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Improvement of Mitochondrial Function of Pituitary Gland of Male Rats by Hydroxytyrosol nanoparticles

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ABSTRACT:

Background :Mitochondria are ubiquitous organelles in eukaryotic cells whose primary function is to generate energy supplies in the form of ATP through oxidative phosphorylation. As the entry point for most electrons into the respiratory chain, NADH:ubiquinone oxidoreductase, or complex I, is the largest and least understoodcomponent of the mitochondrial oxidative phosphorylation system.Hydroxytyrosol (HT) is a major polyphenolic compound found in olive oil with reported anti-cancer and anti-inflammatory activities.

Objectives: identification of neuroprotective effect of HT as well as effect on histopathological study on pituitary gland in rat.

Methodology:In the present study,After 8 weeks of HTextract and nanosespension administration at doses of 50 and 50 mg/kg, the histopathology of pituitary glandmicroscopy observations did not reveal any morphological alteration in the tissue examined.

Results: There were no differences between controls and test item treated animals. Our observations suggest that HT improves mitochondrial function through activation of the AMPK pathway in the pituitary gland in rat.

Conclusions: Hydroxytyrosol (HT) has neuroprotective effect study,hydroxytyrosol and their nanoparticles has not pathological changes on pituitary gland as well as that HT improves mitochondrial function through activation of the AMPK pathway in the pituitary gland in rat.

Keywords : Hydroxytyrosol, nanosespension, Mitochondria, polyphenol

INTRODUCTION

Mitochondriaare organelles exist only in the cytoplasm of the cellsrequirements of adenosine triphosphate (ATP), which is regarded as fuel of the cell, the number of mitochondria varies depending onthe amount of energy consumed by thatcell(McInnes, 2013).Hydroxytyrosol phenol occurs in a natural way in olive (Vilaplana-Pérez *et al.*, 2014) and its hydroxytyrosol, 2-(3,4-dihydroxyphenyl)ethanol structure is regarded as one of the best polyphenols for its ability to scavenge free radicals (Fernández-Bolaños *et al.*, 2012).Moreover, HT also encouraged the action of cells of the immune system, with its high antioxidant capacity protecting neutrophils against oxidation mediated by hydrogen peroxide. (İlhan *et al.*, 2015) In addition, HT effectively protects the DNA of mononuclear blood cells and peripheral monocytes of patients with Alzheimer's disease. (Grossi*et al.*, 2014)**.**
European Science Foundation as 'the science and technology of diagnosing, preventing and treating of clinical disturbance such as relieving pain, traumatic injury, and saving human health, using molecular knowledge and tools of the animal body, there are many of nano-pharmaceuticals like magnetic nanoparticles, quantum dots, dendrimers, liposomes, carbon nanotubes, metallic nanoparticles and polymeric nanoparticles (Martin, 2006; Jasim etal.,2019 b).

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen (Mohanraj and Chen, 2006)

EXPERIMENTAL METHODS

Preparation of hydroxytyrosol - nanoparticles:

Nanoparticles of hydroxytyrosol of were prepared by the nanoprecipitation method according to (Gaonkar*et al.*, 2017 ; jasim *etal.*,2019 a) with mild modification. All steps included

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solvents, dissolved or emulsified were done in a chemical fume hood, which can be listed by following points:

1. The an aqueous solution included 200 mg of hydroxytyrosol was placed in a glass test tube and then transfered 1 ml of de-ionized water solvent, after that the top of the tube was closed with a small piece of aluminum foil, and Parafilm the foil securely, the Parafilm was put tightly opposite to the top edge of the tube.
2. The level of each solvent was tagged on the outside of the test tube and the dissolving hydroxytyrosol was incubated overnight, then vortex on high speed until all hydroxytyrosol powder was entity dissolved (~10 min).
3. An organic phase of Hexane was prepared and added to a 200 ml glass beaker contain aqueous phase of hydroxytyrosol , after that put on a magnetic stir bar at stirring speed 500 rpm for 10 hour until remove hexane compeletly . And then percent 1:1 of 0.06 % of TPGS was added to solution for emulsified solution. Then the test tube was stirried.
4. The emulsified hydroxytyrosol solution was put in the small beaker 20 ml and then directly transfered to the ultrasonicator and immersed in the ice water and sonicate the emulsion for 9 min, pulse on time 15 seconds with pulse off time 15 seconds and 50% amplitude .
5. The emulsified nanoparticle was put in beaker 100ml and then wrapped in aluminum foil, the top of beaker was leaved open to facilitate solvent evaporation.

6.The suspension obtained was filtered (Whatman filter paper 1) to discard any precipitated and then centrifuged at 14,000 rpm at 4 °C .The supernatant containing the unbound drug was discarded; the pellet obtained was washed 2–3 times with distilled water .

EXPERIMENTAL STUDY

Sixty adult male Wister rats were randomly and equally divided into three groups of 20 animals for each. All three groups fed on ordinary pelleted diet and drinking water and subjected to same ventilation and living conditions. The first group, named as control, was daily oral administration distilled water. The second group, labeled as T1,subjected to daily oral administration of HT extract (50 mg/kg) (**Al-Zamely and Sabea, 2019**). The last third group (T2) was daily drenched a treatment of HT nanoparticles (50 mg /kg)

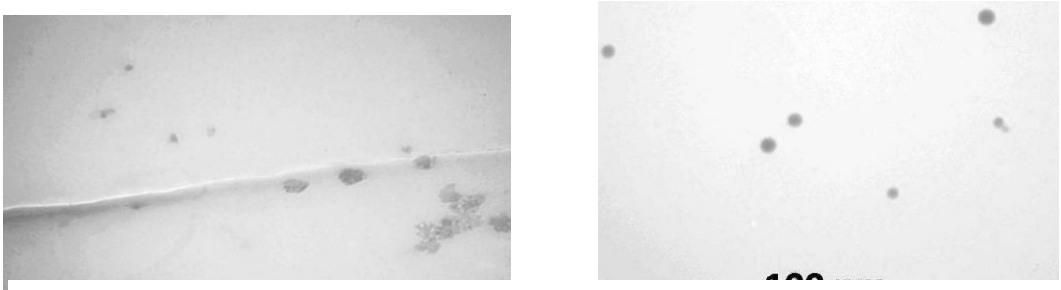
STATISTICAL ANALYSIS

Data are presented as means with their standard errors. The statistical significance of differences among groups were analysed using a one-way ANOVA to be statistically significant.

RESULT AND DISCUSSION

Charctrization of hydroxytyrosol nanoparticles:

The (Fig. 4-3) revealed typical SEM micrograph of chemical and biological SNPs. The morphology of NPs was cubic in shape, uniformly (mono dispersed) without significant aggregation. Scanning electron microscopy has been employed to determine the shape, size and morphology of chemical and biological synthesized SNPs.



Figure(1) Show SEM image of particle size and morphology of hydroxytyrosol nano

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Brookhaven Instruments Corp.
90Plus Particle Sizing Software Ver. 5.34

Date: Sep 14, 2020
Time: 22:22:38
Batch: 0

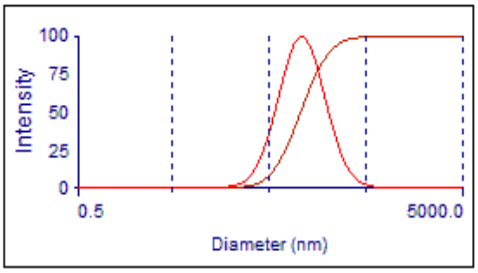
Sample ID **38-2 (Combined)**
Operator ID **9**
Notes **laser**

Measurement Parameters:

Temperature	= 25.0 deg. C	Runs Completed	= 3
Liquid	= Water	Run Duration	= 00:00:30
Viscosity	= 0.890 cP	Total Elapsed Time	= 00:01:30
Ref.Index Fluid	= 1.330	Average Count Rate	= 351.5 kcps
Angle	= 90.00	Ref.Index Real	= 1.590
Wavelength	= 660.0 nm	Ref.Index Imag	= 0.000
Baseline	= Auto (Slope Analysis)	Dust Filter Setting	= 30.00

38-2 (Combined)

Effective Diameter: 107.1 nm
Polydispersity: 0.346
Baseline Index: 5.3/ 97.06%
Elapsed Time: 00:01:30



Lognormal Distribution

Run	Eff. Diam. (nm)	Half Width (nm)	Polydispersity	Baseline Index
1	108.4	62.5	0.332	5.8/ 95.61%
2	102.2	59.1	0.335	4.2/ 95.58%
3	109.6	66.0	0.362	5.4/100.00%
Mean	106.8	62.5	0.343	5.1/ 97.06%
Std. Error	2.3	2.0	0.010	0.5/ 1.47
Combined	107.1	63.1	0.346	5.3/ 97.06%

Figure(2) : particle size of garlic nano suspension.

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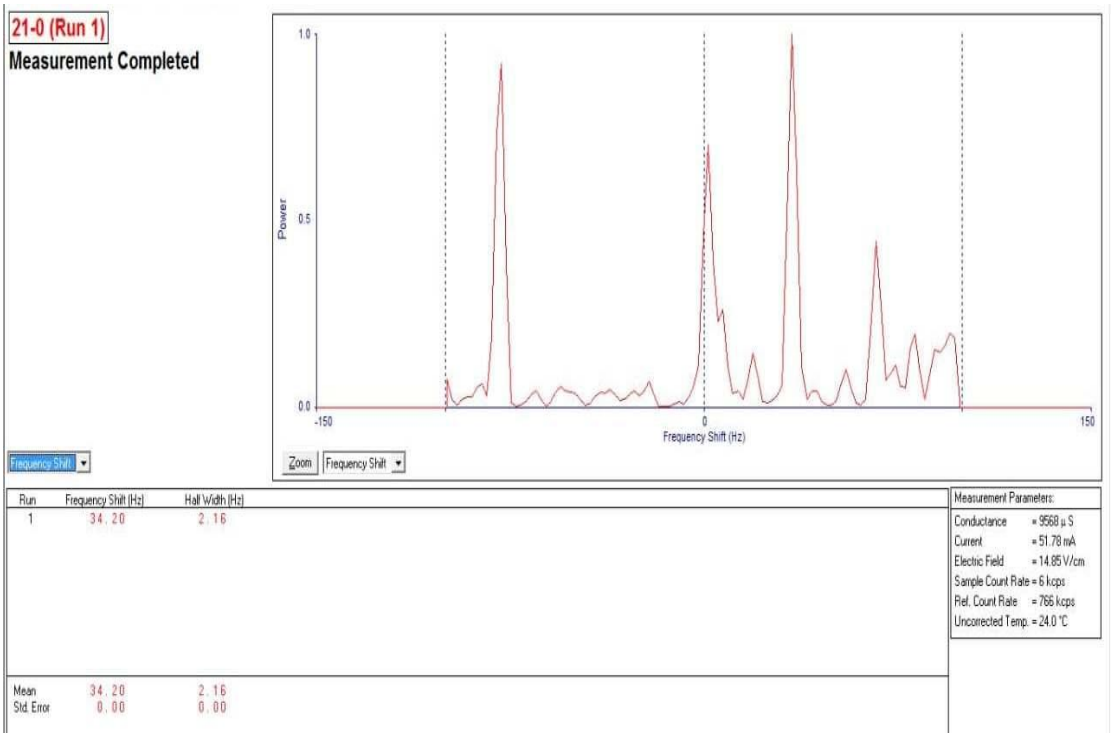

