Protein Isolate | Sunflower Protein hydrolysate (A & F) |
|---|---|---|
| Protein | 95.1 | 99.7 |
| Ash | 1.6 | 0.77 |
| Fibre | 0.7 | -- |

Table 2: Essential amino acid composition of sunflower protein hydrolysate (g/16gN)

| Essential amino acid | Isolate | A & F | FAO* |
|---|---|---|---|
| Lysine | 4.2 | 7.9 | 5.8 |
| Histidine | 2.6 | 3.8 | -- |
| Valine | 5.2 | 3.0 | 3.5 |
| Threonine | 3.5 | 2.3 | 3.4 |
| Methionine +cystine | 4.6 | 8.2 | 2.5 |
| Isoleucine | 5.0 | 2.3 | 6.6 |
| Leucine | 7.1 | 5.6 | 2.8 |
| Phenylalanine + tyrosine | 6.3 | 6.0 | 6.3 |
| Tryptophan | ND | ND | 1.1 |

* FAO/WHO/UNU pattern (1991), A&F: mixed A & F hydrolysate

Villaneuva *et al.* [28] reported the increase in lysine and sulphur amino acids in hydrolysates of soy, peanuts and sunflower, respectively.

**Acute Toxicity Effect:** Examination of rats after 24 hours of injection of high dose of hyrolysate showed no lethality or any manifestations of acute toxicity. This result denotes absence of acute toxicity manifestations and safety of utilization of the tested hydrolysate even in this very high tested dose. In the present study, ingestion of a high dose (200 mg/kg) showed no lethal effect or any toxic manifestations. This result confirms absence of acute toxic effects of the hydrolysate and safety of consumption even in high relative doses. The use of dietary proteins and protein hydrolysates in food products is generally allowed in European countries and has the status of "generally regarded as safe" (GRAS) in the United States of America [29]. In Europe, novel foods are defined as foods and food ingredients that have not been used for human consumption to a significant degree within the European Community before 15 May 1997 (http://ec.europa.eu)[30]. Safety evaluation by external independent experts and approval by competent authorities is necessary before a novel product is allowed on the market [31].

Till date, there has been little concern about safety of food protein-derived BAPs since the body would normally hydrolyze food proteins into peptides [32] and food-grade enzymes and processes are utilized for industrial production of peptides. Numerous studies have shown lack of toxicity of these peptides in cell cultures. The safety aspects of milk-derived peptides have been reviewed [33]. Moreover, a recent study reported that both single dose (2000 mg/kg) and repeated daily dose (1000 mg/kg for 4 wk) of casein hydrolysates containing antihypertensive peptides ($\alpha$s1- casein f 90–94 and f 143–149) resulted in no adverse effects on clinical (blood biochemical, hematology, organ weight ratios, histopathological) parameters or mortality in rats [34]. The sample doses represent peptide amounts that are well above those needed to observe pharmacological activities. BAPs are generally safe but care should be taken to avoid processing techniques that would negatively affect peptide quality and safety [35].

**Antihyperglycemic Effect:** Hydrolysate ingestion has hypoglycemic action as blood glucose before ingestion was 495.2 ± 20.7 mg/dl. After ingestion of dose 1, it declined to 479.2± 24.2 mg/dl. Meanwhile, ingestion of dose 2 led to decrease in the level to 344.6± 10.12 mg/dl (30% decline). Response was dose dependent. Ingestion of anti diabetic drug (Glicazide) led to decline of blood glucose level to 210.0±16.8 mg/dl (57.6% decline) as shown in Table 3. Our results suggest positive antihyperglycemic effect of the high dose (200 mg/dl) of the hydrolysate which equals 30.42% decline in the high blood glucose levels. In a human trial, the concomitant plasma glucose responses were 15 and 12% lower in the patients with type 2 diabetes. The authors conclude that co-ingestion of a case in protein hydrolysate strongly augments the insulin response after ingestion of a single bolus of carbohydrate, thereby significantly reducing postprandial blood glucose excursions in patients with long-standing Type 2 diabetes [36]. An amino acid mixture improves glucose tolerance and insulin signaling in Sprague-Dawley rats. These findings suggest that amino acid supplementation can improve glucose tolerance by increasing skeletal muscle glucose uptake and intracellular disposal through enhanced intracellular signalling [37]. Antidiabetic effects of fermented soybean products on type 2 diabetes was tested, several studies revealed improvements in insulin resistance and insulin secretion with the consumption of these fermented products. Therefore, fermented soybean products may help prevent or attenuate the progression of type 2 diabetes [38].

