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ABSTRACT

This study aimed to reach a comprehensive biological evaluation of Jerusalem artichoke (*Helianthus tuberoses* L.) tubers aqueous extract in adult male albino and to determine its influence on anti-hyperlipidemia protective and sensory properties of chicken and shrimp pops fortified with the extract. Thirty-six adult male albino rats were divided into six groups and treated orally with three concentrations (100, 200, and 300 mg/kg body weight) of *H. tuberoses* extract. Blood and liver samples were collected to measure the biochemical indicators, protein level, antioxidant parameters, and histopathological examination. Chicken and shrimp pops fortified with the extract were evaluated for their sensory characteristics. The results showed that decrease in liver and adipose tissue weights using *H. tuberoses* extract as compared with reference drug (Atorvastatin) group, especially at *H. tuberoses* extract (200mg/kg bwt). Liver aminotransferase enzyme activity (AST and ALT) significantly increased in the control positive group compared to the control negative group. *H. tuberosus* extract-treated groups. Total count of HB, other blood indices, and platelets count were slightly increased in treated groups with extract as compared with the reference drug (Atorvastatin) group. Antioxidant enzymes activities (GPx and SOD) were observed at the highest activities were observed at *H. tuberosus* extract 100 , 300 mg/kg bwt. Administration of the extract induced a significant improvement in histological picture of the liver. Sensory evaluation of chicken and shrimp pops fortified with extract showed acceptability

related to taste, tenderness, and appearance. *H. tuberosus* extract could be used as antihyperlipidemic, properties when used as a food supplement

Keywords: *Hyperlipidemia, Helianthus tuberosus, oxidative stress, ROS, food supplementary, Sensory evaluations*

التأثير الوقائي المضاد لمستخلص الطرطوفة ضد زيادة دهون الدم في ذكور الجرذان

وتقييم تأثيره الحسي على كرات الدجاج والروبيان

المستخلص:

هدفت هذه الدراسة إلى تقييم شامل لمستخلص درنات الطرطوفة على الفعالية الحيوية في ذكور الجرذان البالغة وتحديد مدى تأثيره على الوقاية من ارتفاع الدهون في الدم وتأثيره على الخواص الحسية لكرات الدجاج والروبيان المدعمة بالمستخلص المائي للدرنات. تم تقسيم ستة وثلاثين من ذكور الجرذان البيضاء البالغة إلى ست مجموعات تم تغذيتها بأنظمة غذائية مختلفة تتضمن ثلاث تركيزات من المستخلص (١٠٠، ٢٠٠، ٣٠٠ ملجم/ كجم من وزن الجسم) من مستخلص الطرطوفة. تم جمع عينات الدم والكبد لقياس المؤشرات البيوكيميائية ومستوى البروتين ومضادات الأكسدة والفحص النسيجي. تم تقييم كرات الدجاج والروبيان المدعمة بالمستخلص من حيث خصائصها الحسية. أظهرت النتائج انخفاض في أوزان الكبد والأنسجة الدهنية باستخدام مستخلص درنات الطرطوفة مقارنة مع مجموعة الدواء المرجعي (أتورفاستاتين) وخاصة في مستخلص درنات الطرطوفة (٢٠٠ ملجم/ كجم من وزن الجسم). زاد نشاط إنزيمات الكبد (ast, alt)، بشكل ملحوظ في المجموعة الكنترول الموجبة مقارنة بالمجموعة الكنترول السالب مجموعات مستخلص الطرطوفة. ارتفع العدد كرات الدم ومؤشرات الدم الأخرى وعدد الصفائح الدموية بشكل طفيف في المجموعات المعالجة بالمستخلص مقارنة مع مجموعة الدواء المرجعي (أتورفاستاتين). وقد لوحظت أنشطة الإنزيمات المضادة للأكسدة (الجلوتاثيون بيروكسيداز وسوبر اكسيد ديسميوتاز) في أعلى مستوى الأنشطة التي لوحظت في مستخلص الطرطوفة ١٠٠ و ٣٠٠ ملجم/ كجم من وزن الجسم. أظهرت النتائج أن إعطاء المستخلص أدى إلى تحسن معنوي في الصورة النسيجية للكبد. أظهر التقييم الحسي لكرات الدجاج والروبيان المدعمة بالمستخلص قبولها من حيث الطعم والقوام والمظهر. يمكن استخدام مستخلص درنات الطرطوفة كمضاد لفرط شحميات الدم، ويمكن استخدامها كمكمل غذائي.

الكلمات المفتاحية: فرط شحميات الدم، الطرطوفة، الإجهاد التأكسدي، ROS، المكملات الغذائية، التقييمات الحسية.

Introduction

Helianthus tuberosus L. (*H. tuberosus*) also known as Jerusalem artichoke, is a native North American plant, which has a rich history of cultivation dating back to Native Americans. Its resistance to adverse

growing conditions such as drought, frost, and poor soil quality has made it a valuable crop. Moreover, *H. tuberosus* is a versatile ingredient that can be used in various culinary applications such as cooking, roasting, frying, and processing into tea, flour, French fries, and salads (Michalska-Ciechanowska et al., 2019). The tubers of this plant are mainly composed of water, carbohydrates, and protein and contain little starch and fat, resulting in a relatively low caloric value. Notably, these tubers contain a spectrum of phytochemicals, including polyphenols, which are often formed as secondary metabolites and are present in polyhydroxyphenol complexes. These compounds exhibit a wide range of physiological effects, ranging from antioxidant and antimutagenic to anticancer properties (Bach et al., 2015; Michalska-Ciechanowska et al., 2019; Sokkar et al., 2020). In the context of human health, lipids play a central role, with components such as cholesterol, triglycerides, phospholipids, and lipoproteins performing essential functions. These functions include maintaining the integrity of the cell membrane, participating in the synthesis of vitamin D and steroid hormones, acting as energy stores, and participating in cell signaling processes (Dashty et al., 2014). However, deviations from normal lipid levels, as seen in diabetes mellitus (DM) and dyslipidemia, can pose significant health risks (Achila et al., 2020).

Emerging evidence from clinical and experimental studies highlights the potential of *H. tuberosus* extract in inhibiting key enzymes involved in cholesterol and triglyceride synthesis pathways, such as HMG Co-A reductase and phosphatide phosphohydrolase (Pouyamanesh et al., 2016; Qinna et al., 2012). Additionally, *H. tuberosus* extract contains natural antioxidants, particularly phenolic compounds, which may combat free radicals, bolster immune function, and regulate excessive reactive oxygen species production during infections (Dias et al., 2016; Johansson et al., 2015; Rostami-Mansoor et al., 2021).

