

Protective role of *Salvia officinalis* silver nanoparticles on some physiological and histological aspect in bisphenol a treated female rat

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Abstract

This study was planned to investigate the probable protective role of *Salvia officinalis* have in the nanoparticles and aqueous extracts on female rats which exposed to Bisphenol A. At the end of the experiment the blood samples were collected by heart puncture to evaluate the RBC and WBC count, Hemoglobin, Platelets as well as follicle stimulating hormone (FSH), luteinizing hormones (LH), estrogen, creatinin, urea, aspartate aminotransferase, alanine amino transferees and alkaline phosphatase were estimated furthermore after the rats were scarified Liver, Kidney, Uterus and ovary were collected for histological study. The result shows significant decrease in the RBC, PLT and Hb count in the BPA group in comparison with the control and SOSNPs and SOE groups. In addition, there is a significant increase in WBC count and estrogen level in the BPA treated rats in comparison with control, SOSNP and SOE treated rats. As well as, there is significant increase in the liver enzymes (AST, ALP and ALP) in the BPA treated rats in comparison with control and SOSNP & BPA treated groups. Histological studies show presence of vacuolation with absence of radial arrangement in the hepatocytes in the liver of rats. The histological studies for the uterus of rats Beside ovarian sections BPA reveled mature follicle without oocyte with cyst like structure in the ovary in comparison with control group, these effects were relieved by the role of SOSNP and SOE in alleviating the effects of BPA on studied parameters.

Keywords: Silver nanoparticles; Physiological; Histological; Female Rats

1. Introduction

Bisphenol A is one of chemical compounds found in nature, it can work as estrogenic compound and take its receptor, so it can make disturbance in the endocrine system. Bisphenol A is one of endocrine disturbance diffuse in the environment before 80 years because it's present in many of the products such as food, beverages packaging, epoxy, poly resins and electronic. Bisphenol A is one of xenoestrogens and produced in high quantities exceed 5.5 ton per year [1].

The risk of exposure when polymerization occur, the hydrolysis of ester bond which linked them and which accelerated in the case of increase of temperature and in case of acid contact with these substances. As a result the polycarbonate product are repeatedly washed, or these polycarbonate cans exposed to heat and/or acid or base condition, these condition will lead to hydrolysis of ester bond in the polycarbonate [2].

Naturally estrogen is bound to estrogen receptors. It has responsive elements and induce expression of these cells. these cells in the reproductive organs (vagina, ovary, uterus, oviduct, cervix, testis and epididymis) the mammary gland, tbrain,pituitary, thyroid gland, skeletal and cardiovascular systems, Synthetic estrogen has ability to bind and stimulate

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all these estrogen receptors [3]. BPA also has the ability to stimulate these receptors. BPA can affect the sexual maturation due to diethylstilbestrol similarity and there are legislations introduced to banned the use of BPA in children products as a result of risk on children health [4].

Nanotechnology field is modern technology which focused for research in recent years due to its have wide range of application in filed medicine as well as in energy and electronic industries [5]. Pharmaceutical nanotechnology deal with preparation and development small materials there size in nanometer including nanoparticles, carbon nanotubes, Nano crystal, drug conjugate etc. Synthesis of nanoparticles using biological objects has great beneficial due to rare optical, photo electrochemical and electronic properties [6,7].

Nanoparticles are mass of atoms their scope of size between 1 - 100 nm size which have clear different or better properties depending on their size, distribution and morphology [8,9]. Nanoparticles of Nobel metals recently widely applied in contact with body such as shampoos, detergent and cosmetic products beside medical and pharmaceutical application. Therefore the synthesis of nanoparticles develop environmentally friendly process without toxic chemical. The microorganisms, enzymes and plant or plant extract were used as Biological methods for synthesis nanoparticle which have been suggested as possible ecofriendly choices to chemical and physical methods [10]. Today silver nanoparticles (SNPs) are widely used in many biological applications such as diagnosis, drug delivery, treatment, medical device coating and health care [5].