Table 3: Blood glucose levels

| Group | Mean (mg/dl) | Decline (mg/dl) | Decline (%) |
|---|---|---|---|
| Normal | 110.42±7.02 | -- | -- |
| Hyperglycemic | 495.2 ± 20.7 | -- | -- |
| Glicazide | 210.01±16.79 | 285.23 | 56.60 |
| Dose 1 (100 mg/kg) | 495.17±24.23 | 16.06 | 3.24 |
| Dose 2 (200 mg/kg) | 433.59±10.12 | 150.64 | 30.24 |

Data are expressed as mean ± SE (n=8).

Table 4: Liver enzymes levels

| Serum enzyme level | Toxin alone (TAA) | Toxin +Dose 1 (100mg/kg) | Toxin +Dose 2 (200mg/Kg) |
|---|---|---|---|
| GPT | 319.66 | 247.42 | 249.95 |
| % decline of GPT | -- | (22.60%) | (21.81%) |
| GOT | 623.94 | 613.68 | 619.90 |

Table 5: Effects of treatment (0.4 ml) of sunflower protein hydrolysate on serum cholesterol and triglycerides levels

| Groups | Serum total cholesterol level (mg/dL) | Serum triglycerides level (mg/dl) |
|---|---|---|
| Normal control | 52.52 ± 2.01 | 172.50 ± 1.82 |
| Positive control | 137.47 ± 3.21 | 292.50 ± 22.37 |
| Treatment (0.4 ml) | 111.97 ± 0.20 | 205.63 ± 4.24 |

Data are expressed as mean ± SE (n=8).

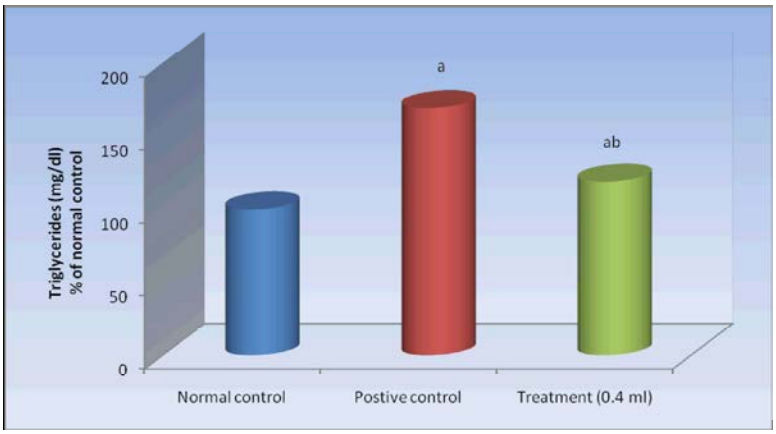


Fig. 1: Effects of treatment (0.4 ml) on serum cholesterol level of hyperlipidemic rats

