ROLE OF AQUEOUS NANOPARTICLES AND PHENOLIC EXTRACT OF SALVIA OFFICINALIS L. ON CYCLOPHOSPHAMIDE-INDUCE SOME PHYSIOLOGICAL DEGRADATION IN ALBINO MICE

Roaa M.H. Shoker¹, Laith M.J. Al-Shamma² and Hazim Ismaeel Abdul-Barrey Al-Ahmed³

¹Pharmacology Department, Medicine Collage, Waist University, Iraq.
²Biology Department, Science Collage, Baghdad University, Iraq.
³Biotechnology Research Center, Al-Nahrain University, Iraq.

Abstract

Objective: The study was aimed to evaluate amelioration effects of aqueous nanoparticles and phenolic extracts of S. officinalis on some physiological degradation which induced by cyclophosphamide (CP). Methods: In the experiment, 40 animals were taken and divided into 5 groups. Each group contain of 8 mice. Group I negative control with normal saline injected intra peritoneal. Each group from (II to V) are injected with CP intra peritoneal on 0.1ml. While group II, III and IV animal administration with 200 mg/Kg b.w. aqueous extracts, 10 mg/Kg b.w. phenolic extract, 10 mg/Kg b.w. NPs aqueous extracts, respectively . Group V positive control without plants extract treated. Blood samples were collected from direct heart puncture after 21 day. Results: The results indicated the total cholesterol, triglycerides, ALT, AST, and ALP were significantly increase at (p<0.05) in mice which treat 40 mg/kg b.w. CP drugs. While when administration aqueous, phenolic, NPs aqueous extract of S. officinalis this parameters reduce significantly at (p<0.05). And the results showed no significant differences in HDL level between all treatments. Conclusions: From this review can be concluded that synthesis AgNPs mediated by S. officinalis aqueous extracts, and the plant have an efficient activity against Hyperlipidemia.

Key words: Medicinal plants, Nanoparticles, Salvia officinalis, Cyclophosphamide drugs, Phenolic Extracts, Lipid Profile.

Introduction

Phytochemical screening of therapeutic plants has added a high deal for finding the new drugs. A several of therapeutic plants have been exposed to developed chemical studies and this has led to the separation of pure bioactive compounds which have been pharmacologically assessed (Kumar et al., 2015). Chemotherapy treat different cancer kinds proficiently, but it cusses acute side effects. Chemotherapy like CP not acts only on the active cells, like cancer cells, but moreover acts on the healthy cells (Abdel-Hafez et al., 2017). Oxidative stress, that is produced by great concentration of free radicals in cells and tissues, may be produced by several negative agent, such as X-ray radiation, UV, gamma, smoking, contaminated diet, alcoholism, harmful ecological factors, drug addiction and exhaustive physical exertion (Serafini and Peluso, 2016; Bi et al., 2017). Several plants have antioxidants constituent such as vitamin C and E, flavonoids, carotenoids, and tannins, etc. that may be utilized to scavenge free radicals from human body (Sharma et al., 2011). “Green nanotechnology” in nanoparticle biosynthesis is increasing between researchers, due to their biological and physicochemical properties, have utilization in several fields, having drug delivery, magnetic devices, sensors, optoelectronics, and focuses on the green synthesis of silver nanoparticles (AgNPs) by utilizing plant sources (Chung, et al., 2016). Aqueous extracts of S. officinalis and R. communis have the ability to reduce silver nitrate ions to silver nanoparticles (Salem et al., 2012). S. officinalis important action on dyslipidemia can be associated to flavonoids found in the plant, this flavonoid increasing mitochondrial DNA content, mitochondrial size, and gene expression associated to
mitochondrial biogenesis (Seo et al., 2015). Dent et al., (2017) showed that sage is polyphenols rich, which indicated have antioxidant activity. S. officinalis L. was present to have relative cardio-protective active by increasing blood HDL cholesterol levels and reducing total triglyceride, cholesterol, and LDL cholesterol levels in hyperlipidemic patients (Kianbakht et al., 2011). In this research effects aqueous nanoparticles and phenolic extracts of S. officinalis were investigated on some physiological degradation which caused by CP.