Numerous studies have underscored the therapeutic potential of *H. tuberosus* tubers in managing various diseases, including diabetes and colon cancer. These tubers are a source of diverse phytochemicals, such as coumarin, sesquiterpenes, chlorogenic acid, and caffeic acid, in addition to proteins, minerals, and carbohydrates (Afoakwah 2022). The primary dietary fiber in *H. tuberosus* tubers, inulin, not only aids in calcium, magnesium, and potassium absorption in the digestive tract but also exhibits hypoglycemic effects, influences liver lipid profiles, improves glucose tolerance, and exerts anti-diabetic effects (Shao et al., 2021).

H. tuberosus tubers have shown anti-inflammatory, antibacterial, anti-cancer, and antioxidant properties of phytochemicals (Zhang and

Kim 2015). This multifaceted health profile has led to the incorporation of Jerusalem artichoke and its inulin as common ingredients in processed foods, including bakery products, confectioneries, and medicinal formulations. In food applications, inulin serves as both a thickening agent and a fat substitute (**Illippangama et al., 2022; Karimi et al., 2015**). Notably, the powder of whole *H. tuberosus* tubers offer high levels of fructooligosaccharides, oligosaccharides, phytochemicals, and inulin, imparting a sweet flavor and eliminating the need for extraction, thereby enhancing its potential as a functional food ingredient (**Afoakwah et al., 2022; Khuenpet et al., 2017; Radovanovic et al., 2015**). Moreover, *H. tuberosus* leaves have been found in medicinal applications due to their bioactive substances like phenolic compounds and flavonoids (**Chen et al., 2014; De Gregori et al., 2018**). These leaves possess analgesic, antibacterial, antifungal, anti-inflammatory, and antispasmodic effects, making them useful for treating various diseases (**Saiki et al., 2022**). Oxygen-reactive species can damage biological macromolecules, leading to cell and tissue damage, but *H. tuberosus* extract with SOD-like activity can scavenge free radicals and protect against oxidative stress (**Wang et al., 2020**).

Despite these promising attributes, *H. tuberosus* tubers remain an underutilized resource, and their full potential remains untapped. This study aimed to assess the anti-hyperlipidemic, properties of the aqueous extract of *H. tuberosus* in male rats using biochemical, and histopathological parameters. Additionally, it will evaluate the sensory properties of *H. tuberosus* extract as a food supplement when incorporated into chicken and shrimp pops. This research aims to shed light on the untapped potential of Jerusalem artichoke in enhancing human health and culinary experiences.

Material and methods

Materials

Plant

Jerusalem artichoke (*Helianthus tuberoses* L) samples were sourced from the Basateen Research Center in Mansoura, Dakahlia, Egypt.

Chemicals

All chemicals and solvents utilized were of analytical grade, with the components for the rat feed, including vitamin and salt mixtures, being sourced from TechnoGene Chemical Co. in El Doki, Egypt. Casein was acquired from Morgan Chemical Co. in Cairo, Egypt. Liver function, and lipid profile kits were sourced from Hiprobiotechnology (City, China). Kits for antioxidant assessments (including glutathione peroxidase (GPx), and superoxide dismutase (SOD)), were acquired from Abcam (City, UK).

Animals

Thirty-six adult male albino rats weighing between 160-180 g were procured from the Institute of Graduate Studies and Research in Alexandria, Egypt. Following a one-week acclimation period, the rats were placed in polypropylene cages filled with wood shavings. The animals were kept in separate cages, with six rats in each cage, in a temperature-controlled room set at $25\pm3^{\circ}\text{C}$ and a 12-hour light/dark cycle. The environment was carefully maintained to be free from any chemical contaminants at the Institute of Graduate Studies and Research in Alexandria, Egypt.

Methods

preparation of plant extract

The extraction of plant material followed a modified procedure described previously (**Kim and Han 2013**). Approximately 50 g of dried tuber powder was mixed with deionized water at a 1:20 weight-to-volume ratio. The mixture was stirred at 45°C for 1 hour and then soaked at room temperature for 24 hours with continuous stirring using a magnetic stirrer. After centrifugation at $3000 \times g$ for 10 minutes at 20°C , the mixture was filtered through Whatman No. 1 filter paper. The remaining material was weighed, and the extraction yield was determined after lyophilization using a Vacuum Freeze Dryer (model: FDF 0350, Korea). The resulting lyophilized powder of the plant extract was then stored at -20°C for further analysis, following the approach outlined by **Vongsak et al., (2013)**.

Reduction power

A spectrophotometric technique was used to quantify the reduction power (**Ferreira et al., 2007**). For this, 2.5 ml of potassium ferricyanide (1%) and 2.5 ml of phosphate buffer (0.2M, PH 6.6) were combined with 1 ml of plant extracts in the appropriate solvents to be tested. The resulting mixture was incubated for 20 minutes at 50°C in a water bath. 2.5 ml of 10% trichloroacetic acid was added after cooling, and if needed, the mixture was centrifuged for 10 minutes at 3000 rpm. After mixing 2.5 ml of distilled water with 0.5 ml of freshly made ferric chloride solution (0.1%), the final layer of solution was let to stand for 10 minutes. At 700 nm, the absorbance was measured. Except for the samples, the control was prepared in a similar way. The calibration curve was constructed using ascorbic acid at various levels as a reference, the reducing power was represented as EC_{50} (mg/ml), which is the effective concentration at which absorbance equals 0.5.

In-vitro hemolytic activity and (IC_{50}) value of *H. tuberosus* extract

The test was performed in 2 ml microtubes of three replicates following the method described by **Farias et al., (2013)** with some

modifications. **1.** A twofold serial dilution of each extract was prepared in saline solution (0.9% NaCl) to create a concentration range of 1,000 µg/ml to 1.9 µg/ml. **2.** A 1% red blood cell suspension (types A, B, and O from rabbit blood) was prepared. Then, 100 µl of this suspension was added to each extract dilution (900 µl) in separate microtubes. These mixtures were incubated at 37°C for 1 h. **3.** After incubation, the microtubes were centrifuged at 3000 x g for 5 minutes. Then, 200 µL of supernatant from each tube was transferred to a 96-well plate to measure absorbance. **4.** To establish reference points for 0% and 100% hemolysis, 100 µl of each red blood cell suspension was mixed with either distilled water (for 100% hemolysis) or saline solution (0.9% NaCl) (for 0% hemolysis). The volume was adjusted to 900 µl with the respective control solution. **5.** The percentage of hemolysis was calculated using the following formula:

$$\% \text{ hemolysis} = \text{Abs test} / \text{Abs pc} \times 100,$$

Where Abs test = Abs540 for the 1% cell suspension treated with the test sample and Abs pc = Abs540 for the 1% cell suspension treated with distilled water.