Salvia officinalis (Sage) is a perennial round shrub in the family of Labiatae/Lamiaceae *Salvia officinalis* is one of the most genera lameaceae by more than 900 species spread through the world. Many species of *salvia officinalis* are important economically which are used as spices and flavoring agents, in the field used in cosmetics and perfumery [10].

Many studies show the *salvia officinalis* (Sage) have carnosic acid and rosmarinic acid which get it potent antioxidant properties [11]. anti-inflammatory, anti-proliferative, antinociceptive, diuretic, antibacterial effects and anticancer. Sage was discussed for the treatment of alzheimer's disease, anti-diabetic [12]. Due to it has aromatic properties its used as flavoring and antioxidant when added to food, Some literatures were used *salvia officinalis* as reducing agent in green synthesis SNPs [13].

In contact to the published finding about the adverse effects of BPA. BPA manufactures persist in describing BPA as a weak estrogen effects and insist there is little concern with human exposure levels. Therefore the present study was aimed to investigate the harmful effect of BPA on the female rats and possible protective effects of *Salvia officinalis* In aqueous extract and NPs. the aim of study is performed via the following objectives [14].

1. Female reproductive hormones (FSH, LH, estrogen).
2. Liver function enzymes (AST, ALT and ALP).
3. Kidney function enzymes (Urea and Creatinine).
4. Histological section for (liver, kidney, uterus and ovary).

2. Bisphenol A (BPA)

Bisphenol A is synthetic organic compound have two equivalent groups of phenol, and its investigated as endocrine disruptor because it have estrogenic activity [15].

2.1. History of BPA

In 1891 bisphenol A synthesized but reported in 1930 's and retriever as compound have estrogenic properties and get good acceptance diethylstilbestrol (DES), and prescribed for women to facilitate the pregnancy, over 30 years and millions of women was taken it before it discovered as carcinogenic and could lead to reproductive defects in girls which burned and their mothers taken DES, because DES have same chemical structure and properties of BPA so it have same toxic effects of BPA [16].

BPA at first it enter in the made of epoxy resins (Plastic Historical Society, 2007). Bisphenol A used in the production of plastic in 1940 's, the production of bisphenol increased in 1950's when the plastic and poly resins used in commercial uses and developed in 1966's (Plastic Historical Society,2007). By accident many of BPA effect was discovered, BPA leaked from autoclaved polycarbonate flask will lead to increase proliferation of breast cancer [17].

2.2. Physical and Chemical Properties

Bisphenol A has common synonyms of BPA or 2,2-bis (4-hydroxyphenyl) propane. These products are light-weight, resistant to high temperatures, tough and adhesive in nature; making BPA a highly useful industrial compound [18]. Its structure consist from two unsaturated rings of phenol like diethylstilbestrol [19]. BPA occurs as a solid at room temperature and standard pressure in the form of prills, flakes and crystals so expect it have low volatility. BPA is considered to have low or moderate hydrophobicity and thus a modest capacity for bioaccumulation [20].

2.3. Production and uses of BPA

2.3.1. Production

BPA is one of the most chemicals which are widely used in the world and produced at 2 million ton per year [21]. Its demand increase per year, the growth demand expected 6- 10%. The increase in the production of BPA made it widely distributed in the world [22,23].

2.3.2. Uses

BPA is used as a hardening agent in plastic, it used in poly carbons and many other types including polycarbonate plastics epoxy resins. polycarbonate used in many materials, especially these are contact directly human, such as food container, baby bottles, etc. as well as it used in the medical equipment, eye glass, digital media (CD, DVD, cell phones) etc [24].

Epoxy resins are used as polymeric binder in protective inner coatings of food and beverage cans industrial learning, adhesives and industrial protective coatings [25]. Few amount of it (10%) used as fire retardants or antioxidant and stabilizer production of rubber and polyvinyl chloride (PVC) and other plastics, some of it may enter environment from sewage or (PVC) production by product [26].

