Research Article

Bicinchonic Acid Assessment for Improvement of Sperms Mitochondrial Functions by Ginseng Extract and Nanoparticales in Rats

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Abstract | The purpose of this study was to evaluate the impact of Ginseng extract and Ginseng nanoparticles on the level of total protein in testicular tissues utilizing the two assays of Western blotting and Bicinchonic Acid. A total of 60 Wister rats were chosen, prepared, and divided equally into three groups: T1, T2, and negative control, which received only distilled water daily and no other treatment. T1 was given a daily dose of (500 mg/kg) of Ginseng extract, while T2 received a daily dose of 250 mg/kg of Ginseng NPs. After a 60-day experimental period, all study animals were put to sleep using chloroform to collect testis samples, which were then homogenized and measured for protein content with a focus on the SDHA proteins. The results showed that, when compared to the value of the control group, the total protein concentration increased significantly in the Ginseng extracts and Ginseng NPs groups. However, there was no discernible difference in the values of the Ginseng extract or Ginseng NP groups. When compared to the control group, the Ginseng extract and Ginseng NP groups showed increased mitochondrial expression of the SDHA protein. Comparison of the values of the therapeutic groups showed that the Ginseng NP group had higher values than the Ginseng extract group. In conclusion, Western blotting proved to be an effective method for identifying and characterizing specific proteins. Additionally, BCA demonstrated a highly sensitive and straightforward immunoassay for detecting elevated complete testicular protein. However, isolating the active components in Ginseng and creating unique NPs based on one of these components can result in the development of novel medicines with distinctive properties. Additionally, there is a clear need for greater research on the enhancement of Ginseng NPs and correct regulation of genes associated to steroid genesis and mitochondrial biogenesis.

Keywords | Succinate dehydrogenase, Ginseng xtract, Bicinchonic Acid Assay , Sperms, Nanoparticales, Rats

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INTRODUCTION

The majority of sperms are composed of significant mitochondrial components. Anifandis and others, 2017. found a link between decreased sperm motility and aberrant sperm mitochondria, proving that mtDNA deletions and other changes can have an impact on sperm functionality and male infertility at the molecular level. Research has showed that, in comparison to control samples, sperm from non- zoo spermic samples has abnormally high levels of certain mtDNA and transcripts that encode mitochondrial proteins that are encoded by the nucleus (Nowicka-Bauer *et al*., 2018; Ferramosca *et al*., 2021). Additional significant discovery was the correlation

between Measurements of the quantity, vitality and activity of sperm as well as the function of enzymes found in mitochondria in sperm, particularly the mitochondrial ETC complex like succinate dehydrogenase (SDHA) (Zhou *et al*., 2019).The citrate SDHA The enzymes included in the Krebs cycle that are nuclear-encoded also showed greatest interactions among each of the investigated enzymes in arrangement as said by many readings (Ehinger *et al*., 2015; Beyramzadeh *et al*., 2017). Additional research revealed that animals lacking the antioxidant cytochrome with a testis- specific subunit produced significantly less sperm than did normal mice (Sinkler *et al*., 2017; Sun *et al*., 2018; Park and Pang, 2021). The effects of these inhibitors on sperm motility are detrimental to male fertility because several studies have shown that several ETC inhibitors may decrease the ability of the sperm mitochondria to move (Davila *et al*., 2016; Aitken, 2017). Ginseng is among valuable Chinese herbal medicine uses herbs. There are up to nine different varieties of ginseng, with the most common being Asian ginseng (*Panax ginseng*), American ginseng (*Panax quinquefolium*) and Japanese ginseng (*Panax japonicus*) which are all named after specific geographical regions. Ginseng has been shown to have a wide range of physiological effects, including those on the nervous, immunological, and cardiovascular systems. It has also been used to enhance sex performance. In countries in East Asia, ginseng. It has been put to use for several thousand millenia in the form of conventional medicine to heal ailments. It's got risen one of the herbs that has been utilized the most frequently during the past 30 years (Yu *et al*., 2017). The distinctive bioactive elements of ginseng are ginsenosides, which are triterpene saponins. But ginsenosides are not the only part of ginseng that has therapeutic properties. The compound's active component, gonintin, was just recently identified (Shao *et al*., 2018; Lee *et al*., 2018; Cho *et al*., 2019). However, the majority of pharmacological and medical studies on ginseng have focused on ginsenosides. He *et al*. (2018) claim that Throughout worldwide. Ejaculated sperms incubated with ginseng extract exhibited significantly higher sperm counts than those treated with a vehicle, according to studies (Wiwanitkit, 2005). In contrast to freshly harvested, untreated, thawed sperm, The movement of sperm or integrity of the membrane are greatly improved after therapy by ginsenoside Rg1 (50 g/ ml) (Kim *et al*., 2013). These findings suggest that the addition of ginseng extract to the cryogen used to preserve sperm could increase fertility. Due to polymeric nanoparticles' (PNPs) increased stability, effectiveness, and security of administering drugs locally and systemically and promote Increased availability is made possible by efficient passage through membrane barriers, and therapeutic location selection is made possible by a change of the surface characteristics (Mittal *et al*., 2019).