### Materials and Methods

#### Collection of Plant Samples

Dry aerial parts of S. officinalis L. was obtained from local markets in Waist Province, Iraq. The plants identified by Dr. Sukeyna Abaas Aliwy Department of Biology/College of Science/University of Baghdad. After collection, ground into a fine powder by an electric grinder, then stored in clean conditions until use.

#### Preparation of Plant Extracts

**A- Aqueous Extract**

Aqueous extract of plants are prepared, weight 100 g of dry powder of plant and place it in conical flask and added 500 ml of distilled water in percentage 1:5 w: v. after that the mixture shacked by electric shaker for 2 hours and left mixture in room temperature. After 24 hours, the mixture was filtered by four layer of gauze and put in tube and centrifuged mixture in centrifuge 2000 rpm to 10 min. The supernatant was filtered by Whatman no. 4 filter paper. Filtrate mixture was concentrated by oven for 72 hours to obtain crude extract. This extract was stored in dark sterile screw bottle 4°C until use (Zheng Mu et al., 1990).

**B- Crude Phenols Extract**

Crude Phenols were extracted according to Harborne (1984). 200 g of plant powder was divided into two equal parts, 300 ml of 1% hydrochloric acid was added to one part, and 300 ml of D.W. was added to the other, the two quantities were transferred to electrical blender for 5 minutes. Then the two mixtures were transferred to boiled water bath for 30-40 minutes, the two mixtures were cooled and filtered through muslin cloth, then transferred to a centrifuge with speed of 3000 rpm for 10 minutes. The two supernatants were mixed. Equal quantity of n-propanol was added to the mixture and sodium chloride was added until the solution was separated into two layers. The lower layer extracted in separating funnel with Ethyl acetate, and the solvent layer was collected and evaporated in a rotary evaporator at 40°C for (1-2) hours. The upper layer was evaporated in a rotary evaporator at 40°C for (1-2) hours the dried material of both layers were mixed and dissolved in 5ml of 96% ethanol, then transferred to oven then the extract was kept in refrigerator until use.

#### Green Synthesis of silver nanoparticles

Silver nanoparticles synthesis were prepared by Veerasamy et al.,(2011).

#### Analysis of phenolic compounds of the plants extracts by HPLC

Analysis of the phenolic compounds was made by injecting 100 µl of the extract of each sample in High Performance Liquid Chromatogram for identification. The procedure that used outlined by Mradu et al., (2012).

#### Liver Enzymatic Assay

**Aspartate aminotransferase AST**

The AST enzyme activity was evaluate in mouse serum by using enzymatic colorimetric kit method produced by (Agappe company) and according to Thefeld et al., (1994).

**Alanine aminotransferase ALT**

The activity of this enzymes was evaluate in blood serum depending on colorimetric method by using enzyme kit produced from (Agappe company) according to Thefeld et al., (1994).

#### Alkaline phosphatase (ALP)

This was intended for in vitro quantitative determination of Alkaline Phosphatase in serum or plasma by Agappe kits.

#### Results

Plants have a great source of natural compounds like vitamins, terpenoids, phenolic acid, alkaloids lignins, cumarins, tannis, quinones, and flavonoids having varied types of secondary metabolites which have antioxidant properties (Thirunavukkarasu et al.,2018). In this work, aerial parts of S. officinalis plants were extracted, for detection of aqueous and phenols extracts. Chemical detection of some active compounds of the aqueous extracts of S. officinalis was investigated. The results showed the tannins, saponins, resins, coumarins, phenols, alkaloids, flavonoids, terpenes, steroid and volatile oil were indicated the positive result of S. officinalis aqueous extracts. While, glycoside absent of this extract. This results also agree with Kadhim et al., (2016) which
detected the alkaloids, phenol, saponins, flavonoids, steroids, tannins, and Vitamin C of *S. officinalis* aqueous extracts. While disagreement with him about glycoside was found in positive results of *S. officinalis* aqueous extract.

**Characterization of synthesized silver nanoparticles (AgNPs)**

Nanoparticles biosynthesis has been considered as a new technique of study. In the continues study, the green chemistry of silver nanoparticles synthesis from aqueous solution as a fast ecofriendly technique was defined (Daoud *et al.*, 2015). In this work, silver nanoparticles were prepared by green method and characterized by using Atomic Force Microscope (AFM). Aqueous extracts of this plants performance as reducing agent through exposure to silver nitrate, silver ions reduction into silver nanoparticles which was observed as a result of the color change. The color change was due to the Surface Plasmon Resonance phenomenon as Fig. 1.

