The present study aimed to investigate the Silver nanoparticles reduces DMBA induced breast and leukemia cancer in Sprague Dawley rats. The anticancer activity of silver nanoparticles was assessed by monitoring the tumor volume, urine parameters, hematological parameters, biochemical estimations (Ferritin, C-reactive protein, ALP and Total protein), and breast tissue estimations (SOD, Catalase, GSH and LPO) at the end of the 90th day. The rats treated with silver nanoparticles 250 µg/kg bw ip and 500 µg/kg bw ip, have shown dose wise significant decreases in tumor volume, increases in RBC and decreases in WBC. Increases in SOD, catalase, GSH, decrease in LPO level when compared with DMBA induced rats. From the results of the study, it is concluded that silver nanoparticles treatment significantly alter the DMBA induced breast cancer and leukemia in rats this activity may be due to antioxidant, cytotoxic and anti-inflammatory activity of the drug.
INTRODUCTION:
Neoplasm or cancer is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal cell and persist in the same excessive manner after cessation of the stimuli which evoked the change.[1] The phrase melanoma came from a Greek words Karakinos to describe carcinoma tumors’ by a healthcare professional Hippocrates (460-370 B.C), but he was no longer the primary to observe this disease. One of the earliest proofs of human bone melanoma was once discovered in mummies in old Egypt and historic manuscripts dates about 1600 B.C. The sector’s oldest recorded case of breast melanoma hails from ancient Egypt in 1500 BC and it was recorded that there used to be no treatment for the melanoma, handiest palliative therapy. In step with inscriptions, surface tumors were surgically removed equivalently as they're removed today.[2]
Mortality produced by the International Agency for Research on Cancer, with a focus on geographic variability across 20 world areas. There can be an estimated 18.1 million new cancer circumstances and 9.6 million melanoma deaths in 2018. Lung cancer is probably the most regularly occurring melanoma and the main intent of cancer dying amongst adult males, adopted with the aid of prostate and colorectal cancer and liver and belly melanoma. Among females, breast cancer is probably the most frequently identified cancer and the main cause of cancer dying, followed by means of colorectal and lung cancer, and vice versa, cervical melanoma ranks fourth for both incidence and mortality.[3]
Cancer is named after the body part in which it originated; thus, breast cancer refers to the erratic growth and proliferation of cells that originate in the breast tissue. A breast is composed of two main types of tissues for example glandular tissues and stromal (supporting) tissues. A glandular tissue house of the milk-producing glands (lobules) and ducts (milk passages). The stromal tissues contain the fatty and fibrous connective tissues of the breast. The breast is also made up of lymphatic tissue-immune system tissue that removes cellular fluids and waste. There are several types of tumors that may develop within different areas of the breast. Most tumors are the result of benign (non-cancerous) changes within the breast. For example, fibrocystic change is a non-cancerous condition in which women develop cysts (accumulated packets of fluid), fibrosis (formation of scar-like connective tissue), lumpiness, and areas of thickening, tenderness, or breast pain.[4]
(DMBA), a polycyclic aromatic hydrocarbon (PAH), has been used extensively as a model carcinogen in cancer research. Studies have focused on the immunotoxicity of DMBA given to experimental animals to induce tumors in skin and mammary gland. It was also established that PAHs such as DMBA require metabolic activation for carcinogenicity. The carcinogenic potency of PAHs correlates with splenic immunosuppressive potency. Thus, PAHs could cause toxicities through two distinct mechanisms, aryl hydrocarbon receptor (AHR)- mediated and metabolic activation. Microsomal epoxide hydrolase (mEH) is an enzyme catalyzing hydrolysis of aliphatic and arene epoxides and these reactions are generally considered the detoxication pathway. The hydrolysis of epoxide derivatives of DMBA and benzo [a] pyrene is, however, required for a major metabolic pathway for activating carcinogens to the ultimate electrophilic derivatives. In the metabolic activation of DMBA, mEH is the only enzyme that transforms DMBA-3,4-epoxide to DMBA-3,4-dihydrodiol (DMBA- 3,4-diol), and then CYP1A1 or CYP1B1 oxidizes DMBA- 3,4-diol to the ultimate carcinogenic form, DMBA-3,4-diol-1,2-epoxide. mEH is expressed not only in liver but also in several extrahepatic tissues including kidney, testis, ovary, lung, thymus and spleen. Thus, mEH is believed to play a critical role in the multiorgan carcinogenesis of DMBA.[5]
A sliver nanoparticle (AgNP) is a soft, white, lustrous transition metal possessing high electrical and thermal conductivity. It has been known longer than the recorded history due to its medical and therapeutic benefits before the realization that microbes are agents for infections.
It is used in many forms as coins, vessels, solutions, foils, sutures, and colloids as lotions, ointments and so forth. It is the foremost therapeutic agent in medicine for infectious diseases and surgical infections. The benefits of silver are more than the risk factors.[6]