MATERIALS AND METHODS

Preparation of ginseng extract and NPs

500g of American ginseng herb was purchased from a powder from a pharmacy store in the city of Babylon, Hilla, and the herbal material was worked on at Al-Qadisiyah University, College of Veterinary Medicine. Where the work was done by taking 100g of the powder of the American ginseng herbal substance and placing it in the thumble of the Soxhlet apparatus in the presence of ethanol (70%) and in this way the American ginseng was extracted. This process is repeated from (10-12) times for two days. After completing the process, the material was filtered by filter paper, then the material was placed in a plate and evaporated with the evaporation device for 2-3 days until the extract was dried. After the fumigation process, the herbal material American ginseng, where the extract of the herbal material was prepared, where it was dissolved in BBS phosphate solution, and the material was placed in the Vortex Mixer for five minutes, The American ginseng extract which prepared in the previous step were dissolved in BBS phosphate solution, and the material was placed in the Vortex Mixer for five minutes, and then it was placed in the Intellingnt ultrasonic processor using a device of UP200ht at a temperature of 50 °C for a period of 30 minutes, a solution was obtained Research to convert the solutions in to Nano sizes .After that stored in 4 °C until use (Aldulemy *et al*., 2021).

Characterization of nanoparticales

UV–visible spectroscopy

The absorbance of the produced nanoparticles was measured using UV-visible spectrophotometer at wavelengths ranging from 200 to 800 nm with 1 nm wavelength interval (Agilent-Cary 60, United States).

Scanning electron microscopic analysis (SEM)

The form, surface morphology, Size of the nanoparticles were determined using scanning electron microscopy. To summarize, the produced nanoparticle dispersion was air dried before being loaded into sample containers. The sample was then coated with gold using a sputter, coater in a vacuum, and images were obtained using a SEM at 20 kV and various magnifications.

Infrared (FTIR) fourier transform spectroscopy

The chemical composition was determined using Fourier transform infrared (FTIR) spectroscopy. It is one of the methods used to characterize nanoparticles by gathering information on the maximum values in particular ranges of surface activity, determining the inhomogeneous nature of the absorption and reflection spectra, and interpreting the information using the wavelengths that are present in the

spectrum. The actual through sampling is done by Fourier transform (a mathematical operation), examining the vibrational structures of the materials Also, to determine the structure of the solid is done using the FTIR technique (Chen *et al*., 2015). And FTIR is used Crystallinity and nanoparticle sizes (Kumar and Kumbhat, 2016).

X-ray diffraction (XRD) analysis (XRD)

X-ray diffraction is a widely used technique as a useful characterization tool for a study, The chemical and crystalline composition as well as the physical properties of the material are non - destructive. And you use Also to characterize the crystal structure of Alicia. XRD analysis was performed to detect the composition (Zhang *et al*., 2016) for the crystalline compound.

Study design and sample collection

Sixty Wister rats were randomly selected and subjected for preparatory period; during which, they kept at 23 ± 2ºC, fed on basal laboratory diet and tap water, and exposed to 12/12 light and dark conditions. After one week, the study animals were equally divided into three groups as following:

1. First group (Negative control group): Animals of this group remain without any treatment and received only daily distilled water and give (2ml) from phosphate buffer solution (PBS)
2. Second groups (T1): Animals of this group were treated by Ginseng extract received (500 mg/kg) (2ml) orally by stomach tube for 60 day.
3. Third groups (T2): Animals of this group were treated by Ginseng NPs received (250mg/kg) (2m) orally by stomach tube for 60 days (Kamel *et al*., 2019).

At the ending of an experimental period, all study animals were a euthanized with chloroform and subjected for collection of testis tissue samples.