2.4. Exposure to BPA

BPA A is a pseudo-persistent chemical, even it have short half-life it too diffuse because it continuous release in the environment [27]. The exposure to the BPA is increased due to increase use and production in the environment. the exposure to BPA is directly in the food or chronically from beverage packaging and storage container. The BPA leaches from the plastic when exposed to the heat or acidic environment so the exposure to the BPA increased [28].

2.4.1. Environmental exposures to BPA

BPA is ubiquitous chemical in the environment, so it famous contaminant. it has been found in the waste water from factories that produce and has been identified as the compound responsible for the majority of estrogenic activity of landfill leachate. BPA documented in the US in waters, private wells soil, ambient air, canned food and plastic packaging [29]. Bisphenol A is found in soil contaminated such as industrial soils. Bisphenol exposure in the environment occur in two ways, occupational exposure which occur in the workers in companies and the other way by consumer products [30].

BPA pollution is not only occur from the factories that produce BPA but occur from factories that use bisphenol in their products. BPA releases to the environment can also occur from sites where thermal fax paper is recycled. Discharges to air expected to break down fairly rapidly, but discharges to water are likely to be more persistent and more of a problem [31].

2.4.2. Exposure through Breast feeding

BPA was measured in the colostrum in the milk of many healthy women after three days of delivery, the BPA concentration range 1-7ng/ml so the colostrum and breast feeding they are route for BPA exposure for infants. As well as its can pass to the embryo through the placenta [32].

2.5. Timing of exposure

After exposure to BPA will cause effects on the body, some of these effects are organizational and continue through the life of the exposed animal. While some organizational effects are not detectable in the time of exposure but subsequently appear at the adulthood [33].

There is critical period of exposure, which give most potent effect, this effect occur when exposure occur in the time of development. The exposure to BPA also effects on embryo which delivered from mother exposed to BPA, the females embryo especially affected by bisphenol A. It affects the spermatogenesis in animals, decrease motility, concentration, morphology changes as well as sperm DNA damage [34].

2.6. Toxokinetics of BPA

After exposure of human and animals to BPA following oral exposure the absorption occur. The main metabolites dose in the blood. Bisphenol A and its metabolites are widely distributed in humans and animals body. More than 90% of non-metabolized BPA is reportedly bound to plasma protein [35].

Many studies have attempted to describe the inability to detect unconjugated serum BPA in one experiment conducted with a limited sample size and a relatively insensitive inspection. as an indication that all administered BPA is completely metabolized during its first pass through the liver. New findings show the kinetics of BPA metabolism are very similar in humans, monkeys, and mice and also suggest greater human exposure than earlier estimated [36].

The European Food Safety Authority (EFSA) count on pharmacokinetic evidence to conclude that rodent toxicity data are not directly relevant to human risk assessment. Further, the EFSA debate that rapid metabolism will result in negligible exposure during the perinatal period because of BPA glucuronidation in pregnant women or sulfation in newborns [37]. These causes fail to consider the deconjugation of BPA glucuronide in utero by β -glucuronidase, an enzyme that is present in high concentrations in placenta and various other tissues. Further, arylsulfatase C which reactivates endogenous sulfated estrogens develops early in life and so may deconjugate BPA sulfate in newborns [38]. Bio monitoring studies and laboratory experiments document free BPA in rat and human maternal, placental, and fetal tissues, indicating that human BPA exposure is not negligible. The pattern of these detections is consistent with deconjugation in the placenta, resulting in fetal exposure [39].

2.6.1. Absorption

BPA is absorbed through skin and GIT.the absorption of bisphenol in the GIT is rapid and cause severe damage to the liver, kidney and other organs (sax 1985). the absorption of BPA in the colon is more than in the jejunum [40].

