

# Evaluation Protective Role of *Salvia officinalis* Silver Nanoparticles Against Toxic Effect of Bisphenol a in Female Rats

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## Abstract

Bisphenol A ( BPA ) is chemical compound found in the environment and used in the manufacturing of plastic , which known as deleterious part of different body parts . *Salvia officinalis* ( SO ) which is perennial round shrub in the family of Labiatae /Lamiaceae. It's has potent antioxidant and anti-inflammatory effects . The present study was conducted to evaluate the protective role of SO as nanoparticles against harmful effect of BPA in female rats . Eighteen adult female rats were used and divided equally and randomly into three groups as following : first group received DMSO orally and served as control group while second and third groups were gavaged either with BPA alone or in combination with SOSNP respectively , all the treatments extend 30 days . The result revealed harm effect of BPA by significantly increase the level of estrogen , AST, ALT , ALP , urea and creatinine with significantly reduction in the FSH and LH . SOSNPs show ameliorative effect on all above parameters .

**Keywords :** *Salvia officinalis* , Bisphenol A , FSH , LH

## Introduction

BPA consider one of the most famous xenoestrogens in the nature before 80 years and enter in many product such as packaging of beverages and food , epoxy , poly vinyl carbon and electronic . It's mimic estrogenic effect via its ability to stimulate estrogen receptors ( ER) , so it's can cause disturbance in the endocrine system <sup>1</sup> . The polymerization increase the risk of exposure , by acceleration the hydrolysis of the ester bond due to heating or acidic environment so the risk of exposure will increase . As a result to frequent wash of the polycarbonate and the temperature may be high or acidic medium ,this will increase the risk of hydrolysis <sup>(2,3)</sup> . The estrogen receptors are diffused in different organs such as reproductive organs ( ovary , uterus , oviduct and ovary ) as well as mammary , pituitary , thyroid glands and many other organs , so the binding of BPA with these receptors will lead to increase expression of these cells <sup>(4,5)</sup> . BPA has similar effects of diethylstilbestrol on sexual maturity so its banned to use in the baby milk bottles <sup>6</sup> Nanotechnology is modern technology , this technology focused by the researchers recently due to wide range of applications in energy , electronic purposes as well as in medicine <sup>7</sup> . The nanotechnology

recently enter to pharmaceutical line which used in the preparation and development Nano scales particles in the formation of drugs conjugates , Nano crystals , etc <sup>(8,9)</sup> . Nanoparticles are cluster of atoms their size range from 1 – 100 nm , due to its size have noble properties as well as morphology and distribution <sup>(10,11)</sup> . It have novel properties and functions due to its small size or intermediate , nanoparticles give high efficiency than traditional method for treatment due to mentioned properties as well as decrease the dose and the side effects of chemicals <sup>12</sup> *Salvia officinalis* rich in canosic acid and rosmarinic acid so it have antioxidant properties <sup>(14,15)</sup> , anti-inflammatory , antiproliferative <sup>16</sup> , antibacterial <sup>17</sup> , and anticancer <sup>18</sup> , as well as , sage used as anti-diabetic <sup>19</sup> . Due to aromatic properties the sage used as flavoring agent and antioxidant additive for canned food <sup>20</sup> . *Salvia officinalis* good reducing agent , so it used as reducing agent in the preparation of nanoparticles <sup>21</sup> .

## Materials and Method

### The tested compound is bisphenol A ( BPA )

BPA ( cas 80 – 05 – 7 , > 97 % ) was obtained from high media company ( India ) , Dimethyl sulfoxide ( DMSO ) assist as vehicle for suspension of BPA as

well as used as control substance. *Salvia officinalis* herb buy from local market of karbala.

### Animals of study

This study was carried at the college of veterinary medicine / university of Kerbala, eighteen adult female rats. Their ages ranged from 14 to 16 weeks old and the average of weight (200 – 250 gm). After 15 days of accommodation in metal cages under suitable environment conditions at temperature  $23 \pm 2$  °C and 12 h light / 12 h dark, with ad libitum feeding and water daily, divided randomly into 3 groups 6 animals on each group.

### Experimental design :

First group administrated 0.25 ml DMSO and, the second group administrated 250 mg / kg BPA<sup>22</sup> and the third group co administrated 250 mg / kg BPA and 150 mg / kg SOSNPs<sup>23</sup>.

### Extraction of salvia officinalis

After collection of sage plant, wash it by double distilled water (DDW) then cut and grind until powdered it. Weighs fifty grams of powdered SO then mixed with 250 ml from DDW and boiled for 5 minutes, then cooled the aqueous extracts and filtered by filter paper<sup>24</sup>.

### Preparation of salvia officinalis silver nanoparticles (SOSNPs)

At first prepare 1nM of silver nitrate solution, this solution prepare by adding 0.0170 grms of AgNO<sub>3</sub> to 100 ml of DDW by carefully addition, then store in dark colored bottles to avoid auto oxidation after that put the silver nitrate solution and adding it gradually to 10 ml of SO aqueous extract then heat the mixture to 40 °C until see the color from pale yellow to brown<sup>25</sup>.