Bicinchonic acid assay

The total protein contents in homogenized testicular tissues were measured using the Bicinchoninic Acid Assay Kit (Elabscience, China) according to the manufacturer's instructions. A standard curve for bovine serum albumin (BSA) was created using BSA dilutions in RIPA lysis Buffer according to manufacturer's instructions. All samples were diluted using RIPA Lysis Buffer. Duplicate BSA or sample lysates were pipetted into plate. BCA reagents were used and from the mixture were added to each well of a well plate. The well plate was covered with aluminum foil, rocked using a plate shaker, and then incubated. After the incubation time, the absorbance value Optical Density (OD) of each well was measured using a Microplate reader at 562 nm. The linear standard curve of mean absorbance (two values) of BSA was used to measure the concentration of protein in the samples.

Western blotting assay (SDS-PAGE)

Protein Sample Preparation (Extraction of Proteins from Tissue Samples). carefully wash the tissue with pre-cooled PBS Buffer ((0.01 M) to (pH 7.4)) and remove any debris. The tissues should be weighed and broken up before being lysed with (RIPA) Lysis Buffer (E BC R327) at the proper ratio (add (10 L) PMSF and (10 L) Na 3a VO 4 to each (1mL) to RIPA Lysis of Buffer).-According to the tissue weight ratio, homogenization is advised: RIPA Lysis Buffer volume = 3:10. To 0.3 g of tissue the specimen, add 1 milliliter of RIPA Lysis Buffer; the precise volume can be adjusted to suit the needs of the experiment. Shakeandl yseonice for (30) minutes homogeneity. To completely lyse the cells and reduce the sample's density, sonicate sample for 1 minute (in an iceewater bath) using 2 s of sonicat and 2 s of spacing time. A centrifuge at 4 °C for 10 minutes at 12,000 revolutions per minute (rpm). Using the BCA technique, measure the protein level in the dregs. Electrophoresis based on molecular weight of the target protein prepare an appropriate separation gel. In a reserve well, add 5 L of Precision Plus Protein TM Dual Color Standards Marker (161-0374, Bio-Rad, UK) to confirm target molecular weights and the degree membrane of transfer. With Electrophoresis Buffer (EBCR331) begin with the electrophoresis process.45 minutes of electrophoresis at 100 volts while the samples are in the stack gel followed by 50 minutes at 150 volts when the blue wave enters the gel that separates the samples. Bromophenol blue electro phonetically migrates across the gel in around 1.35 hours. Transfer Membrane (Wet Transfer) Depending on the molecular r weight of the target proteins use the (PVDF) Membranes (EBCR266) with a 0.45 m pore size. The PVDF Membrane must first be activated by soaking in methanol for one minute before being placed in transmembrane Buffer (EBCR333). Additionally, the Transmembrane Buffer must be pre-soaked with fiber mat and filter paper before usage. Put the parts in the following sequence: (negative electrode) black plate, fiber mat, filter paper, and gel PVDF The wet transference tank contains the membrane filtration paper fiber mate white plate(positive electrode) in the following order discharge bubbles, clamp, and place in wet transfer tanks. Depending on the molecular mass of the chosen protein, modify the transmembrane conditions. Make sure that low temperatures are used during the transmembrane procedure. Following the transmembrane, gently remove the (PVDF) Membrane and washed with (TBST) Buffer for a minut. Incubation of Antibodies (Ab):-Soak the PVDF The membrane for 1.5 hours at room temperature in TBST Buffer (EBCR335) containing 5% bovine serum albumin (BSA) as a blocking buffer. Incubate Overnight at 4 °C, soak the (PVDF)* Membrane in the primary antibody working solution. and then give it a gentle shake. The primary antibody should be diluted as directed in

the antibody manual (Anti-SDHA Antibody and Beta Actin; 0.5L: 1mL BSA). Using TBST Buffer, wash the PVDF Membrane three times for 15 seconds each. Use a TBST Buffer containing 5% BSA to dilution the secondary anti body's (EAB 1003, EAB c1001) at a ratio of 1:5000 in accordance with the recommended secondary antibody concentration. Incubate at room temperature for an hour off a shakers (PVDF) Membrane Cleaning on three occasions (15 minutes) each with TBST Buffer. Detection: (EBC R347) excellent chemiluminescent substrate detection kit contains the following components: combine A and B in a (1:1) ratio as a working liquid. Take the (PVDF) Membranes out of the (TBST) Buffer and immerse it with a filter papers in the solution. Place the (PVDF) Membrance within the X-ray cassette, continuously saturate it with the ECL work solution, expel the air bubble, and detect the result using X-ray film. 3. To get the best photograph, adjust the exposure time.