2.6.2. Distribution and metabolism

Sulfation and glucoridation these are the major pathways for BPA metabolism. microsomal cytochrome p 450 also work in BPA metabolism Where it can be metabolized BPA via hydroxylation into catechol (5- hydroxyl BPA) (5-oH – BPA) that was further converted to bisphenol-o-quinine. bisphenol o-quinone is BPA product which produced as a result of BPA metabolism, bisphenol o-quinone bind irreversibly to DNA and result covalent modifications that cause hepatotoxicity reported that the glucuronidation of BPA was mediated by UGT2B1, an isoform of UDPGlucuronsyl transferase (UGT), in the rat liver with the production of BPA–monoglucronide metabolite. Sulfation of BPA by sulfotransferases in the liver is a metabolism pathway in mammals [41].

The major part indicated in plasma radioactivity after oral administration of BPA is the monoglucuronide conjugate of BPA in the plasma of rat BPA degrade by rat liver into 4-methyl-2,4- bis (p-hydroxyphenyl) pent-1 ene (MBP) which it have potent estrogenic activity BPA require both microsomal and cytosolic for metabolic activation in rat liver as well as in monkey, human and mouse liver S9 fraction. 4-Methyl-2,4-bis(4hydroxyphenyl)pent-1- ene (MBP)is the active metabolite of BPA wich it more active than BPA about 1000 times [42].

2.6.3. Excretion and / or retention

Despite BPA can be administrated by different ways, different dose and animals, BPA excreted in feces about (56% - 82 %) and in the urine at the range (13% - 28%). Inoue *et al.*,(2003) [40] said the BPA after absorption will be glucuronidated in the liver and excreted through bile. BPA can accumulate in the follicular fluid of female [43].

2.7. BPA as an Endocrine- disrupting chemicals

2.7.1. 1BPA is a xenoestrogen

At first bisphenol A was considered weak environmental estrogen 1000 once less than estradiol based on binding on alpha and beta receptors in the nucleus. while another recent studies said that the low doses of bisphenol may give high estrogenic effects such as estradiol [44]. BPA have ability to bind to the cellular estrogen receptors and give some estrogenic activity on the cells, and activate G protein receptors [45].

In addition of these effects some of BPA metabolites are more potent estrogens than its parent. BPA also can pass through the placenta and affect the neonate where exposed to BPA through fetal life. The rat exposed to BPA in early days of life may suffer from prostate cancer [46].

2.7.2. Effect of BPA on reproductive system

Depending on the dose and the period of exposure to the BPA the effect determined, estrogenic effect should affect female reproductive system. In utero exposure to environmentally relevant doses of BPA induced morphological and functional alterations in the reproductive tract in mice that were revealed during adulthood (at 3 months of age). These findings included decreased weight of the vagina and endometrial lamina propria, increased proliferative activity of epithelial cells in the endometrial glands and increased expression of estrogen receptors ER α and progesterone receptors PR in the luminal epithelium of the endometrium and sub epithelial stroma [47].

Naturally rat exposed to bisphenol A will reduce primordial follicle pool due to increase proliferation rate by estrogenic pathway. The long term exposure to BPA to the female mice will cause carcinogenic effect and reproductive adverse effects when it occurs in critical period of differentiation [48].

Pre natural development mice exposed to BPA will undergo from endometriosis like structure with the presence of both glands and stromal and expressing estrogen receptor cystic ovaries and adenomatous hyperplasia, the endometriosis which may lead to infertility. Also the rat exposed to bisphenol A at low doses, there hypothalamus will decrease its estrogen receptors [49].

Early BPA exposure has marked effects on mammary gland development, and it also promotes the development of intraductal hyperplasia and precancerous mammary lesions in adult rodents. Also the exposure to BPA will lead to decrease in the fertility and affect the reproductive tissue [50].