Basal diet preparation

Protein (10%), corn oil (10%), vitamins mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and corn starch (69.5%) are included in the basic diet made following the formula provided by **AIN (1993)**. While the salt mixture used was prepared by **Hegsted et al., (1941)**, the vitamin mixture component utilized was advised by **Campbell, (1963)**.

High-fat diet

The elements and methods for preparing a high-fat diet as indicated by **Reed et al., (2000)**

Biological experimental design

Animals were divided into two primary groups as follows: Group 0: Negative Control Group (six rats received one ml sterilized saline 0.9% orally by gavage tube daily during the entire trial period for four weeks with normal feed). The second main group comprised (30) rats these rats were further segregated into 5 matched subgroups (Groups 1–5). All Groups (1-5) fed high-fat diet for four weeks to inject hyperlipidemic and it was divided into Group 1: Positive Control Group (high-fat diet for four weeks). Group 2: Reference drug Atorvastatin (Atro) for four weeks. Groups 3, 4, and 5: Treatment Groups (received one ml of *H. tuberoses* 100, 200, and 300 mg/kg body weight, respectively, dissolved in sterilized saline 0.9% daily for four weeks orally by gavage tube. The animal groups and corresponding interventions are presented in Table (1). All ethical principles governing the treatment and utilization of laboratory

animals were strictly adhered to. The methodologies and guidelines implemented in the current study have received validation from the Ethical Review Board (Approval reference: IACUC 82-3B-0223). The subjects were provided with a standard laboratory diet and access to water ad libitum. The experiment was continued for one month after the one-week acclimation period. Rats at the end of the experiment, rats were sacrificed.

Table (1). Animal groups and treatments

Code	Animal grouping	Intervention
GP0	Negative Control Group	one ml sterilized saline 0.9% orally
GP1	Positive Control Group	high-fat diet for four weeks
GP2	Reference drug	Atorvastatin
GP3	Treatment Group 1	one ml of <i>H. tuberoses</i> 100 mg/kg body weight
GP4	Treatment Group 2	one ml of <i>H. tuberoses</i> 200 mg/kg body weight
GP5	Treatment Group 3	one ml of <i>H. tuberoses</i> 300 mg/kg body weight

Hematological Biochemical analysis

Blood collection

Four weeks afterward, after an overnight fast, rats were anesthetized by diethyl ether, and blood was extracted via blood pressure puncture. Glass centrifuge tubes that were reduced dry and clean were used to collect blood samples. This procedure was used to separate the serum at 4000 rpm for 15 minutes at room temperature (Sharma et al., ٢٠١٤). Before analysis, the serum was thoroughly extracted, put into antiseptic cuvette tubes, and frozen at -2٠ °C (Malhotra, ٢٠٠٣). Hematological analyzers were utilized to determine the CBC automatically using blood samples that were collected in EDTA tubes (Hussain et al., 2018)

Biochemical analysis

Another blood sample was collected from each rat without anticoagulant for serum separation. Serum was separated using centrifugation at 4°C for 10 min at 1000×g. Serum samples were collected in Eppendorf tubes and stored at -20°C until analysis. The collected serum was used for the estimation of liver function test: AST and ALT using an automated blood chemistry analyzer (Huang et al., 2006), alkaline phosphatase (ALP) (Lee et al., 2015), and lipid profile: triglycerides, total cholesterol, LDL and HDL (Jung et al., 2022).

Body organs (liver and tissues) samples and enzymatic analysis

Following blood collection, the anesthetized rats were humanely sacrificed, and the liver samples were promptly excised from each animal and weighed according to Mee-Young et al., (2013)'s formula., thoroughly rinsed with ice-cold saline, and instantly preserved at -20 °C for subsequent biochemical analysis. Another sample of the liver was used for histopathological examination. To prepare the homogenate, the

liver sample was weighed and homogenized in 0.1M phosphate buffer (pH 7.4) at a ratio of 10 % (w/v) and homogenized using Potter Elvehjem type glass Teflon homogenizer. The homogenate was centrifuged at $10,000 \times g$ for 20 min at $4^{\circ}C$ (Ashok and Sheeladevi 2014) and the supernatant was used for the determination of protein, GPx, and SOD. Protein level was measured in the liver homogenates according to Bradford (1976) using bovine serum albumin (BSA, 1- 60 μg) as a standard. The experiment involved protein solutions containing 10 to 100 μg of protein in a maximum volume of 0.1 ml. These solutions were pipetted into test tubes. The volume in each test tube was then adjusted to 0.1 mL using an appropriate buffer. Next, 5 ml of protein reagent were added to each test tube, and the contents were thoroughly mixed with a vortex mixer. The absorbance of the solutions was measured at a wavelength of 595 nanometers after 2 minutes and again before 1 hour using 3 ml cuvettes. A reagent blank was prepared using 0.1 ml of the same buffer and 5 ml of protein reagent. The absorbance readings were measured against this blank. Finally, a standard curve was created by plotting the weight of protein in each sample solution against its corresponding absorbance. This standard curve was then used to determine the protein concentration in unknown samples.

Determination of oxidative markers

Determination of glutathione peroxidase (GPx) activity in the liver homogenate

GPx activity was assessed by measuring the reduction of glutathione (GSH) using cumene hydroperoxide (CumOOH) as the substrate. The reaction produced oxidized glutathione (GSSG), which was then reduced back to GSH. The remaining GSH was quantified by reacting it with DTNB to form a yellow compound. Absorbance measurements were carried out at 412 nm. GPx activity was calculated using the formula:

$$GPx \text{ activity} = \frac{(Ac - As) \times 6.2}{13.1 \times 0.05 \times 10 \times 10}$$

Where (Ac - As) represents the difference in absorbance between the control and test samples. This method allowed for the precise determination of GPx activity in the samples. See supplementary file for detailed method (Paglia and Valentine 1967).

Determination of superoxide dismutase (SOD) in the liver homogenate

SOD activity was determined by monitoring the inhibition of pyrogallol auto-oxidation at alkaline pH, which generates superoxide anion radicals (O_2^-). SOD in the sample scavenges O_2^- , leading to the formation of hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). In a quartz cuvette, homogenate (test) or buffer (reference), and pyrogallol

were mixed with a buffer solution, and absorbance was measured at 420 nm after 30 and 90 seconds. The percentage inhibition of pyrogallol auto-oxidation by the homogenate was calculated, and SOD concentration in the samples was determined using a calibration curve. See supplementary file for detailed method (Marklund and Marklund 1974).