Statistical analysis

All data come from at least three independent experiments. Values were represented as per (Mean ± Standard Error (M±SE)). Satiating significance be there calculated using SAS using the one way ANOVA. Differences were considered significant atP< 0.05 (*) and P<0.01 (**) considered significant (Stroup*et al.*, 2018).

RESULTS AND DISCUSSION

Characterization of ginseng NPs

The Araliaceae family includes the perennial plant ginseng. In the current study, we efficiently and affordably biosynthesized gold nanoparticles utilizing ginseng aqueous extract. The analysis includes UV_V is spec HR, TEM, XRD, FTIR and SAED. analyses were used to evaluate the produced ginseng gold nanoparticles (GNPs). The surface plasma on resonance peak at (538 nm), is observed was detected by UV-Vis spectroscopic investigation, and it doesn't fade until 30 days had passed since incubation. The produced gold nano particles have a shape is spherical crystalline nature are varying sizes are confirmed by the result of (HRTEM XRD and SAED). According to FTIR data, the biological elements in ginseng reduced gold ion to create gold nanoparticle.

UV-visible spectroscopy analysis

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path length and loaded with UV probe software was used for the recording of spectra and measuring absorbance for method development and validation study.

The wave length of nanoparticles were measured from 200 to 1100 nm for wave length by recording UV-visible spectrum of prepared nanoparticales solution. Maximum absorbance (λ max 1.372) was shown at 245 nm Figure 1.

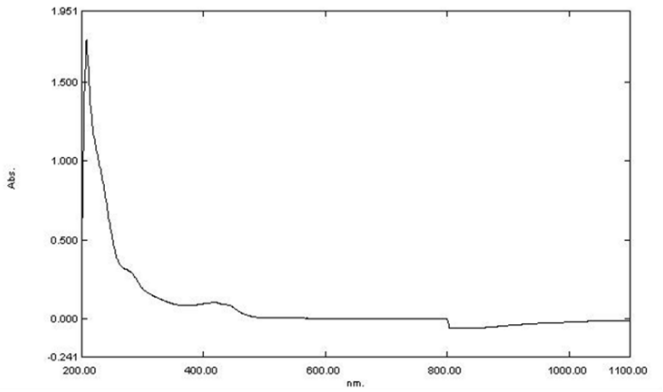

Figure 1: Maximum absorbance (λ max 1.372) was shown at 2.

SEM analysis

The morphology of nanoparticles was also confirmed in terms of crystallite size utilizing scanning electron microscopy (SEM). The SEM images shown in Figure 2. Exhibited those particles have spherical shape with a smooth exterior. The average diameter of the obtained NPs evaluated by SEM was 98.24 nm at range from 49 to 132 nm.

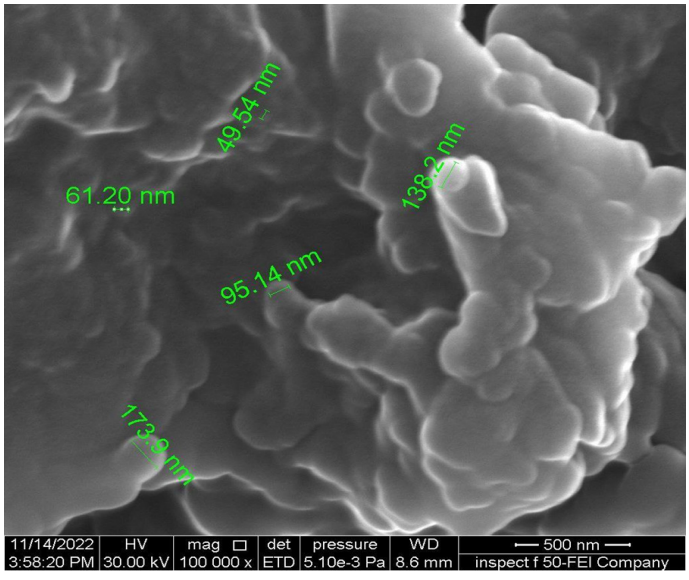

Figure 2: SEM of nanoparticles of ginseng appear round to oval shape of nanoparticles with particle size around 100 nm associated with, including cm^{-1}.