### Identification of silver nanoparticles.

#### Spectrophotometry

The spectrophotometry was evaluated by using UV-visible double beam (lapomed) were used to identify the stability and formation of silver nanoparticles. The wavelength for this apparatus ranged from (100 – 800) nm, 480 nm was the optimum wave length for SOSNPs solution (26, 27).

### Scanning Electron Microscope

By using scanning electron microscope (SEM) (Hitachi), the solution was tested to show the size and shape of the silver nanoparticles. This procedure was done by put one drop of solution on the aluminum – covered by copper grid and films on the SEM system, then let it for 2 minutes, by using blotting paper remove the extra solution then drying the grid.

### Blood sample collection

At the end of experiment, The animals anesthetized, this done by putting the rats in closed jar containing soaked by chloroform. Then the blood collected by heat puncture. the blood gelled tube and centrifuged for 15 minutes (300 RPM). The supernatant serum was drawn and kept by eppendorf tubes and store at (-4 °C) until analyzed.

### Statistical Analysis

The results was collected and analyzed by SPSS program version 13, the mean and standard error were dependent in this analysis<sup>55</sup>.

### Hormonal assay

The (FSH, LH and estrogen) hormones was evaluated at the end of experiment. Assessing the concentration of FSH and LH in the blood is a valuable tool in diagnosing equilibrium in regulating fertility across the pituitary-pituitary gonadal axis. As well as, estrogen evaluation generally considered useful tool in the diagnosis follicular growth and development. The FSH, LH and estrogen concentration was evaluated according to ELISA kit of Monobind incorporation (USA)<sup>28</sup>.

### Biochemical tests

#### A- Liver enzymes

Liver enzymes (AST, ALT and ALP) were determined to evaluate the toxic effect of BPA on the liver and the protective effect of SOSNPs, these tests was done by ELZA depending on the (Agappe diagnostic, spectrophotometer kit, India)<sup>29</sup>

#### B- Kidney enzymes

The kidney enzymes (creatinine and urea) were evaluated to determine the renal toxicity caused by the effect of BPA and the protective effect of SOSNP,

these tests was done depending on ( Agappe diagnostic , spectrophotometer kit , India ) kit <sup>29</sup>

get it high reductive effect <sup>30</sup> and make the SO suitable for preparation silver nanoparticles ( <sup>31, 32</sup> ) .

## Results and Discussion

### Synthesis of Silver nanoparticles by using *saliva officinalis* extract ( SOSNPs ) :

*saliva officinalis* extract was used to prepare the silver nanoparticles as reductive material , the change in the color is the indicator for silver nanoparticles reduction , this reduction occur due to high content of carnosol, carnosic acid, flavonoid glycosides, niacin, nicotinamide, flavones and estrogenic substances which

### Identification of silver nanoparticles

The silver nanoparticles were identified by UV identification and SEM scanning . Depending on the optical absorbance of UV radiation ((Labomed Spectro UVD-3200 spectrophotometer ) , the result show the peak of absorption at 480 nm ( figure 1) when the color changed to brown this effect by the shape and size of nanoparticles and due to increase in the surface Plasmon resonance ( SPR ) <sup>33</sup> , the change in the color occur due to the reduction of nanoparticles by SO <sup>34</sup> .