**MATERIALS AND METHODS**

**Chemicals**

7,12-dimethylbenz (a) anthracene (DMBA) were procured from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. Remaining all the chemicals used in the present study was purchased from Himedia, India. Biochemical kits were procured from Erba Mannheim Pvt. Ltd., Bangalore, India.

**Animals**

Healthy Female Sprague Dawley (150-250g) of 40-45 days old were procured from Biogen laboratory animals facility, Bengaluru, Animals were acclimatized for one week after procurement and then fed with normal pellet diet and water *ad libitum* throughout the experiment period. Standard laboratory conditions were maintained under controlled atmosphere (12:12 h light/dark cycles with an ambient temperature of 25±2 0C and humidity at 50±10%). Committee for the purpose of control and supervision of Experiments on Animals (CPCSEA) guidelines were followed in this study and the protocol was approved by the Institutional Animal Ethics Committee (IAEC approval no: PESCP/IAEC/82/2018) of PES College of Pharmacy Bengaluru, India. The animals were provided with standard pellet diet Amrut Laboratory Animal Feed, Mysore Feeds Limited, Bangalore, India.

**Induction of mammary carcinogenesis**

DMBA (Sigma Aldrich) was dissolved in refined soya oil and stirred slowly using glass rod with heating up to 900C to make it completely dissolved. Solubilized DMBA suspension was cooled and administered into the animals for the period of 90 days. All the groups of animals were administered through the intragastric gavage. The animals were regularly examined by palpation for the development of tumors. The animals were consistently monitored for the feed and water intake.[7]

**Preparation of silver nanoparticles solution (AgNP)**

The Silver nanoparticles were procured from Amnium Technologies private Limited (Kothrud, Pune, India). The stock of silver nanoparticles solution (10mg/ml) by dissolving in sodium citrate solution (0.02 mg/mL) and in 0.1 mM phosphate-buffered saline respectively. The solution of AgNPs was kept at 4 °C until use.

**Experimental design**

The total rats were divided into 5 groups and each group contained 8 rats. Group I animals were received the normal saline (2ml/kg bw po). Group II animals were received the 20 mg/kg intragastric gavage given DMBA in refined soya oil. Group III animals were received DMBA and Standard drug Cyclophosphamide (150mg/kg bw) given intraperitoneal. Group IV and V were received DMBA and Silver nanoparticles medium and high dose (250µg/kg & 500µg/kg bw) given by intraperitoneal for 90 days. Animal’s tumor volume weekly beginning on 35th day until the end of the study using Vernier caliper.

**Measurement of mammary tumor volume**

The mammary tumour volume was calculated using digital Vernier calipers. Tumour volume = \( V = \frac{W^2 X L}{2} \),

Where \( W = \) Tumour width, \( L = \) Tumour length.[8]

**Urine analysis:** Urine was collected through metabolic cages for the analysis. Urine was then analyzed for the levels of creatinine ratio by ERBA Kit method in auto-analyzer.

**Hematological analysis.** Blood was collected last day of the treatment through retro-orbital puncture. Blood was then analyzed for the hematological parameters of RBC, WBC and Differential counts.

**Serum analysis**

Blood was collected last day of the treatment through retro-orbital puncture and processed...
into serum by centrifugation at 3000 rpm for 15 min. Serum was then analyzed for the levels of serum ferritin, Alkaline phosphate (ALP), C-reactive protein and total protein, by ERBA kit method in auto-analyzer.

**Breast tissue estimation**

Animals were sacrificed the breast tumor tissue is washed thoroughly and rinsed with ice. Then tissue were gently blotted between the folds of a filter paper and weighed in an analytical balance. 10% homogenate was prepared in 0.05M phosphate buffer (pH 7) using a homogenizer at 4°C. The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was used for the estimation of SOD, GSH, Catalase and LPO.

**Statistical analysis**

All data were expressed as the mean ± standard deviation (SD). Statistical significance was determined by one way ANOVA followed by Dunnett’s Multiple Comparison Test.