The analysis of ginseng nanoparticles was conducted using FTIR (fourier transform infrared spectroscopy)

Fourier Transform Infrared Spectroscopy (FTIR) spectroscopy of ginseng exhibited the presence of bending vibration of the aliphatic CH bond at the peaks 781.70 and 2919.18cm^{-1}. While the wave numbers 977.80, 963.09,

1577.34, 1540.23 and 1510.24cm^{-1}, which are may be attributed to the stretching and blending of the double carbon bond of the C=C. As for the peaks at numbers 1627.76, 1602.58, 1281.88 and 1233.44 cm^{-1}, they may be due to the presence of a vibrational bending of C=O and C-O, due to the aromatic amine group, respectively. As OH was found at the wave numbers 3778.20, 3687.97, 3658.73, 2956.30, 2919.18, and 2850.02cm^{-1}, was attributed to the carboxylic groups. According to the above, the FTIR spectrum of pure extract of ginseng showed a number of peaks, including the wave numbers 1627.76 and 1602.58 cm^{-1} that are attributed to the CH, in addition, there are other groups in the solution that confirm the presence of ginseng molecules associated with, including cm^{-1}, respectively Figure 3.

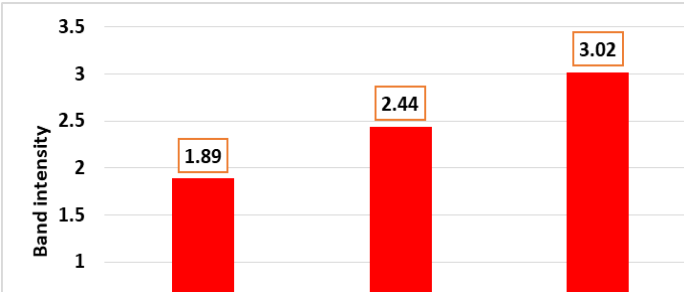

Figure 3: showed that FTIR spectra of ginseng nanoparticls.

The text describes an X-ray diffraction analysis

The Paul Scherrer equation is utilized for determining the size of powder crystals, expressed as Dp = (0.94 X λ)/ (β X Cos θ). The average crystallite size (Dp), line broadening (β), Bragg angle (θ), and X-Ray wavelength (λ) are all important factors to consider. The present data of XRD Ginseng recorded four main peak 2 Theta at 26.231, 42.048, 54.374 and 77.229 with their FWHM 0.363, 0.777, 0.767 and 0.572 to recorded size at 22, 11 and 15 nm, respectively Figure 4.

Bicinchonic acid assay

The total protein concentration data revealed a substantial increase ($p<0.05$) in values of both Ginseng extracts. (2.44±0.07) and Ginseng NP (3.02±0.05) groups when compared with the value of control (1.89±0.05) group. However, no significant variationwas found between values of both Ginseng extract and Ginseng NP groups Tables 1, 2 and Figure 5.

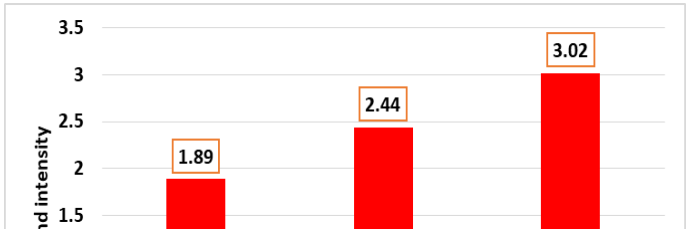

Figure 4: A very broad peak.

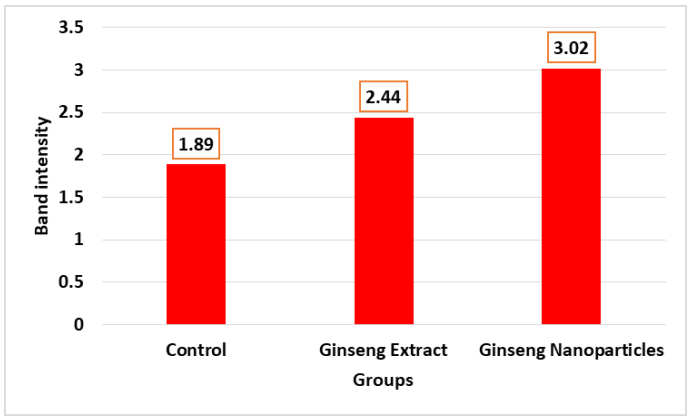

Figure 5: SDHA/B-Actin Right testis tissue in Nano, extract and control.