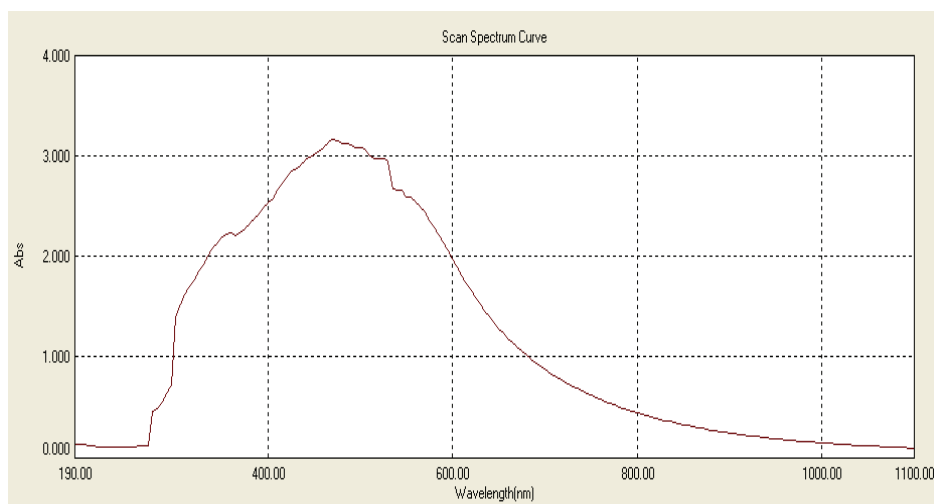


Figure ( 1 ) show the peak of wave length for SOSNPs UV test

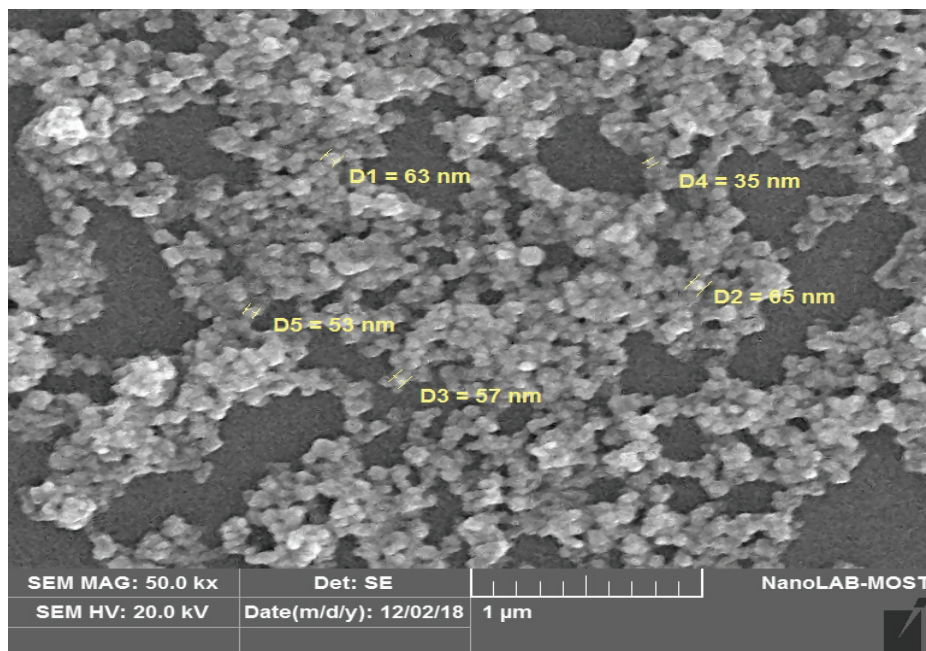


Figure ( 2 ) SEM image for SOSNP reveal the shape and size of silver nanoparticles

Protective effects of SOSNPs on the reproductive hormones of rats exposed to BPA .

The statistical analysis for the rats reproductive hormones table ( 1 ) show significance increase (  $P \geq 0.05$  ) in the estrogen level of the rats exposed to BPA in comparative with control group , due to the effect of BPA at hypothalamus pituitary regulatory circuit <sup>36</sup> by interfering with estrogen receptors (  $\alpha$  and  $\beta$  ) in the hypothalamus – pituitary- gonadal axis ( 37 , 38 ) while in the rats treated with SOSNPs combined with BPA show significance reducing the level of estrogen lower than BPA treated rats to be clear to normal level due to antioxidant properties of SO <sup>30</sup> .

**Table 1 statistical analysis for the effect of BPA on reproductive hormones and the protective effect of SOSNPs (Means  $\pm$  SE) .**

| GROUPS                  | FSH                        | LH                         | Estrogen                    |
|-------------------------|----------------------------|----------------------------|-----------------------------|
| G1<br>DMSO              | 3.07<br>$\pm$<br>0.04<br>A | 2.47<br>$\pm$<br>0.09<br>A | 47.97<br>$\pm$<br>0.26<br>B |
| G2<br>BPA               | 1.77<br>$\pm$<br>0.08<br>C | 1.27<br>$\pm$<br>0.11<br>C | 51.82<br>$\pm$<br>0.39<br>A |
| G3<br>BPA<br>+<br>SOSNP | 2.59<br>$\pm$<br>0.05<br>B | 2.03<br>$\pm$<br>0.07<br>B | 48.29<br>$\pm$<br>0.55<br>B |

**Effect of BPA on the liver enzymes and the protective effects of SOSNPs**

The statistical analysis for liver enzyme results table ( 2 ) show significance (  $P \leq 0.05$  ) increase in the liver enzymes ( AST , ALT and ALP ) as effect of BPA administration in comparison with control group , these enzymes were tested to evaluate toxicity in the liver, which leakage into the tissue. The toxic effect of BPA come from oxidative stress effect, in addition to cellular damage which come from reactive oxygen species ( ROS ) this may lead to increase in the liver enzymes level .

**Table 2 statistical analysis for the effect of BPA and the protective effect of SOSNPS on liver enzymes (Means  $\pm$  SE) .**

| GROUPS                  | Creatinene                  | Urea                        |
|-------------------------|-----------------------------|-----------------------------|
| G1<br>DMSO              | 1.40<br>$\pm$<br>0.014<br>C | 28.08<br>$\pm$<br>0.35<br>C |
| G2<br>BPA               | 1.72<br>$\pm$<br>0.008<br>A | 37.59<br>$\pm$<br>0.54<br>A |
| G3<br>BPA<br>+<br>SOSNP | 1.52<br>$\pm$<br>0.011<br>B | 31.16<br>$\pm$<br>0.46<br>B |

- The different litters refer to the significant change between groups .
- No: 3

## Conclusion

From the result of present study we conclude that the BPA cause deleterious effects on liver, kidney and reproductive hormones and SOSNPs reduced the harmful effects of BPA.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Veterinary Medicine / University of Kerbala - Karbala, Iraq and all experiments were carried out in accordance with approved guidelines.

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