Histopathology

The liver samples were fixed in 10% neutral formalin and dehydration steps were followed in ascending grades of ethanol. The tissue cleared in xylene and embedded in paraffin wax. Sections (3-5 microns thick) were then stained with hematoxylin and eosin (H&E) to prepare for histological examination (Bancroft and Gamble 2008) and subjected to the light microscope for the histopathological examination.

Preparation and sensory properties of both chicken and Shrimp pops fortified with *H. tuberoses* extract

Chicken breast, raw shrimp, wheat flour, corn starch, rusk, table salt, spices, skim milk powder, sugar, black pepper powder, chicken egg, and vegetable oil were obtained from a local hypermarket in Alexandria, Egypt. Chicken and shrimp pops were prepared according to Monjurul et al., (2013) and Ismail et al., (2019). Ingredients and their percentage used in the chicken and shrimp pops are presented in Table (2). Chicken breast chunks and fresh shrimp meat were ground separately twice in a meat grinder (Moulinex - Model ME605131). After chopping the mixture separately, each of them was weighed and formed into pops (balls) 10 g weight and diameter ranging from 1.2 to 1.5 cm). The formulated pops were pre-dusted (with wheat flour and starch) then with batter (Table 3), covered with the mixture, and then covered with breadcrumbs. The pieces were fired in sunflower oil at a temperature of $175 \pm 5^{\circ}\text{C}$ for 5 min. Fried balls were drained on absorbent paper towels and allowed to cool to room temperature ($24 \pm 5^{\circ}\text{C}$) for sensory evaluation.

Table 2. Ingredients used for the preparation of both chicken and shrimp pop formulas as (g/100g).

Ingredients	Treatments (T)			
	T ₀	T ₁	T ₂	T ₃
Chicken breast meat/Shrimp meat	80	80	80	80
Wheat flour	8	8	8	8
Skim milk powder	8	8	8	8
Table salt	1	1	1	1
Spices mixture	2	2	2	2
sugar	0.7	0.7	0.7	0.7
Black pepper powder	0.8	0.8	0.8	0.8
<i>H. tuberoses</i> extract %	-	10	20	30

Table 3. Ingredients and their percentage used for batter preparation

Ingredients	Percentage (%)
Wheat flour	15
Corn Starch	5
Rusk	15
Sodium chloride	1
Spices	1
Chicken egg	19
Water	44

Sensory evaluation both of chicken and shrimp pops samples

The sensory assessment of fried chicken and shrimp pops was conducted by a panel of 30 experts from the Home Economy Department, Faculty of Education at Alexandria University, Egypt. Panelists were instructed to rate various characteristics, including appearance, taste, color, odor, tenderness, and general acceptability. The evaluation was carried out using a numerical hedonic scale, ranging from 1 (indicating very poor) to 9 (representing excellent), by the method outlined by **El-Anany et al., (2020)**. The assessments took place in individual sensory booths within the Food Sensory Laboratory. This study was conducted with approval from the Human Ethics Committee (Protocol approval number IACUC 0306304).

Statistical Analysis

Data analysis was conducted by employing ANOVA (one-way analysis of variance) with Duncan in SPSS® version 16.0. A statistical significance level (p-value) below 0.05 suggests a significant variance among groups (**Steel and Torrie 1960**).

Results and Discussion**Reducing power (EC₅₀) value of sample extracts**

The reducing power (EC₅₀) value of *H. tuberosus* extract (highest EC₅₀) was more effective than ascorbic acid (lowest EC₅₀) as shown in Table (4). Additionally, the reducing power (EC₅₀) values with *H. tuberosus* extract (44.05 µg/ml) significantly surpassed ascorbic acid (16.82 µg/ml). Furthermore, *H. tuberosus* extract exhibited substantially higher reducing power (EC₅₀) values than ascorbic acid. This aligns with a prior study showing that *H. tuberosus* extract contain the highest total phenolic content and demonstrated excellent free radical neutralization abilities (266.69 ± 2.51 mg GAE/g dry extract) (**Showkat et al., 2019**). *H. tuberosus* extract exhibited a significantly higher reducing power (EC₅₀) value compared to ascorbic acid (vitamin C). This indicates a greater ability of the extract to scavenge free radicals. This finding aligns with the reported high total phenolic content in *H. tuberosus* extract (**Showkat et al., 2019**). Phenolic compounds are well-known for their antioxidant properties. Their ability to donate electrons allows them to

neutralize free radicals, preventing cellular damage. The present study suggests that the high phenolic content in *H. tuberosus* extract is likely responsible for its superior reducing power compared to ascorbic acid. A study by (Yin et al., 2013) investigated the antioxidant activity of various vegetables and found that artichoke extracts exhibited strong reducing power due to the presence of phenolic compounds like cynarine. Similarly, another study by (Wang et al., 2010) demonstrated that mulberry leaf extract had a higher reducing power than ascorbic acid, which the authors attributed to the extract's rich phenolic profile.

Table 4. Reducing power (EC₅₀) value of sample extracts

Extracts	Reducing power EC ₅₀ (mg/ml)
Ascorbic	16.82±0.07 ^b
<i>H. tuberosus</i>	44.05±0.11 ^a

- EC₅₀ (mg/ml): effective concentration at which the absorbance is 0.5. Means in the same column followed by different lower-case letters are significantly different (p<0.05)

In-vitro hemolytic activity and (IC₅₀) value of *H. tuberosus* extract

In the current study, hemolytic activity and IC₅₀ values of sample extract were determined, and the results showed that increasing hemolytic activity and IC₅₀ values were dependent on concentration as shown in Table (5). When *H. tuberosus* extract was compared to aspirin as a positive control, it had the highest IC₅₀ value indicating a high safety profile. When *H. tuberosus* extract was compared to ascorbic acid, which had the lowest IC₅₀ values, it was found to be substantially more effective in scavenging free radicals. Previous studies have suggested a correlation between total phenolic concentration and overall antioxidant activity (Rahim et al., 2022). Furthermore, *H. tuberosus* extract exhibited substantially higher reducing power (EC₅₀) values than ascorbic acid. This aligns with a prior study showing that *H. tuberosus* extract contain the highest total phenolic content and demonstrated excellent free radical neutralization abilities (266.69 ± 2.51 mg GAE/g dry extract) (Showkat et al., 2019).

Table 5. In-vitro hemolytic activity and IC₅₀ value of *H. tuberosus* extract

Concentration (µg/ml)	5	10	20	40	60	80	100	200	400	600	IC ₅₀ (µg/ml)
% of Hemolysis	1.5	6.9	8.2	9.7	11.2	12.6	13.2	15.3	18.4	20.6	1456.7±0.1

Values are mean ± SD of 3 replicates. IC₅₀ (µg/mL): The extracted concentration of that causes hemolysis in 50% of RBCs.