3. Nanotechnology and nanoparticles

Nanotechnology is the science that deals with the matter at the size 1 billionth of a meter (10^{-9} m = 1 nm) and also could be defined as the study of manipulating matter at the molecular and atomic scale. The typical size is ranged between 1 nanometer to 1 micrometer. Nanoparticle is the most essential constituent in the manufacture of Nano structure and is too smaller than the world of everyday objects that are described by the law of motion that generated by Newton, but bigger than atom or simple molecule that are measured by quantum [51,52].

Nanotechnology field the modern science of material and is the most active fields. Nanoparticles show specific properties and improved characteristics such as distribution, size and morphology. The Nano material's and nanoparticles have new application which are progressing rapidly [53].

Due to huge application of nanomaterial in the field of physics, chemistry, biology and medicine there are many researches made on synthesized Nano materials and their characterization is an emerging field of Nano technology from the past years. They can be synthesized by using many methods including chemical and physical, electrochemical thermal method and Photochemical [54].

Recently the green method for synthesis of Nano particles have been emerged due to ecofriendly economic method. In this method the plant extract are used reducing and stabilizing agent. A number of plant extracts used in the synthesis of SNPs such as *Elephantopus scaber*, *Thymbra spicata* *Salvia officinalis* [55].

3.1. Silver nanoparticles

Silver nanoparticles (SNPs) are very important metal used as nanoparticles among other types of metals. The SNPs which have size smaller than 100 nm containing about 10000- 15000 silver atoms. The synthesis of silver nanoparticles has attracted enormous attention due to their diverse various properties like electrical conductivity, optical, catalysis, magnetic polarize activity as well as antimicrobial activity [56].

3.2. Pharmacokinetics

The AgNP absorption in the GIT then distributed in different organs and accumulated in reticulo endothelial organs, particularly spleen, liver and lung. Several investigation in vivo recorded the liver as the main site of silver particulate accumulation beside brain and testis, Another research consider the lung are the route of uptake. The SNPs distributed

by the blood stream and accumulated in the liver, kidney, spleen, brain and lung. SNPs may enter some cells such as renal tubular epithelial cells and hepatic cells as well as blood brain barrier [57].

3.3. Medical application of silver nanoparticles

Silver nanoparticles (SNPs) are widely used in the medical applications. SNPs have been used in the treatment of virus, bacteria as well as eukaryotic microorganisms [58]. SNPs are of value in textile coatings, food storage, water purification systems, cosmetics and electronics. SNPs are available as alternative to antibiotics also in the treatment of burns, healing of wounds and prevent infections. SNPs also used as promotion of growth in the poultry [59].

Silver nanoparticles work as broad-spectrum antimicrobials by destroy their membrane. SNPs may work as antimicrobial by inhibiting bacterial reproduction, It can in ' inactivate proteins, stumbling respiration and electron transfer, and thence inactivating bacteria. The smaller size of silver nanoparticles give the highest activity against bacteria due to it have greater surface area, so will give the better effect [60]. The combination of silver nanoparticles with some antibiotics such as ampicillin, kanamycin, erythromycin and chloramphenicol will give synergistic effects as bactericidal against Gram-positive and gram-negative bacteria. Silver nanoparticles may have harm effects and cause cytotoxic effects on body cells [61,62].

4. *Salvia officinalis* (SO)

Salvia officinalis (sage) is one of the largest and important medical plant genera of the Lamiaceae family and it have about 900 species, widespread throughout the world, sage is the common name also used to refer to *salvia officinalis*. Sage is distributed in the Mediterranean countries beside other regions which it found. Family: Lamiaceae or mint family, Sub family: Nepetoideae, Common name: garden sage, common sage or Dalmatian sage. Local name in Mediterranean countries: Alremeian [63,64].