**Fig. 1**: Nanoparticles samples.

1- *S. officinalis* aqueous extract, 2- *S. officinalis* NPs Aqueous extract. After stirring 20min at 75°C.

The results in this work agree with other researcher, Salem *et al.*, (2012) showed that the aqueous extracts of *S. officinalis* and *R. communis* have the ability to reduce silver nitrate ions to silver nanoparticles. The color change was yellowish-brown due to the Surface Plasmon Resonance phenomenon. Rajendran and Prabha, (2016) showed the reduction of silver ions to silver nanoparticles might be a periodical color change. AFM was one of the primary tools for determining imaging and manipulating matters at the nanoscale (Li *et al.*, 2011). Homogeneous distribution of silver nanoparticles was observed AFM three dimension images. Fig. 2 shows the AFM images of AgNPs appear the globally spherical shape and nanoparticles agglomeration which prepare by *S. officinalis*.

Granularity distribution chart was obtained from AFM software for biological methods which gives the particles size distribution of AgNPs (Fig. 3), diameter average of the biosynthesis AgNPs particle was (63.24 nm). Ismail (2019) showed the average dimeter for biosynthesis AgNPs particle from aqueous extracts of *Trigonella foenum-graecum* (fenugreek) was about 56.12nm.

**Fig. 2**: Topography and three-dimensional images showing the globally spherical shape of the prepared particles of *S. officinalis*.

Chemical constituents of the plants extracts

Results of HPLC analysis indicated the presence of 4 phenolic compounds in plants. All the isolated compounds appeared to have different retention time. These constituent concentration showed in table 1 and Figs 4, 5, 6, 7 and 8.

Blood biochemical parameters

**Lipid Profile**

Cyclophosphamide was a common anticancer drugs utilized for the treatment of numerous malignancies. However, upon treatment, it makes cute toxicity due to
Table 1: Types and concentration of phenolic constituent in plant extract.

<table>
<thead>
<tr>
<th>Phenolic compounds (µg/ml)</th>
<th>S. officinalis</th>
</tr>
</thead>
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<tr>
<td>Epigenin</td>
<td>276.54</td>
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<tr>
<td>Kaempferol</td>
<td>146.50</td>
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<tr>
<td>Quercetin</td>
<td>368.34</td>
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<tr>
<td>Catechin</td>
<td>1254.53</td>
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<tr>
<td>Total concentration (µg/ml)</td>
<td>3045.91</td>
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Fig. 4: HPLC profile of Phenols Standard (1) epigenin.

Fig. 5: HPLC profile of Phenols Standard (2) kaempferol.

Fig. 6: HPLC profile of Phenols Standard (3) quercetin.

Fig. 7: HPLC profile of Phenols Standard (4) catechin.

Fig. 8: HPLC profile of Salvia officinalis Phenols (1) epigenin, (2) kaempferol, (3) quercetin, (4) catechin.

Administration CP drugs in experimental mice for 21 days make a marked changes in blood lipid profile such as total cholesterol and triglycerides which was studies in table 2. Total cholesterol and triglycerides were significantly increased at (p<0.05) in mice which treated CP drugs (170.73±3.40 mg/dl), (167.12±6.40 mg/dl) as compare with the control (90.71±6.29 mg/dl), (59.44±4.18 mg/dl) respectively. Several classes of drugs to be considered as common causes of altered lipid profiles (Varghese et al., 2017). Results showed aqueous extracts of S. officinalis reduced significantly at (p<0.05) total cholesterol and triglycerides (147.79±4.45 mg/dl), (140.49±3.56 mg/dl) compare with CP group. Total cholesterol and triglycerides were reduced significantly at (p<0.05) in group of mice which were treated with phenolic extracts (140.95±4.88 mg/dl), (109.79±53.85 mg/dl) respectively. These results agree with previous study which showed S. officinalis L. was decreased total triglyceride, cholesterol, and LDL cholesterol levels in hyperlipidemia patients (Kianbakht et al., 2011). Alenzi et al., (2016) mention treatment with N. sativa L oil which have active constituent, thymoquinone, may decrease cyclophosphamide-induced toxicity. In this study nanoparticles were teste to reduce side effects of anticancer drugs. The results show higher reduce significantly at (p<0.05) of total cholesterol and triglycerides in group of mice which were treat with NPs aqueous extracts (105.47±7.30 mg/dl), (100.57±8.59 mg/dl) respectively. While results indicate no significantly of high density lipoproteins (HDL) in all treatment. The coated AgNPs with S. officinalis have promising possible as a source for the progress of chemotherapeutic drugs in future (Baharara, et al., 2017). Ismail, (2019) showed aqueous extract and green AgNPs of T. foenum-graecum seed which reduced significantly (p<0.05) triglyceride, cholesterol, and LDL-cholesterol level in serum of Streptozotocin diabetic mice. No-significant differences in HDL level between all studied.