**RESULTS**

**Tumor volume**

Data represented in the Table 1, DMBA control group showed increases the tumor volume p<0.0001 considered as significant compared to normal control. Silver Nanoparticle treated (250µg/kg bw and 500 µg/kg bw) group showed significant response of ***p<0.0001 and ***p<0.0001 respectively when compared to DMBA control group by decreases in tumor volume levels. The standard drug cyclophosphamide has been showed a significant decreases response ***p<0.0001 as compared to DMBA control group.

**Table No 1: Effect on tumor volume in normal rats and treated rats.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor volume in mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0</td>
</tr>
<tr>
<td>DMBA Control</td>
<td>770.48±201.26***</td>
</tr>
<tr>
<td>DMBA + CYP</td>
<td>366.08±91.47***</td>
</tr>
<tr>
<td>DMBA+ AgNP (250µg)</td>
<td>424.51±56.13***</td>
</tr>
<tr>
<td>DMBA+ AgNP (500µg)</td>
<td>252.36±82.95***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± SD of 8 animals in each group and significantly different P<0.05.

**Fig 1:** (a) Mammary tumors development DMBA induced female Sprague Dawley rats. (b) Measuring of tumor volume using Vernier caliper in treated rats
Urine parameter

**Creatinine:** Data represented in the Table 2, DMBA control group showed significant increases in the levels of urine creatinine of p<0.0001 as compared to Normal group. Silver nanoparticle (250µg/kg bw and 500 µg/kg bw) group showed significant response of ***p<0.0001 and ***p<0.0001 respectively when compared to DMBA control group by decrease in creatinine levels. The standard drug Cyclophosphamide has been showed a significant decrease response ***p<0.0001 as compared to DMBA control group.

**Table No 2: Effect of creatinine levels in normal rats and treated rats.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.15±0.033</td>
</tr>
<tr>
<td>DMBA Control</td>
<td>0.30±0.0596***</td>
</tr>
<tr>
<td>DMBA + CYP</td>
<td>0.19±0.0212***</td>
</tr>
<tr>
<td>DMBA+ AgNP (250µg)</td>
<td>0.20±0.0229***</td>
</tr>
<tr>
<td>DMBA+ AgNP (500µg)</td>
<td>0.16±0.0433***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± SD of 8 animals in each group and significantly different P<0.05.

**Hematological parameter**

**RBC:** Data represented in the table 3, DMBA control group showed significant Decreases in RBC counts when compare with normal control. Silver nanoparticle (250µg/kg bw and 500 µg/kg bw) group showed significant response of ***p<0.0001 and ***p<0.0001 respectively when compared to DMBA control group by increases in RBC Counts. A standard drug Cyclophosphamide has been showed a significant increases response **** p<0.0001 as compared to DMBA control group.

**WBC:** Data represented in the Table 3, Rats treated with silver nanoparticles (250µg/kg bw and 500 µg/kg bw) group showed significant response of ***p<0.0001 and ***p<0.0001 respectively when compared to DMBA control group by decrease in the WBC counts. A standard drug of cyclophosphamide have shown a significant increase response ***p<0.0001 as compared to DMBA control group.

**Table No 3: Effect of RBC and WBC counts in normal rats and treated rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>RBC Count</th>
<th>WBC count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>8.11±0.1814</td>
<td>10847±813.77</td>
</tr>
<tr>
<td>DMBA Control</td>
<td>5.42±0.4734***</td>
<td>20291.87±1190.51***</td>
</tr>
<tr>
<td>DMBA + CYP</td>
<td>6.94±0.5667***</td>
<td>11711.87±860.01***</td>
</tr>
<tr>
<td>DMBA+ AgNP (250µg)</td>
<td>7.16±0.1858***</td>
<td>12694.37±1321.03***</td>
</tr>
<tr>
<td>DMBA+ AgNP (500µg)</td>
<td>7.36±0.3125***</td>
<td>11474.57±568.14***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± SD of 8 animals in each group and significantly different P<0.05.
Biochemical parameters
Data represented in the table 4, Fig.2  DMBA control group showed decreased in the (total protein, ferritin) and increased in the (C-reactive protein, Alkaline phosphate) ***p<0.0001 and ***p<0.0001 considered as significant compared to normal group. AgNP treated (250µg/kg bw and 500 µg/kg bw) group showed significant response of ***b and C*** with p<0.0001 and p<0.0001 respectively when compared to DMBA control group by increased in the (total protein, ferritin) and decreased in the (C-reactive protein, Alkaline phosphate). A standard drug of cyclophosphamide has been shown a significant response a***with p<0.0001 as compared to DMBA control group.