Body organs weight (g) of rats in different studied rat groups

The findings indicated that rats in a control group (high-fat diet) had significantly increased liver and adipose tissue weights compared to the negative control group. However, treatment with *H. tuberosus* extract,

particularly at a dose of 200 mg/kg body weight, significantly reduced liver and adipose tissue weights compared to a control and reference drug groups as shown in Table (6). *H. tuberosus* is a good source of dietary fiber (Oszmiański et al., 2021). Dietary fiber promotes satiety and reduces overall calorie intake, potentially leading to weight loss and decreased fat storage in adipose tissues. *H. tuberosus* extract might influence how the body processes fat. It could potentially promote fat burning (lipolysis) and reduce fat synthesis (lipogenesis) in the liver, resulting in lower liver weight. Chronic inflammation is associated with obesity and can worsen fat accumulation in the liver and adipose tissues. *H. tuberosus* extract may possess anti-inflammatory properties that contribute to its weight-reducing effects (Liu et al., 2016). Studies on other dietary fiber sources support the findings of this research. For instance, a study by Guess et al., (2015a) demonstrated that inulin supplementation led to weight loss and reduced hepatic fat content in human participants. This aligns with the observed decrease in liver weight with *H. tuberosus* extract administration in the current search. Interestingly, the study found that *H. tuberosus* extract (200 mg/kg) was more effective in reducing liver and adipose tissue weights compared to the reference drug (Ator). This suggests that *H. tuberosus* extract might offer a promising alternative for managing obesity and its associated health risks.

Table 6. Body organs (liver and tissues) weight (g) in different studied rat groups.

Body organs	Weight (g)	
	Liver	Adipose
GP0	5.36 ± 0.21 ^d	2.5 ± 0.23 ^d
GP1	10.61 ± 0.27 ^a	8.5 ± 0.18 ^a
GP2	6.19 ± 0.33 ^c	3.07 ± 0.21 ^c
GP3	8.95 ± 0.25 ^b	5.93 ± 0.25 ^b
GP4	5.76 ± 0.19 ^d	2.25 ± 0.11 ^d
GP5	6.43 ± 0.28 ^c	1.76 ± 0.14 ^e

Values are mean ± SD of ten replicates. The means for the same ore followed by different lowercase letters are significantly different ($P < 0.05$). GP0 (negative control), GP1 (control positive), GP2 (Reference drug, ATOR), GP3 (*H. tuberosus* extract 100 mg/kg bwt), GP4 (*H. tuberosus* extract 200 mg/kg bwt), and GP5 (*H. tuberosus* extract 300 mg/kg bwt)

Oxidative stress

The results demonstrated that administration of the extract, particularly at doses of 100 mg/kg and 300 mg/kg body weight, significantly increased the activities of two key antioxidant enzymes, glutathione peroxidase (GPx) and superoxide dismutase (SOD), compared to the control and reference drug (ATOR) groups as shown in Table (7). The observed increase in antioxidant enzyme activity suggests

that *H. tuberosus* extract might work through several mechanisms: Enzyme Induction: The extract might stimulate the production of GPx and SOD by the body's cells. Free Radical Scavenging: As discussed earlier, the extract exhibits strong antioxidant properties. By directly scavenging free radicals, it could reduce the workload on existing antioxidant enzymes, allowing them to function more effectively. A study by **Amarowicz et al., (2020)** explored the antioxidant properties of Jerusalem artichoke tubers and found that they effectively increased GPx and SOD activity in human endothelial cells. Similarly, **Tang et al., (2017a,b)** reported that *H. tuberosus* extract displayed preventive effects against oxidative stress and inflammation in mice. Elevated oxidative stress is implicated in various chronic diseases, including heart disease, diabetes, and cancer. If future studies confirm the ability of *H. tuberosus* extract to enhance antioxidant enzyme activity in humans, it could potentially serve as a complementary therapy for managing these conditions. Studies by (**Amarowicz et al., 2020a; Amarowicz and Pegg 2020b; Biel et al., 2020a,b**) investigated the effects of Jerusalem artichoke tubers on oxidative stress markers in rats with colitis. The results showed that the extract administration significantly reduced oxidative stress markers like malondialdehyde (MDA) and myeloperoxidase (MPO) activity while also increasing the activity of the antioxidant enzymes GPx and SOD. (**Chen et al., 2018**) explored the antioxidant properties of Jerusalem artichoke extracts in vitro and found that they exhibited strong free radical scavenging activity and inhibited lipid peroxidation. These findings suggest the extract's potential to protect cells from oxidative damage. (**Li et al., 2016**) examined the anti-inflammatory and antioxidant effects of Jerusalem artichoke fructosyl oligosaccharides (JFOs) in mice with induced colitis. The study demonstrated that JFOs, a type of fiber abundant in Jerusalem artichokes, effectively reduced inflammation and oxidative stress markers while also enhancing antioxidant enzyme activity. As discussed earlier, *H. tuberosus* extract possesses strong antioxidant properties. This could protect red blood cells from oxidative damage, potentially contributing to improved function and lifespan, as noted in a review by **Chen et al., (2018)**.

Table 7. Antioxidant parameters in different studied rat groups

Sample code	GPx (U/g protein)	SOD (U/g protein)
GP0	45.63±0.18 ^d	0.89±0.01 ^b
GP1	18.32±0.12 ^f	1.45±0.01 ^a
GP2	52.40±0.13 ^b	0.58±0.03 ^d
GP3	40.91±0.10 ^c	0.62±0.01 ^c
GP4	51.02±0.14 ^c	0.49±0.01 ^c
GP5	59.02±0.14 ^a	0.41±0.01 ^c

Values are mean ± SD of ten replicates. The means for the same ore followed by different lowercase letters are significantly different ($P < 0.05$). GP0 (negative control), GP1 (control positive), GP2 (Reference drug, ATOR), GP3 (*H. tuberosus* extract 100 mg/kg bwt), GP4 (*H. tuberosus* extract 200 mg/kg bwt), and GP5 (*H. tuberosus* extract 300 mg/kg bwt).