Liver Enzyme

CP was induced severe liver damage which showed by elevated serum ALT and AST accompanied by increased hepatic oxidative stress and inflammatory biomarkers (Mansour et al., 2017). Even low doses of CP may causes significant hepatotoxicity in humans which...
often act as a barrier against its clinical uses (Cuce et al., 2015). ALT, AST, and ALP showed increased significantly at (p<0.05) in CP groups (68.570±2.622 mg/dl), (78.466±3.598 mg/dl), and (232.48±7.52 mg/dl) as compared with control (31.39±2.759 mg/dl), (51.144±3.653 mg/dl), (203.81±11.65 mg/dl) respectively, (Tables 3). Shokrzadeh et al., (2014) observed serum ALT, AST, and ALP levels were increased significantly at (P<0.001) in all mice injected with 200 mg/kg of CP when compared to the untreated control animals, the serum ALT, AST, and ALP levels of the CP groups were (123.78±4.24 IU/l) (96.18±2.07 IU/l) (185.86±5.37 IU/l) while the control groups (42.37±1.46 IU/L) (31.39±1.48 IU/l) (52.28±1.29 IU/l), respectively. Dina et al., (2019) showed the hepatic enzymes ALT, and AST in serum were significantly increased at (P<0.05) in CP-treated rats when compared with the control. ALT, AST, and ALP activity reduced significantly at (P<0.05) in aqueous and phenolic extracts of *S. officinalis* plants groups, when compared with CP group. ALT levels were (53.260±6.869 mg/dl), (40.704±3.703 mg/dl) within a serum normal range. While AST levels (68.304±3.357 mg/dl), (61.122±3.759 mg/dl). And ALP levels (219.298±2.771 mg/dl), (200.84±2.73 mg/dl) frequency. Results indicated present significant different between aqueous and phenol extracts. Results showed higher effects to reduced ALT, AST, and ALP activity of *S. officinalis* NPs aqueous extract were (29.298±2.771 mg/dl), (50.372±4.280 mg/dl), and (170.46±3.22 mg/dl) respectively when compare with CP group (68.570±2.622 mg/dl), (78.466±3.598 mg/dl) (232.48±7.52 mg/dl). These plants act as hepatoprotective agent, due to they contained compounds and vitamins with antioxidant activities. *S. officinalis* have hepatoprotective activity and so utilize this plant for treating liver disorders (Parsai et al., 2014). Ibrahim, (2014) showed ALT, AST, and ALP levels significantly increased at (P<0.05) in rats group which treated with Mncl₂ as compared with a control group, and ALT, AST significant decrease at (P<0.05) in rats groups treated with Mncl₂ and aqueous extracts of *S. officinalis* compared with Mncl₂ group. ALP decreased significantly (P<0.05) in groups treated with the extract compared with Mncl₂ group. Hong et al., (2015) showed several phytochemicals and medicinal herbs have been examined as complementary and alternative treatments for acute liver diseases. Due to hypolipidemic drugs are extensively in addition to these drugs have other adverse effects. In India approximately 150 medicinal plants have been utilized as herbal drugs against Hypolipidemic. Due to herbal drugs is significantly have sight side effects compared with the synthetic hypolipidemic drugs (Srivastava and Srivastava, 2018).

References

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<th>Groups</th>
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<th>HDL mg/dl (mean±SD)</th>
<th>TC mg/dl (mean±SD)</th>
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Shokrzadeh, M., A. Ahmadi, F. Naghshvar, A. Chabra and M.