Salvia officinalis: is a perennial evergreen subshrub with woody stems, grayish leaves, and blue to purplish flowers. It is one of the family Lamiaceae and is native to the Mediterranean region, and it has naturalized in many other places hroughout the world. It has medicinal and culinary use, and in recent times as an decorative garden plant. More than 900 species of salvia spread throughout the world and used in the folk medicine. *Salvia officinalis* was described by C. Linnae in 1753 and it name come from Latin word " salve ", to save, to behealthy. to refer medical value of this plant [65,66].

4.1. Historical review

Salvia officinalis (SO, Sage) was used as medical plant to treat evil and snake bites, promote women's fertility, as well as it used as skin local anesthesia and diuretic. *Salvia officinalis* is one of ancient plants used by humans as a medical herb and considered as global panacea. Sage also recommended to be cultivated in the monastery gardens during Carolingian empire and early middle ages. It has been grow for centuries in the old world for it healing and food properties, in old herbs was often have prodigious properties [66,67]. Many species of *salvia officinalis* native in the Mediterranean region. In the past sage was used for mitigation fever, wasp sting, mental changes, insect bites and hair care [68,69].

Salvia officinalis used internally (as tea or directly chewed) for the treatment some respiratory disorder such as upper respiratory tract infection, asthma as well as dermal disorder and GIT. The sage leaves can be collected then crushed and boiled in water to inhale the fumes. *Salvia officinalis* essential oils have many pharmacological responses such as ant oxidative, antimicrobial, Anti-Proliferative and anticancer [70].

4.2. Composition of *salvia officinalis*

Salvia officinalis contain many of biologically active compounds such as essential oils, phenylpropanoid, flavonoid, glycosides phenolic acid and tannins. *Salvia officinalis* contain blend of monoterpenes, ditrepenes, sesquiterpenes with (branched, ilnear as well as saturated and unsaturated) aliphatic hydrocarbons, as well as many researchers recorded it have essential oils [71,72]. The camphor and 1,8-cineol also found in this plant. Sage leaves have many compounds such as tannin, triterpenoids, estrogenic substances and volatile oil, with vitamins C and A [73,74].

4.3. Medicinal use of *salvia officinalis* (sage)

Sage incorporated as flavoring substance with list of substances by food and drug administration (FDA), and some species are important economically which are used as flavoring of food, cosmetic, perfumery as well as it used in the folk medicine. Sage have antidiuretic and hypoglycemic effects on diabetes induced rats [75].

Essential oils are mixture of volatile substances which is formed as secondary products from metabolism of sage, these compounds were used as hair and skin care as well as healing of wound, In addition its used in the cosmetics, food and pharmaceutical industries. Sage also used as ecofriendly antiseptic properties which it mimic bacterial and fungal activity. Essential oils have anti-inflammatory effects on mucous membranes of upper part of respiratory system and mouth [76,77].

Sage also possess diuretic effects, as well as it used to treat coronary heart diseases, cerebrovascular diseases in addition for these effects sage have antimicrobial and antifungal anti-inflammatory activities. Anti-inflammatory, hypoglycemic, improvement of learning and memory, all these effects were seen in the *salvia officinalis* [78,79, 80].

Salvia officinalis have phenolic acid and flavonoids compound which make it have decrease weight and anti-obesity effects so it decrease triglycerides level in the blood and decrease hyperlipidemia. as well as the sage investigated for the treatment of the alzheimer's disease. *Salvia officinalis* improve the immune system by increase interleukin, cytokines production, so this develop it's antiviral effects. In addition for these the sage have other pharmacological effects such as antioxidant, antibacterial, antifungal, anti-inflammatory, the sage also have anti proliferative effects on cancer cell [81, 82, 83].

5. Conclusion

From our data, we conclude the BPA has harm effect on the body systems especially on the a-reproductive system, which causes disturbance in the level of hormones. b- cause changes in the blood picture. BPA cause hepatorenal toxicity. *Salvia officinalis* have protective effects against harmful effects of BPA which case ameliorative effects in the tested parameters. Nanoparticles get better effect than water extract.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this document.

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