Effect on hematological parameters (CBC)

The present study observed that *H. tuberosus* extract administration slightly increased various hematological parameters (CBC) in rats compared to the control and reference drug (ATOR) groups as shown in Table (8). This suggests a potential effect of the extract on red blood cell function. *H. tuberosus* is known to contain iron. Increased iron availability could stimulate hemoglobin production, leading to higher Hb and HCT levels. The extract might contain vitamins and other nutrients essential for red blood cell production and maturation, such as Vitamin B₁₂ and folic acid. As discussed earlier, *H. tuberosus* extract possesses strong antioxidant properties. This could protect red blood cells from oxidative damage, potentially contributing to improved function and lifespan. Limited research directly investigates the impact of *H. tuberosus* extract on hematological parameters. However, studies on other plant extracts rich in iron and antioxidants have shown positive effects on red blood cell health. *H. tuberosus* is known to contain some iron, though the exact amount can vary (Xu et al., 2017). Increased iron availability could stimulate hemoglobin production, leading to higher Hb and HCT levels. Additionally, research by Awika et al., (2009) explored the inulin content of Jerusalem artichoke tubers and its potential health benefits. Inulin is a prebiotic fiber that can stimulate the growth of beneficial gut bacteria, which may indirectly influence red blood cell health through improved nutrient absorption.

Table 8. Hematological parameters (CBC) in different studied rat groups

Hematological parameters	Control negative	Control positive	Reference drug (ATOR)	THE 100 mg/kg bwt	THE 200 mg/kg bwt	THE 300 mg/kg bwt
HB (g/dL)	12.8±1.3 ^e	17.5±0.9 ^a	12.8±1.8 ^f	14.8±0.8 ^c	14±0.4 ^d	15.5±0.6 ^b
HCT (%)	34±2.5 ^e	47.3±1.9 ^a	34±3.1 ^e	38±2.7 ^c	34.8±1.8 ^d	40.3±2.5 ^b
MCV (fl)	56.5±3.4 ^b	59.6±2.5 ^a	56.5±3.8 ^b	55.2±1.7 ^c	53.9±2.1 ^e	54.8±3.2 ^d
MCH (pg)	21.2±0.9 ^c	22±0.3 ^a	21.2±0.7 ^c	21.4±0.5 ^b	21.6±0.8 ^b	21±0.6 ^c
MCHC (g/dL)	37.6±1.1 ^c	36.9±0.8 ^d	37.6±0.5 ^c	38.9±1.2 ^b	40.2±0.2 ^a	38.4±0.7 ^b
RDW-CV (%)	13.4±0.01 ^b	13.9±0.05 ^a	13.4±0.02 ^b	13.3±0.04 ^b	12.4±0.01 ^d	12.8±0.07 ^c
Platelets (10 ³ /ul)	540±23.5 ^b	434±24.1 ^d	540±27.1 ^b	393±19.8 ^e	566±25.2 ^a	518±21.9 ^c

Values are mean ± SD of ten replicates. The means for the same ore followed by different lowercase letters are significantly different ($P < 0.05$). *H. tuberosus* extract (HTE), Haemoglobin (Hb), Haematocrite (HCT), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin), MCHC (Mean Corpuscular Haemoglobin Concentration), Red cell distribution width (RDW).

Effect on liver function parameter

Liver aminotransferase enzyme activity (AST and ALT) significantly increased in the control positive group compared to the control negative group. *H. tuberosus* extract-treated groups, especially at doses of 200 and 300 mg/kg b.w, exhibited significant decreases in AST and ALT activity compared to the Reference drug group (Ator), rats fed a high-fat diet containing extract from *H. tuberosus* showed a significant decrease in there with levels of HDL increasing and LDL, triglycerides, and cholesterol decreasing as indicated in Table (9). When compared to the reference drug group (Ator), the inclusion of *H. tuberosus* extract resulted in lower liver and adipose tissue weights. This was especially true at the dosage of 200 mg/kg bwt of *H. tuberosus* extract. These findings align with a more recent work (Yanru et al., 2023), where dietary inulin derived from *H. tuberosus* extract was found to significantly lower serum cholesterol levels in rats fed an inulin-enriched diet for three weeks, leading to notable reductions in serum glucose, triglycerides, and total cholesterol. *H. tuberosus* tuber, owing to its dietary fiber content, offers anti-obesity benefits when included in the diet of obese rats, and inulin supplementation in humans resulted in significant weight loss compared to control subjects, accompanied by reduced hepatic muscle fat content (Guess et al., 2015b; Oszmiański et al., 2021). These results collectively underscore *H. tuberosus* extract's efficacy as a functional food with anti-obesity properties, capable of mitigating cholesterol accumulation, and glucose levels, as corroborated by previous studies (Heier et al., 2021; Wang et al., 2020). *H. tuberosus* reduces blood LDL while increasing HDL in rats with hyperlipidemia (Amin et al., 2019). Santos et al., (2018) reported that *H. tuberosus*

extract reduces the atherosclerosis index and lipid profiles in hyperlipidemic patients.

Table 9. Liver function enzymes in different studied rat groups

Groups	AST	ALT	CHOLEST	TRIGLYC	HDL	LDL
GP0	63±0.01 ^f	82±0.00	122±0.01 ^f	164±0.01 ^e	51±0.00 ^c	40±0.01 ^c
GP1	238±0.05 ^a	84±0.01	420±0.00 ^a	503±0.01 ^a	40±0.01 ^f	279±0.02 ^a
GP2	188±0.01 ^c	92±0.01 ^a	186±0.01 ^b	464±0.01 ^b	48±0.01 ^e	41±0.01 ^d
GP3	235±0.01 ^b	89±0.02	124±0.01 ^d	189±0.02 ^c	53±0.02 ^b	39±0.02 ^e
GP4	170±0.01 ^d	66±0.01	123±0.01 ^c	168±0.01 ^d	49±0.01 ^d	38.5±0.01 ^f
GP5	67±0.01 ^e	83±0.00	130±0.00 ^c	136±0.01 ^f	63.5±0.01 ^a	41.7±0.01 ^b

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GP0 (control negative) GP1 (control positive), GP2 (Reference drug, ATOR), GP3 (*H. tuberosus* extract 100 mg/kg bwt), GP4 (*H. tuberosus* extract 200 mg/kg bwt), and GP5 (*H. tuberosus* extract 300 mg/kg bwt).