Figure (3) : Zeta potential of hydroxttyrosol nanoparticles refer to stability of nanoparticles

SNPs size was determined by dynamic light scattering. The size of nanoparticles distribution analysis of chemical method revealed the average of particles size was approximately 107 nm (107 ± 3) (Fig. 4-4)

The activity of SNPs influence of its size particles, the small particles well known more effective than large one (Ghotaslou*et al*., 2017). Many previous studies reported that activity based on particles size of SNPs (Martinez-Castanon*et al*., 2008).The nanoprecipitation technique produced nanoparticles (NPs) of approximately 107 nm in size, which is optimal for the ability of NPs to be used for oral administration , this result was in an agreement with (Rafiei and Haddadi, 2017). The present study and others have used a variety of emulsifying and stabilizing agents, including vitamin E-TPGS and hexane (McCall and Sirianni, 2013).Our study used a 1:10 drug-polymer ratio, this result is in agreement with results reported by Gaonkar *et al.* (2017) who demonstrated that a 1:10 ratio is optimal for a PLGA delivery system for Garcinol loading drug rather than 1:20.

Herein, the protocol used is focused on synthesis of nanoparticles with vitamin E TPGS as the emulsifying agent and hexane as the solvent. However, alternative solvents and/or emulsifying agents are feasible(Jasim et al.,2021). In general, the experiments have appeared that hexane produces smaller and more uniformly sized nanoparticles because hexane is water-miscible, it is excluded.Our data were concord with many reports showed that hexane reduced size of HT nanoparticles and prevent nanoparticles aggregation and sedemination that generate a significant increase in particles due to water immscible and asignificant particles aggeregation that result from incompatable with emulsifier vitamin E-TPGS(Song *et al.*,2006; Yalcin*et al*., 2017). On the other aspect elevation of nanoparticles size reduced the uptake by cell and limitation the distribution in the body due to mononuclear phagocytic system tag and expell the drug size of more than 1000 nm (Gustafson *et al.*, 2015, Behzadi*et al.*,2017) .

The size of NPs had a direct influence on mechanisms of particle internalization by cells, as well as their distribution in tissue, which can be resulted in dramatic differences in delivery effectiveness(Mundargi *et al.*, 2008). Delivery of small NPs might also be facilitated passive targeting of vessels via the enhancing permeation and retention effect as well as reducing drug administration frequency(Zhang *et al*., 2018). In the present study the nanoprecipitation technique produced a drug

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encapsulation efficiency varies widely depending on the properties of the specific drug, the size of the particle, and the emulsifier. Encapsulation of hydrophobic drugs via a single emulsion may be supported the generation of ultra-small NPs compared to the double-emulsion method recorded by (Zhang and Feng,2006;Madani *et al.*, 2018) .

The histopathological examination of the pituitary gland of the male rats section in the control group showed normal adenohypophysis, mediastanium and neurohypophysis. Figure 4.7, 4.8 shows the structure of the control rat's pituitary gland. The first treatment group which subjected to dose of hydroxytyrosol 50 mg/kg for 8 weeks showed no lesion, as shown in figure 4.11, 4.12. Whereas the second treatment group which subjected to of hydroxytyrosol nanoparticle 50 mg/kg for 8 weeks showed no clear lesion figure 4.9, 4.10. Microscopy observations did not reveal any morphological alteration in the organs or tissues examined. There were no differences between controls and test item treated animals.