Table No 4: Effects of serum parameters (Ferritin, C-reactive protein, Alkaline phosphate and Total protein,) in normal, DMBA induced Breast cancer and treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ferritin (µg/l)</th>
<th>CRP (mg/l)</th>
<th>ALP (IU/l)</th>
<th>Total protein g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>81.65±1.924</td>
<td>4.21±0.2998</td>
<td>68.06±4.697</td>
<td>6.49±0.5250</td>
</tr>
<tr>
<td>DMBA Control</td>
<td>42.87±2.309***</td>
<td>13.58±0.7179***</td>
<td>117.75±6.337***</td>
<td>4.09±0.320***</td>
</tr>
<tr>
<td>DMBA + CYP</td>
<td>73.22±3.402***</td>
<td>6.91±0.5882***</td>
<td>70.56±2.175***</td>
<td>5.95±0.229***</td>
</tr>
<tr>
<td>DMBA+ AgNP (250µg)</td>
<td>80.36±2.027***</td>
<td>12.09±0.5001***</td>
<td>65.62±3.452***</td>
<td>5.54±0.246***</td>
</tr>
<tr>
<td>DMBA+ AgNP (500µg)</td>
<td>85.53±2.206***</td>
<td>9.12±0.8115***</td>
<td>58.43±3.258***</td>
<td>5.13±0.239***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± SD of 8 animals in each group and significantly different P<0.05.
Fig. 2. Effect of AgNP on Serum parameters. (a) Ferritin, (b) CRP and (c) ALP: DMBA group

*** p<0.0005 compared to the normal control; Cyclophosphamide, AgNP (250µg/kg) and AgNP
(500µg/kg) a*** P<0.0005, b*** P<0.0005, c*** P<0.0005 compared to the DMBA control. (d) Total
protein: DMBA group *** p<0.0005 compared to the normal control; Cyclophosphamide, AgNP
(250µg/kg) and AgNP (500µg/kg) a*** P<0.0005, b*** P<0.0005, c*** P<0.0005 compared to the
DMBA control.

Evaluation of breast tissue antioxidants enzymes

DMBA induced breast cancer rats shows the effect of silver nanoparticles in antioxidants by enhanced oxidative stress. The given Table no:5 and fig.3 indicated the activity of superoxide dismutase, catalase, glutathione and Lipid peroxidation.

The levels of enzymatic antioxidants, like SOD and CAT in the breast tissue of DMBA control showed decreased response when compared to the normal control. AgNP treated (250µg/kg bw and 500 µg/kg bw) rats improved the activity of SOD and CAT levels showed significantly increases in both of doses when compared to DMBA control. Silver nanoparticles suppressing the oxidative stress prompted by DMBA. Though, cyclophosphamide treated rats also manifested improved effect on SOD and CAT activity showed increases when compared to DMBA control rats.
The levels of non-enzymatic antioxidants of GSH in breast tissue of control and experimental rats. In untreated DMBA control rats the levels of GSH were significantly decreased when compared to normal control. AgNP treated (250µg/kg bw and 500 µg/kg bw) rats, the levels of GSH were significantly increased when compared with DMBA control group. Cyclophosphamide rats showed significantly increased when compared to the DMBA control groups.

The levels of lipid peroxidation antioxidants levels in breast tissue of control and experimental rats. In untreated DMBA control rats the levels of lipid peroxidation were significantly increased when compared to normal control. AgNP treated (250µg/kg bw and 500 µg/kg bw) rats, the levels of LPO were significantly decreased when compared DMBA control group. A standard drug of cyclophosphamide rats showed significantly decreases when compared to DMBA control groups.

Table No 5: Effects on antioxidants enzymes (SOD, CAT, GSH and LPO) in normal

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD U/mg</th>
<th>Catalase U/mg</th>
<th>GSH U/mg</th>
<th>LPO nMol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>13.64±0.694</td>
<td>68.71±1.4240</td>
<td>18.79±1.340</td>
<td>14.33±1.097</td>
</tr>
<tr>
<td>DMBA Control</td>
<td>7.57±0.6573***</td>
<td>40.25±1.179***</td>
<td>8.446±0.937***</td>
<td>48.56±1.010***</td>
</tr>
<tr>
<td>DMBA + CYP</td>
<td>12.57±0.6901***</td>
<td>65.65±1.613***</td>
<td>17.63±1.168***</td>
<td>20.38±1.037***</td>
</tr>
<tr>
<td>DMBA+ AgNP (250µg)</td>
<td>12.06±0.930***</td>
<td>61.83±1.454***</td>
<td>17.17±0.714***</td>
<td>22.35±1.225***</td>
</tr>
<tr>
<td>DMBA+ AgNP (500µg)</td>
<td>13.02±0.620***</td>
<td>64.23±1.194***</td>
<td>18.20±0.880***</td>
<td>18.54±1.132***</td>
</tr>
</tbody>
</table>