Liver Histopathological results

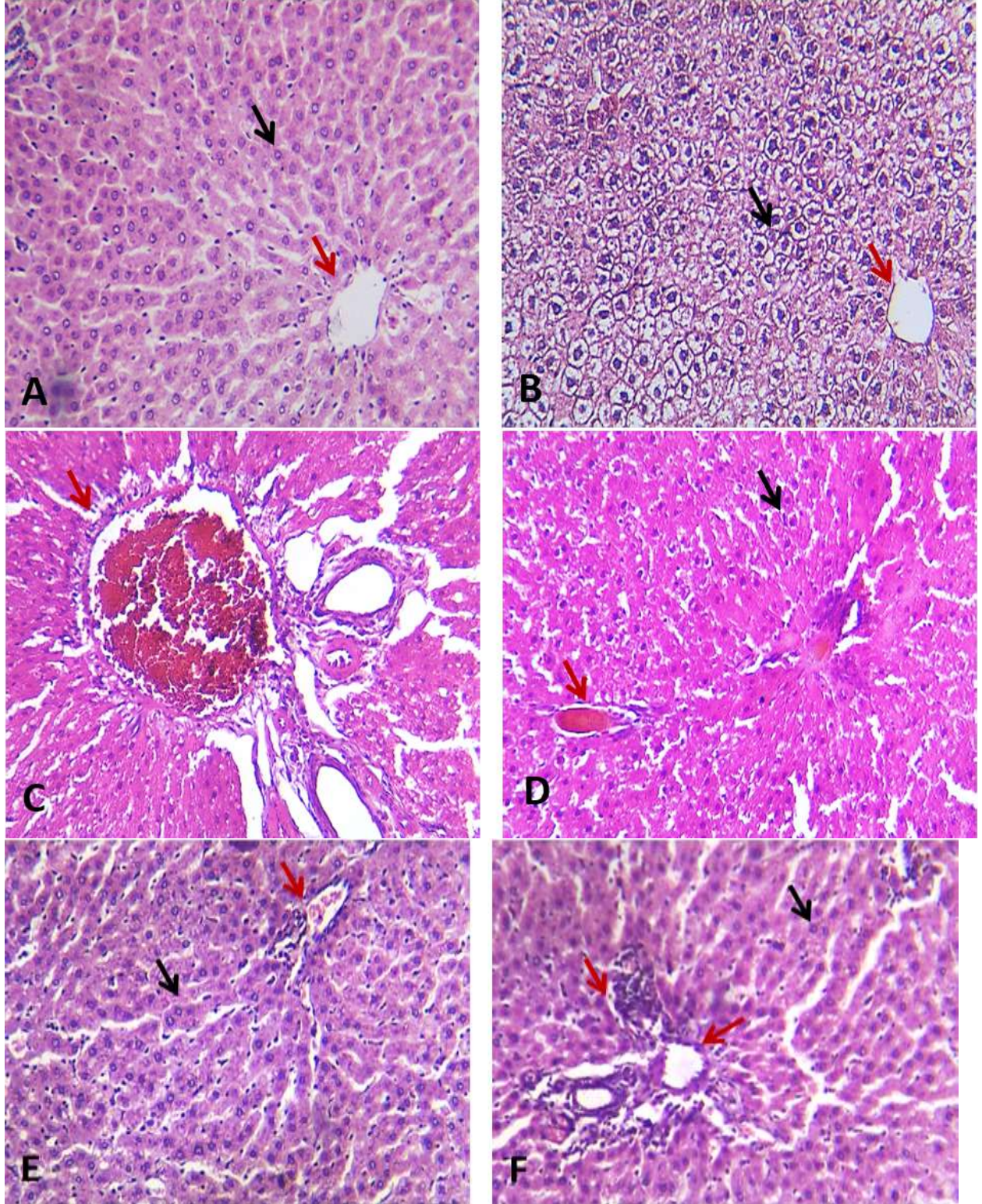
The histopathological examination and scores were shown in Table (10). Histological scores in the liver such as vacuolar and hydropic degeneration, hemorrhage, and necrotic hepatocytes disappeared in Group 4 and Group 5. Overall, all the histological scores were decreased in all studied groups as compared with the positive control. Furthermore, histological assessments of liver tissues revealed significant improvements in various scores, including vacuolar and hydropic degeneration, hemorrhage, and necrotic hepatocytes. These improvements were particularly notable in Group 4 and Group 5, receiving *H. tuberosus* extract at doses of 200 mg/kg b.w. and 300 mg/kg b.w., respectively. Overall, all histological scores decreased in Group 2 (Reference drug, ATOR), Group 3 (*H. tuberosus* extract 100 mg/kg bwt), Group 4 (*H. tuberosus* extract 200 mg/kg bwt), and Group 5 (*H. tuberosus* extract 300 mg/kg bwt) compared to Group 1 (control positive), as summarized in Table 10 and Figure 1.

Table 10. histopathological scores in different studied rat groups

Group s	Sinusoidal dilatation	Vacuolar and hydropic degeneration	Hemorrhage	Mononuclear cells infiltration (Inflammation)	Necrotic hepatocytes
GP0	+	-	-	-	-
GP1	-	+++	+++	-	+++
GP2	+	-	++	-	++
GP3	+	+	-	+	+
GP4	+	-	-	++	-
GP5	+	-	-	+	-

- There is no histological change. + Histopathology in less than 20% of fields. ++ Histopathology in 20 to 60% of fields. +++ Pathological anatomy in more than 60% of fields.

GP1 (control positive), GP2 (Reference drug, ATOR), GP3 (*H. tuberosus* extract 100 mg/kg bwt), GP4 (*H. tuberosus* extract 200 mg/kg bwt), and GP5 (*H. tuberosus* extract 300 mg/kg bwt).



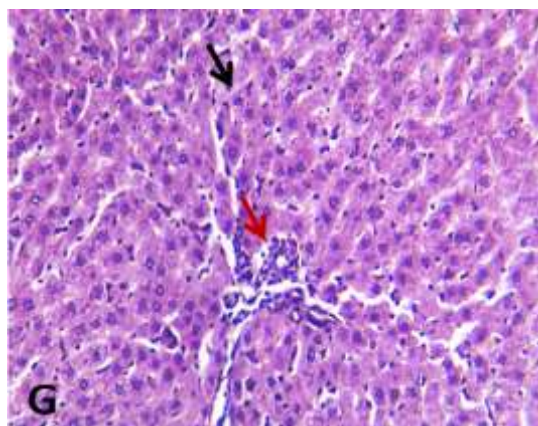


Figure 1. Hematoxylin and eosin (H&E) - Macular histopathology of liver samples from rats of the different groups studied.