The antioxidant and anti-inflammatory activity of hydroxytyrosol has given a protection to pituitary gland tissue, the current results were highly agreeable with the work of Schaffer (Schaffer *et al.*, 2007) who conducted that hydroxytyrosol rich olive mill waste water extract has a powerful antioxidant, anti-inflammatory, and antithrombotic activities. Moreover, the pituitary gland shows three parts separated by adense connective tissue septae that contains blood as it was also reported by Mahmood (Mahmood, 2014, Mahmood, 2015). Under physiological conditions, the normal pituitary gland receives most of its blood supply through the capillary network of superior and inferior hypophyseal (portal) vessels in the infundibulum (Stanfield, 1960, Gorczyca and Hardy, 1988, Goyal *et al.*, 2018). Previous study about the neuroprotective effect of hydroxytyrosol on the Rat´s brain tissue revealed normal brain tissue with normal neuron and blood vessels. Moreover, there is no detectable changes in tissues of brain compared with the control group, which were also have normal brain tissues. These results lead to the conclusion that the antioxidant activities and neuroprotective ability of hydroxytyrosol improves the brain tissues, following acute and subchronic oral administrations of HT (Tamemi, 2018). Furthermore, the daily administration of 100 mg/kg /BW of HT is safe and has no toxic effect on rodents. This study agreement with D'Angelo et al.2001. Administered a single dose of 2 g/kg of body weight in rats and found an absence of toxic effects or macroscopic alterations in tissue of pituitary.

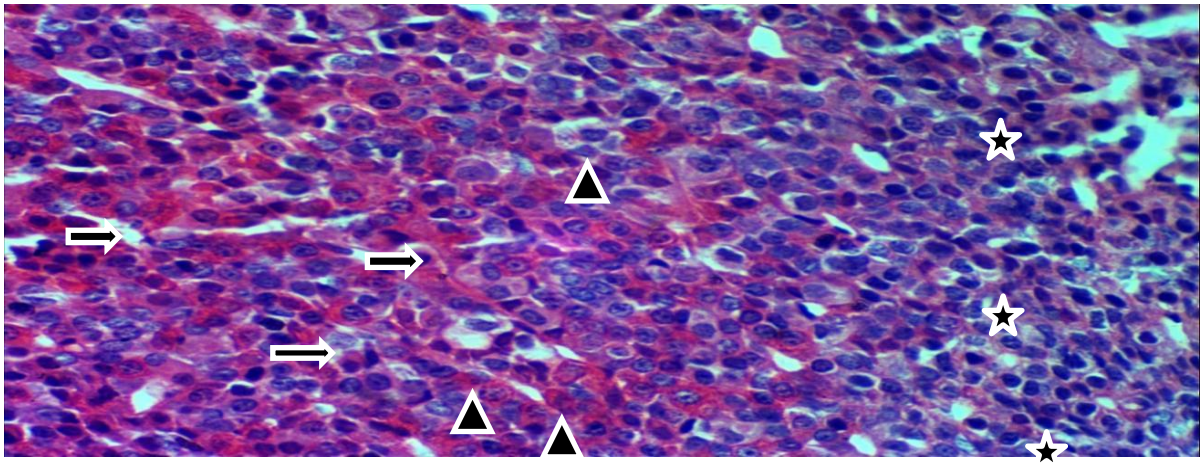

Figure 4.8. Histopathological section of pituitary gland of control group rat.

Normal histological architecture of pituitary gland. Note the acidophil cells (arrows), basophils cells (star) and chromophobe cells (triangles).H&E. X400.