The values were expressed as mean ± SD of 8 animals in each group and significantly different P<0.05.
DISCUSSION
In the present study, Breast cancer is the most common type of cancer and the leading the cancer of death. Breast cancer is induced by intragastric gavage of 7,12-Dimethyl Benz (A) Anthracene (DMBA) in experimental female Sprague Dawley rats. DMBA is a well-known potent carcinogen which has been used to induce carcinogenesis in the mammary gland or skin of experimental rodents such as rat and mouse. Breast tissue may be a major target for the toxicological effects of a variety of lipophilic carcinogens such as polycyclic aromatic hydrocarbon (PAH), if such compounds are not bio transformed to hydrophilic metabolites that are easily excretable. Metabolic activation of DMBA, the member of the PAH family, produces radical cations, free radicals and oxygenated metabolites. DMBA can induce substantial oxidative damage in various bodily organs (in particular liver and breast), a property which has made DMBA a suitable and useful agent for generating in vivo models of rat.(12,13) During the puberty, pregnancy, and lactation, the mammary gland undergoes a very significant change because of the mammary gland is one of the several organs that always experience changes the development of birth.(14) Cyclophosphamide is most commonly used chemotherapeutic agent in the treatment of cancer. It is also used in the treatment of some connective tissue and autoimmune diseases, minimal lesion glomerulonephritis and for the control of organ rejection after transplantation.(15) Cyclophosphamide was given intraperitoneal (i.p) for
(1-12 weeks), also known as immunomodulatory effect, changes in tumor growth may also be attributable to alternate mechanisms. Glutathione (GSH) is a major endogenous antioxidant; with important roles in detoxifying free radicals and reactive oxygen species. Decreased GSH and/or increased oxidative stress may impact expression of vascular endothelial growth factor (VEGF), the major angiogenic factor during epithelial carcinogenesis in a large number of human cancers and metastase. Results showed that the intraperitoneal injection of AgNP showed anticancer activity against the DMBA induced mammary carcinoma and leukemia in rats was confirmed by following measures and estimation. The AgNP treated animal exhibited decrease in tumor volume when compared with DMBA control group this activity is indication of cytotoxic effect of AgNP. RBC and WBC counts treated of AgNP showed the RBC count increased when compared to the DMBA control rats. WBC counts decreases the AgNP treated rats when compared to the DMBA control. Hence this activity indicates the cytotoxic potential of the AgNP. Urine creatinine levels in AgNP treated rats showed the significantly increases when compared with DMBA control. The treatment drug indicates the Nephrotoxic agent. Silver nanoparticles exhibited decrease in C – reactive protein level and alkaline phosphate when compared with the DMBA treated animal. This activity is indication anti-inflammatory potential of the Silver nanoparticles. Ferritin and total protein levels of rats treated with silver nanoparticles exhibited increases in ferritin and total protein levels when compared with the DMBA rats. Breast Tissue antioxidant enzymes: The animal treated with the Silver nanoparticles has showed increase in SOD, catalase, GSH activity and decrease in LPO level when compare to DMBA treated rats. Hence it reveals that Silver nanoparticles could restore the activity of these antioxidant enzymes and possibly could reduce generation of free radicals and cellular damage. Silver nanoparticles significantly reduced the toxic elevated level of Malondialdehyde. This conformed that the oxidative stress in the tissue was reduced.

CONCLUSION

The present study demonstrated that that DMBA administration was associated with the development of breast tumor, elevated levels of tumorigenicity and oxidative stress markers. Silver nanoparticles administration promoted the decrease of tumorigenicity and oxidative stress levels. From the results, it is evident that silver nanoparticles is capable of protecting the breast tissue against oxidative. Further, the showed the ability of silver nanoparticles in inhibiting cell proliferation, inflammation and tumor development. Overall, finding of our study confirm the chemopreventive potential of silver nanoparticles against DMBA induced breast cancer in female Sprague Dawley rats.

CONFLICT OF INTEREST

Declared as none

ACKNOWLEDGEMENT

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REFERENCE