Western blotting assay

The results of the current study showed the level of SDHA protein in Ginseng extract group and Nano group compared to non treated control group. SDHA protein increased significantly in both treated group in compare with control group. Moreover, theGinseng nanoparticls goup showed highly significant increased (3.029 ± 0.058): $P < 0.0001$) in level of SDHA compared with Ginseng extract group(2.443 ± 0.079): $P < 0.0001$), respectively (Figure 6A, B, C). The image showing the level

Table 1: Total protein content in homogenized tissue in Ginseng Nanoparticles and other group.

Standard concentrations of BSA1 (mg/mL)	2 Reading data (replication) absorbance unite (nm)		Samples codes	2 Reading data (replication) Absorbance unite (nm		Final concentration of total protein (µg/µL)
2	1.135	1.191	C1	3.129	3.331	16.9
1.5	0.904	0.96	C2	3.303	3.346	15.3
1	0.672	0.733	C3	1.838	1.948	25.1
0.75	0.501	0.546	E1	3.225	3.111	20.8
0.5	0.431	0.527	E2	2.921	3.095	21.4
0.25	0.298	0.361	E3	3.151	3.234	17.4
o.125	0.192	0.246	N1	3.156	3.247	13.8
0.025	0.157	0.183	N2	3.107	3.026	19.6
5	0.121	0.15	N3	3.001	3.281	18.1

[1]Bovine serum albumin standard concentrations. [2]Absorbance reading using Micro plate reader at 562 nm wavelength.

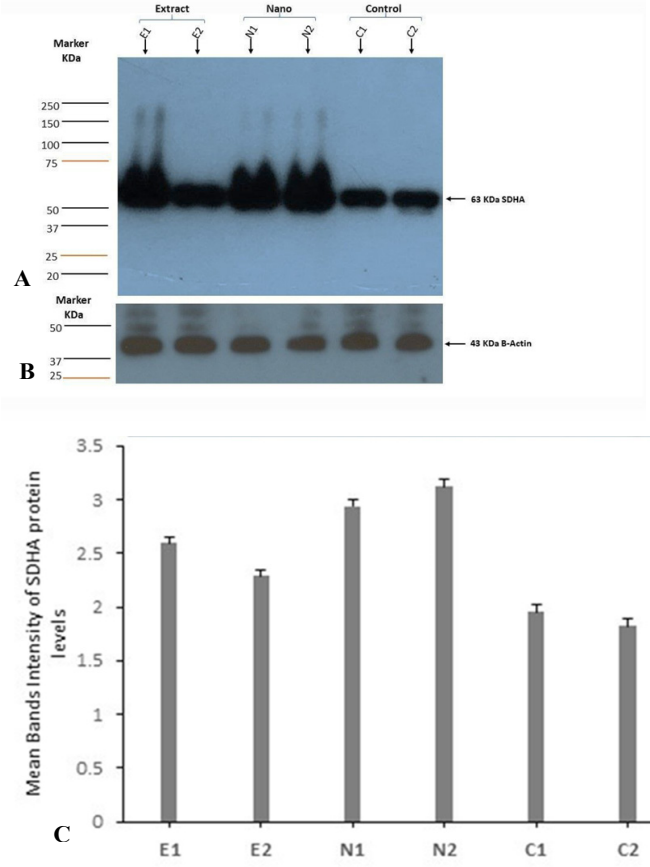

Figure 6: Mitochonderial expretion of SDHA protein in treated group with extract (E1 and E2) or nanoparticls (N1 and N2) compeared to non treated group (C1 and C2) of mature wister male rat epididmus during spermatogenesis.

Table 2: SDHA/B-Actin concentration in ginseng Nanoparticles and other group.

Group	Conc- (Mean ±SE)
Control	1.89±0.05A
Ginseng Extract	2.44±0.07B
Ginseng Nanoparticles	3.02±0.05C
P Value	<0.0001
LSD(P<0.05)	0.192

Different letters between any two means denote to the significant difference at P<0.05

of SDHA protein in extract group and Nano group compared to non treated control group. SDHA protein increased significantly in both treated group in compare with control group. (B) The image showing the level of B-Actin as a housekeeping gene that presents the equelizing of pipetting error and non effecting of cell homeostasis during treated process for all animals of experiment. (C) The bar chart showing the columns of means, which are based on band intensity analysis data for each group.

UV-visible spectroscopy analysis

For the method development and validation study, a double beam UV-visible spectrophotometer (Shimadzu, model 1800) with two matched quartz cells with 1 cm light path length and loaded with UV probe software was used to record spectra and measure absorbance. recording spectra and measuring absorbance. Wave length of nanoparticles was measured (Gibis *et al.*, 2014).