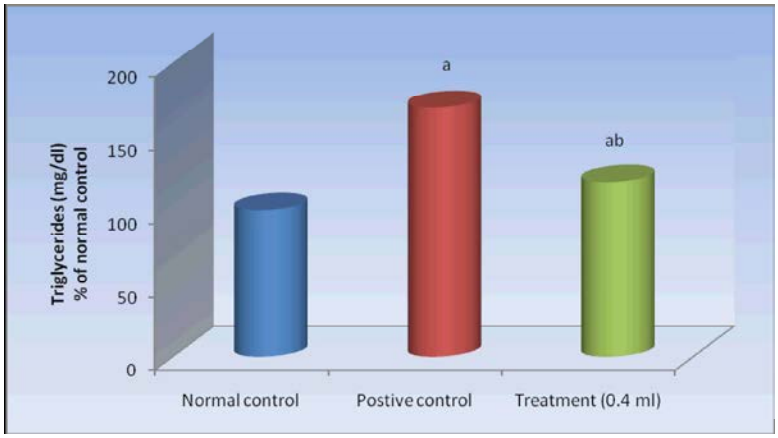


Fig. 2: Effects of treatment (0.4 ml) on serum triglycerides level of hyperlipidemic rats

**Hepatoprotective Effect:** Hydrloysate ingestion in a dose of 100 mg/kg led to decline of serum glutamate pyruvate transaminase enzyme level (GPT) from 319.7 to 247.4, higher dose has no better effects. As shown in Table 4. Glutamate pyruvate transaminase (GOT) showed no improvement in its level. It is well known that GPT is

specific for liver damage and is much more pathognomonic for liver monitoring. This result declares the liver support action of the used hydrolysate. The hepatoprotective effect in this study is suggested by 22.60% decline of serum GPT. This data points to the supportive role of the utilized hydrolysate. In patients with chronic liver disease, complex alterations in the metabolism of proteins occurs and nutritional support is essential in the athogenesis and treatment of this disease. Patients with chronic liver failure have high plasma levels of aromatic amino acids (AAA) and methionine and low levels of branched-chain amino acids (BCAA) [39, 40]. While, casein hydrolysates are commonly used for nutritional applications in patients with chronic liver disease, a protein source with a higher level of BCAA is more desirable. Sunflower globulins have been suggested as excellent protein sources for the development of protein hydrolysates with high levels of BCAA [41]. Sunflower protein hydrolysate is recommended for enthral, parenteral and oral nutrition of liver disease patients, being hypoallergenic, having low bitterness and providing a high Fischer ratio (BCAA: AAA) of approximately 75[42].

Oral feeding of porcine plasma protein hydrolysate (PPH) to male rats (200 mg/kg of body weight orally once daily for 12 days) significantly lowered ($P<0.01$) the serum levels of hepatic enzyme markers (aspartate transaminase and alanine transaminase) compared with the $CCl_4$-only treatment group. Levels of hepatic superoxide dismutase, glutathione peroxidase, catalase and total antioxidant capacity were significantly increased and the malondialdehyde levels were sharply decreased ($P<0.01$) in rats treated by PPH. Histological examination of the liver showed that lesions, including necrosis, lymphocyte infiltration and fatty degeneration, were partially healed by treatment. These data suggest that in rats, PPH can protect the liver against $CCl_4$-induced oxidative damage [43]. Bioactive peptides from salmon pectoral fin protein were tested for alcoholic liver injury. Oxidative stress was significantly ($p<0.05$) increased serum markers of liver damage, such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase; however, these activities decreased significantly ($p<0.05$) after hydrolysate administration. Results indicated that the hydrolysate had a protective effect against ethanol-induced hepatotoxicity that was comparable to that of silymarin, which was supported by evaluating liver histopathology in the rats [44].

**Hypolipidemia Effect of Sunflower Protein Hydrolysate:** As for the effect of the hydrolysate on hyperlipidimic rats, it is clear in the present work, that administration of sunflower hydrolysate resulted in significant decrease in both serum total cholesterol (18.55%) and triglyceride (29.70%) levels in induced hyperlipidemic rats ($p<0.01$) (Table 5).