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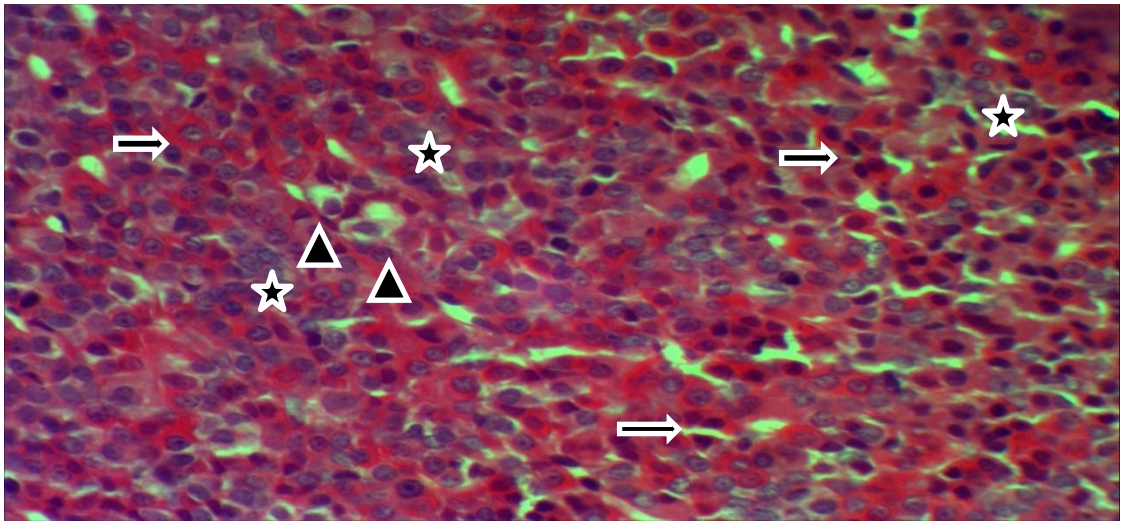

Figure 4.10. Histopathological section of pituitary gland of nanosespension treated group rat.

Normal histological architecture of pituitary gland. Note the acidophil cells (stars), basophils cells (arrow heads) and chromophobe cells (triangles).H&E. X400.

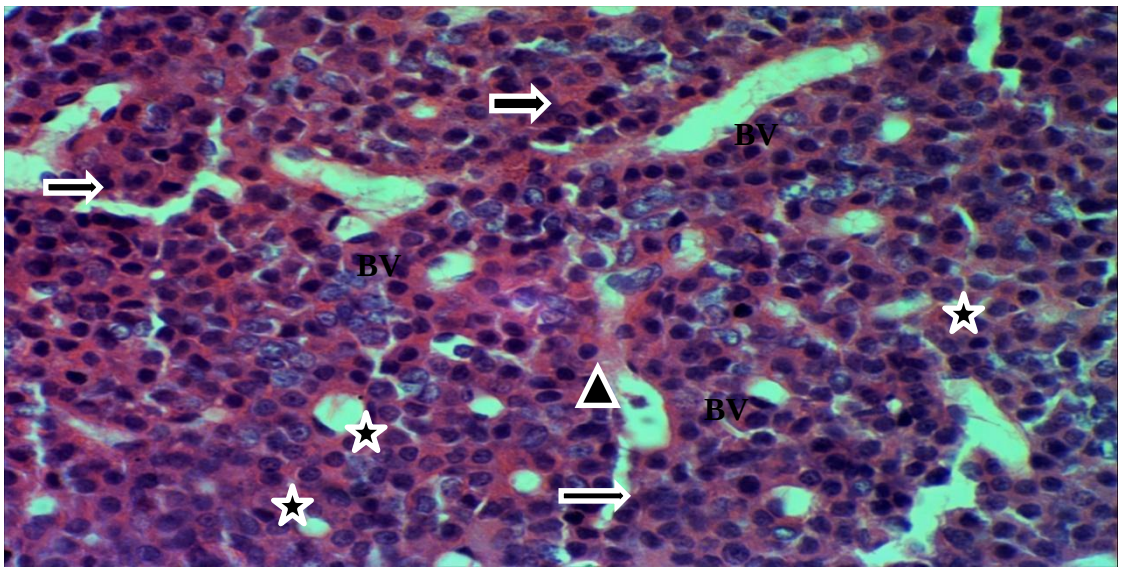

Figure 4.12. Histopathological section of pituitary gland of hudroytyrosol extract treated group rat.Normal histological architecture of pituitary gland. Note the acidophil cells (stars), basophils cells (arrow heads) and chromophobe cells (triangles).BV: Blood vessels. H&E. X400.

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CONCLUSIONS

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