SEM analysis

The morphology of nanoparticles was also confirmed in terms of crystallite size utilizing scanning electron microscopy (SEM). exhibited those particles have spherical shape with a smooth exterior (Olatunad *et al.*, 2020).

The analysis of ginseng nanoparticles was conducted using FTIR (Fourier Transform Infrared Spectroscopy)

The FTIR spectra for ginseng showed appear new the study uses FTIR results to predict the chemical and biological activities of biomolecules, providing insights for effective plant product use. Exhibited the characteristic bands at 1384.04 and 1224.17 cm-1 indicating the presence of N-H stretching aliphatic primary amine group, at 2959.01 cm-1 presence of strong broad N-H stretching amine salt. Moreover, the carboxylic acid O-H was confirmed at 1384.04 cm-1. While strong - C-O for primary alcohol and sulfoxide was recorded at 1056.99 and 1033.85 respectively. The presence of esters (S-OR) group at 703.77 cm-1 (Pimentel-Moral *et al.*, 2019).

The text describes an x-ray diffraction analysis

In common, the material in crystalline state appears The XRD analysis revealed the crystalline nature of ginseng extracts within the liposome, indicating better solubility in aqueous medium. The ginseng extracts were found to be interposed between phospholipid bilayer chains, enhancing flow ability (Rasmussen *et al.*, 2020).

Bicinchonic acid assay

It should be highlighted that the detection of- producing proteins of normal spermiogenesis, suggesting a potential target pathway for further investigation. In seminal plasma, the cytoskeleton and intracellular proteins have been seen in earlier investigations (Pilch and Mann, 2006; Batruch *et al.*, 2012). These proteins may come from spermatogenesis, epididymal fluid, or prostasomes, secretory vesicles seen in seminal plasma (Gerena *et al.*, 1998; D'Amours *et al.*, 2012; Pilch and Mann, 2006). Which have been demonstrated to transport particular proteins to spermatozoa in the male reproductive tract and so modify sperm activity, are particularly abundant in the epididymal fluid. Ginseng appears to enhance sperm quality in guinea pigs exposed to dioxin, according to recent research (Hwang *et al.*,

2004). The results of numerous clinical experiments to increase sperm quality and quantity have been mixed (Chaney, 1979). In the current study, ginseng-treated rats had considerably higher epididymis sperm counts than the rats in the control group. The use of ginseng also increased sperm motility, although it had little effect on testicular or body weight. This shows that ginseng might be suggested as a therapy for male infertility linked to spermatogenesis. Expanding the therapy options for male factors in infertility might benefit from a mechanistic knowledge of how ginseng promotes spermatogenesis (Park *et al*., 2007). According to reports, oxidative stress contributes to infertility in people with UMI (Doshi et a., 2015; Mayorga-Torres, 2014) and has an impact on spermatozoa function. According to reports, sperm protein expression levels are changed when under ongoing oxidative damage examined by (Agarwal *et al*., 2014) Canonical pathways like Cellular compromise, cell death, and survival are all interconnected processes. We have previously shown that infertile males has a significant reactive oxygen species content have variable expression of proteins related with oxidative stress, protein changes, and energy consumption and control (Sharma and Kiran, 2013). Additionally, in spermatozoa under oxidative stress, the proteins linked to the antioxidant system, such as peroxiredoxin, are under expressed (Sharma and Kiran, 2013; Hamada *et al*., 2012). The process of fertilization depends heavily on sperm proteins that are specifically relevant to reproductive function. The study on infertile males with normozoospermia revealed that several proteins required for fertilization were under expressed (reviewed by Bracke *et al*., 2018). In patients with in vitro fertilization failure, Li *et al*. (2018). The study has established a connection between the fertilization process and (ACRBP (acrosin-binding protein)) and (ZPBP1 (zona pellucida- binding protein)) (Liu *et al*., 2018).