Using protein hydrolysates (PH) from jellyfish decreased total serum cholesterol and triglyceride and increased high-density lipoprotein cholesterol in rats fed with high-fat diet. The reduced TC and TG significantly, compared with the high-fat diet control group. TG reduction was comparable to that of the positive standard atorvastatin [45]. The hydrolysate contained high levels of Glycine, Glutamic acid, Aspartic acid and Alanine. Similar result was observed by Manso *et al.* [46] by consumption of egg white hydrolysate in spontaneously hypertensive rats which proved to have lowering triglycerides and total cholesterol without changing HDL levels. Freshwater clam (a gellyfish) hydrolysate increases relative bile acid-binding capacity and inhibition of micellar solubility of cholesterol with subsequent decline in total cholesterol level [47].

**Anti-Hypertensive Effect of Sunflower Protein Hydrolysate:** Hypertension is a common and serious chronic health problem and identified as a major risk factor for the development of arteriosclerosis, stroke, myocardial infarction and end-stage renal disease, affecting 15-20% of adults in the world [48]. In recent years, food-derived antihypertensive peptides have attracted much attention from both researchers and consumers. ACE inhibitors of protein hydrolysate origin have been obtained from various food sources, including bovine casein [49], fermented foods [50] and rice hydrolysate [51]. Unfortunately, the tested protein hydrolysate did not exert any anti hypertensive effect on induced hypertensive rates (Table 6). The effects of different enzymolysis conditions, the relative molecular weight and amino acids composition all have an effect on the ACE inhibitor activities of the produced hydrolysate or peptide prepared [52]. Perhaps more investigation is needed for this experiment, or perhaps sunflower protein hydrolysate does not possess this property.

**Evaluation of Weight Losing Effect of Sunflower Protein Hydrolysate:** Another negative effect was attained with the results of the effect of the hydrolysate on weight loosing effect on obese rats (Table 7). There was no statistical difference between body weight gain in hydrolysate treated rats (28.00±1.99) compared to control group (30.17± 3.11).

Table 6: Effect of oral administration of sunflower protein hydrolysate (40mg/kg/day) on systolic blood pressure in L-NAME-treated rats

| Systolic blood pressure (mm Hg) | | | | | |
|---|---|---|---|---|---|
| Week | | | | | |
| Group | 0 | 1 | 2 | 3 | 4 |
| Normal control (distilled water) | 98.14 ±5.2 | 97.155±6.4 | 100.3 ±3.3 | 109.50 ±5.3 | 102.73 ±3.5 |
| Positive control L-NAME (50mg/kg) | 99.78±6.1 | 129.85±3.5 | 144.26±4.1 | 159.69±3.6 | 178.02±3.1 |
| L-NAME (50mg/kg) + ext.(40mg/kg) | 100.95±4.8 | 127.5±3.7 | 144.72±4.8 | 162.85±4.2 | 193.84±5.7 |

Data are expressed as mean ± SE (n=8)

Table 7: Evaluation of weight losing effect

| Group | Body weight gain (g) after 3 weeks |
|---|---|
| Group 1: Control obese (Cafeteria diet) | 30.17± 3.11 |
| Group 2: Cafeteria diet+ Silbutramine (3gm/kg), 3 weeks | 16.33± 1.37 |
| Group 3: Cafeteria diet+ tested extract (.4 ml/rat), 3 weeks | 28.00± 1.99 |

Data are expressed as mean ± SE (n=8).

## CONCLUSION

The results confirm absence of acute toxic effects of our hydrolysate and safety of consumption even in high relative doses. Also, the results suggest positive antihyperglycemic effect of the high dose (200 mg/dl) of our hydrolysate which equals 30.42% decline in the high blood glucose levels. The hepato-protective effect in our study is suggested by 22.60% decline of serum GPT. This data points to the supportive role of the utilized hydrolysate. Sunflower hydrolysate resulted in significant decrease in both serum total cholesterol (18.55%) and triglyceride (29.70%) levels in induced hyperlipidemic rats ($p<0.01$). The protein hydrolysate doesn't exert any anti hypertensive effect on induced hypertensive rats. The tested protein hydrolysate doesn't have weight losing effect on obese rats; probably further investigation is needed for the antihypertensive and weight loosing effect.

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