Images (40x) show the following: (a) Group (1): showing normal hepatic cords with normal hepatocytes (black arrows), and clear central vein (red arrows) (b, c) Group (2): showing complete loss of Typical architecture, in which all hepatocytes show necrosis with atretic nuclei and severe aqueous and vacuolar degeneration (black arrows). In addition, there is a large degenerated area characterized by central lobular congestion and dilated portal tract congestion with hemorrhage (red arrows). (d) Group (3): Illustration of loss of normal architecture, with some hepatocytes displaying round nuclei (black arrows). The degenerated area is occupied by centrilobular congestion, crowded dilated portal spaces, and hemorrhages (red arrows). (e) Group (4): indicates loss of normal structure, as some hepatocytes suffer necrosis with hypnotic nuclei (black arrows). The focal area is infiltrated by mononuclear cells (red arrows), accompanied by a mild degenerating area characterized by centrilobular congestion, dilated portal tract congestion, and hemorrhage (red arrows). (F) Group (5): showing fairly normal hepatocytes with active dilated sinusoids between them (black arrows). A small degenerated area is occupied by infiltration of mononuclear cells (red arrows), with a clear central vein also visible (red arrows). (G) Group (6): characterized by normal hepatocytes (black arrows) along with a focal area infiltrated by mononuclear cells (red arrows) and a clear central vein (red arrows). (H & E X 40)

Sensory evaluation of chicken and shrimp pops fortified with *H. tuberosus* extracts

A sensory evaluation was carried out on the final product (Figure 2) to assess the influence of added *H. tuberosus* extract at 3 different concentrations on the following selected attributes: appearance, color, taste, odor, tenderness, and overall acceptability. Table (11) shows the sensory score for each of the attributes in all treated samples. All treatments exhibited favorable sensory attributes, including color, taste, odor, tenderness, appearance, and overall palatability, with panelist scores consistently above 6. There were no notable differences between all the treatments, whether in chicken or shrimp pops formulation. As food selection is a multifaceted process influenced by sensory and non-sensory factors (Lester et al., 2022), *H. tuberosus* offers valuable nutrients like fructose, inulin, and oligofructose (Shoaib et al., 2016). Inulin, present in

these artichokes, serves as a fat substitute, improving food texture (Menegas et al., 2013). It can enhance gastrointestinal health, calcium absorption, and impact physiological processes, potentially reducing the risk of diseases like cardiovascular disorders (Abed et al., 2016). In the food industry, inulin and oligofructose are favored as low-calorie and functional food ingredients (Jackson et al., 2023). With a rising focus on nutrition and calories, consumers increasingly seek healthy, innovative, and convenient food choices, leading to greater consumption of light and diet foods (Mihafu et al., 2020; Zhang et al., 2020).

Table 11. Sensory evaluation of chicken pops and shrimp pops fortified with *H. Tuberosus*

Samples	The Ratios	Sensory attributes of chicken pops					
		Appearance	Color	Taste	odor	Tenderness	Overall Acceptability
C. C.P	control	8.20±0.96	8.17±0.87	8.07±0.98	7.83±1.09	8.00±1.11	8.07±0.91
C.P. T.H	(10%)	8.22±0.75	8.33±0.88	8.10±0.76	8.00±1.05	8.07±0.98	8.13±1.01
C.P. T.H	(20%)	8.30±0.79	8.43±0.86	8.27±0.58	8.07±1.02	8.20±0.85	8.17±0.91
C.P. T.H	(30%)	8.47±0.86	8.53±0.82	8.30±0.75	8.23±1.01	8.27±0.91	8.33±0.88
F		0.590	0.993	0.674	0.761	0.475	0.447
P		0.623 ^{ns}	0.399 ^{ns}	0.570 ^{ns}	0.518 ^{ns}	0.700 ^{ns}	0.720 ^{ns}
		Sensory attributes of shrimp pops					
C.S.P	Control	8.00±1.17	7.90±1.06	7.83±1.39	7.43±1.22	7.70±1.09	7.97±1.09
S.P. T.H	(10%)	8.13±1.01	7.97±1.13	7.93±1.02	7.57±1.22	7.73±1.17	7.77±1.17
S.P. T.H	(20%)	8.17±1.02	8.03±1.03	8.00±0.91	7.60±1.28	7.83±1.09	7.90±1.09
S.P. T.H	(30%)	8.33±0.76	8.17±0.95	8.27±0.58	7.93±1.14	8.10±0.71	8.00±0.95
F		0.562	0.356	0.997	0.915	0.930	0.274
P		0.641 ^{ns}	0.785 ^{ns}	0.397 ^{ns}	0.436 ^{ns}	0.429 ^{ns}	0.844 ^{ns}

F.F test One-Way (ANOVA), Values are expressed as mean ± SD for each chicken pops and shrimp pops fortified with *H.tuberosus* samples. ns: Statistically not significant. C.C. P= Control chicken pops, C.P. T.H = chicken pops fortified with *H.tuberosus*. C.S. P= Control shrimp pops, S.P. T.H = Shrimp pops fortified with *H.tuberosus*.

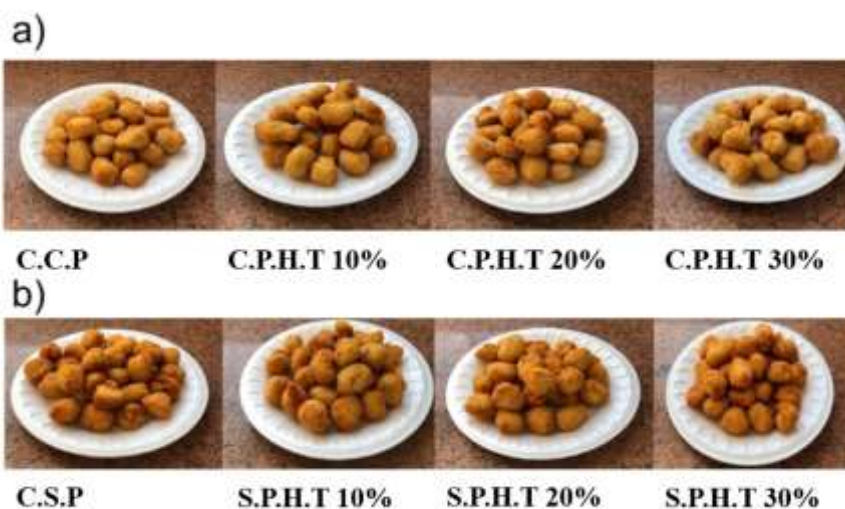


Fig. 2. Sensory evaluation of a) chicken and b) shrimp pops fortified with *H. tuberosus* extract (H.T).

Conclusion

This comprehensive study sheds light on the remarkable *H. tuberosus* potential in addressing a spectrum of health-related concerns, notably in the realm of obesity and metabolic health. Moreover, the study illuminates *H. tuberosus* extract's hepatoprotective prowess, with evidence of its effectiveness in mitigating oxidative stress, enhancing liver function, and improving histological outcomes. Lastly, the study reveals that the inclusion of *H. tuberosus* extract substantially enhances the sensory appeal and overall quality of chicken pops and shrimp pops products, making them more palatable and well-received. These findings collectively emphasize *H. tuberosus's* versatility and potential applications across various domains, including nutrition, marking it as a multifaceted and promising resource.

Ethics approval and consent to participate: The study was approved by Ethics Committee for the care and use of animals, microorganisms, and living cell cultures in education and scientific research (Protocol approval number IACUC: 82-3B-0223; and 0306304)

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