Western blotting assay

By controlling redox proteins in elderly rats Korean red ginseng, also known as Panax ginseng Meyer, is a type of ginseng native to Korea has been shown to improve testis efficiency Sperm maturation is a crucial process in the reproductive cycle. The purpose of this study was to see Korean red ginseng water extract (KRG-WE) regulates changes in enzymatic and non-enzymatic antioxidants in aged rats' testes, affecting spermatogenesis, sex hormone receptors, and enzymes controlling oxidation, histone deacetylation, and growth-related activities. Additionally, the enzyme-modified *P. ginseng* (Pectinase-treated) reduced the oxidative stress caused by hydrogen peroxide in GC-2 sperm cells, as well as altered expression of testicular genes in elderly rats (Kopalli *et al*., 2015). Ginseng nanoparticles demonstrated a noticeable improvement in mitochondrial enzymes through increased intestinal absorption of extract as well as highly cellular uptake by tissue, prolonged circulation with sustained released the statement is currently inactive. compound of extract, and synergistic interaction between extract to potentiate the body immune system with improved normal function. On the other hand, immunohistochemical analysis of SDHA in Ginseng semen corroborated the notion of a considerable rise in SDHA gene expression and protein expression of mitochondria in rats given red ginseng. The NPs group found 95% of semen in the epididymis lumen, and SDHA was significantly expressed in every epididymal epithelial cell in the study by Wang *et al*. (2020). Compared to the group of Ginseng extract semen, where SDHA appears to be involved in approximately. It has been discovered that Spermato motility and, consequently, fertilization potential are connected with Spermato mitochondrial activity. Additionally, a number of studies have shown in which the mitochondrial tablet directs a wide range of waving proteins to the average-piece and may thus be essential for spermatozoa function (Lu *et al*., 2017; Khalid *et al*., 2020; Gallo *et al*., 2021). The study found that Ginseng extract and Ginseng NPs significantly increased SDHA protein levels, as confirmed by the western blotting assay results. Italiano *et al*. (2012) noted a decrease in SDHA protein expression in tumor cases compared to non-tumor instances, stating that this was to be expected given that tumor-related mutations can result in shortened SDHA proteins. The SDHA gene encodes the primary catalytic subunit, and germline mutations in SDHA are linked to neurological conditions like early- onset encephalopathy, ocular atrophy, ataxia, and myopathy (Ghaoui and Sue, 2018; Di Donato, 2009). Ginsenosid is the primary active component of ginseng. Ginsenoside Rb1 controls ROS generation and levels in mitochondria to control mitochondrial activity (Li *et al*., 2012; Zhou *et al*., 2019). According to research, Lycium barbarum polysaccharides greatly enhance mitochondrial activity, plasma membrane integrity, and sperm survival (Zheng *et al*., 2022). By blocking the antioxidant action of the caspase pathways, angelica polysaccharides decrease apoptosis and ROS, safeguard mitochondrial integrity, and enhance energy metabolism (Zhuang *et al*., 2020). The primary active component isolated from Epimedium, Icaridin is frequently used to treat male erection problems. By promoting cell proliferation and obstructing mitochondria-dependent apoptotic pathways, as well as by exhibiting protective actions against testicular dysfunction, it cures sperm abnormalities in rats (He *et al*., 2021). According to Yin *et al*. (2010) psyllium polysaccharide may be helpful as a natural antioxidant. According to Shin *et al*. (2011) Cuscuta sinensis is a TCM herb with liver and kidney tonic qualities, can enhance kidney function. According to Loh *et al*. (2010) the active ingredient in *Leonurus japonicus* leonurin can suppress the synthesis of

mitochondrial ROS and restore mitochondrial function and redox state. Curculigo orchioides improves sexual dysfunction brought on by excessive sugar levels and supports Yang; it also increases sperm parameters and count in rats (Thakur *et al*., 2012). TCMs frequently have several components, multiple potencies, multiple targets and multiple action pathway.

CONCLUSIONS AND RECOMMENDATIONS

By utilizing the Western Blotting and Bicinchonic Acid tests, this work offered fresh insights into the mitochondrial expression of SDHA proteins. Both ginseng extract and ginseng NP treatment indicated a considerable protein expression, with ginseng NP use in particular showing no adverse effects during the course of the trial. However, isolating the active Ginseng components and creating specialized NPs based on one of these components can result in novel treatments with distinctive properties. It is also important to continue researching the enhancement of ginseng NPs and proper control of genes associated to steroidogenesis and mitochondrial biogenesis.

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NOVELTY STATEMENT

The novelty of the study is pocus on physiologically activity of Ginseng Nanoparticales in improvement of rats sperms mitochondrial function which means possibility for improvement of sperms functions and then increase animal reproduction.

AUTHOR'S CONTRIBUTION

These authors each contributed equally.

Ethical approval

The current study was conducted with the permission of the Departments of Physiology, Biochemistry, and Pharmacology. College of Veterinary Medicine, Al-Qadisiyah University (Al-Qadisiyah, Iraq).

Conflict of interest

The authors have declared no conflict of interest.

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