CISSUS QUADRANGULARIS EXTRACT MEDIATED GREEN SYNTHESIS OF SILVER NANOPARTICLES: PHARMACOLOGICAL APPLICATIONS

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Abstract
The present investigation was focused with much attention to investigate the pharmacological activity of Cissus quadrangularis plant extract mediated silver nanoparticles. Anti-inflammation activity of the silver nanoparticles of the plant Cissus quadrangularis in mice model was studied using formalin induced paw oedema method. The anti-obesity effect of nanoparticle synthesized plant extract Cissus quadrangularis with fat rich diet of egg feed induced in albino mice was also assessed. Nanoparticles were also studied for its immune response against SRBC with hydrocortisone induced mice model. The oral administration of plant extract (100mg/kg) exhibited increased the percentage of inhibition paw oedema with 50% at 4 hours while the standard drug paracetamol (100mg/kg) revealed a less percentage of inhibition paw oedema with 37.5% after 4 hours. It was found out that, the amount of SGOT and SGPT enzymes present in the serum of Hydrocortisone treated mice revealed 184U/ml and 149 U/ml. The results of hypolipidemic effect revealed that, the nanoparticle alone significantly reduced the total cholesterol to 128 ± 4.1 mg/dl rather compared to other groups. The nanoparticles obtained from Cissus quadrangularis exhibited significant anti-inflammatory, immunomodulatory and hypolipidemic activities without any serious side effects

Keywords:
Cissus quadrangularis, silver nanoparticles, antiinflammation, anti-obesity, immunomodulation

Introduction
Cissus quadrangularis is the most common species, belonging to the family Vitaceae, commonly known as “Hadjod” in Hindi or bone setter due to its bone fracture healing property. [1] It is one among the most frequently used medicinal plants in India which can be found throughout the country. In Siddha system of medicine, it is used for healing bone fracture, piles, as an anti-aging herb, in Asthma, cough and gonorrhea. [2] Inflammation is a normal protective response of living tissue to injury caused by physical trauma, Hypersensitivity, noxious chemicals or Etiologic agents. It involves a well organized cascade of fluid and cellular changes within living tissue.[3] It is characterized by redness (rubor), swollen joint that is warm to touch, joint pain, its stiffness and loss of joint function. [4] The free radicals especially, the reactive oxygen species (ROS) creates oxidative stress in the cells leading to inflammatory and infectious condition. Phagocytic cells including polymorphonuclear leukocytes (neutrophils, eosinophils) and mononuclear cells (macrophage and lymphocytes) produce excessive amount of ROS which play an important role in the host defense mechanism. Besides their defensive effects these excessively produced ROS deregulate the cellular functions causing cellular and tissue damage, which in turn augments the state of inflammation.[5] Cellular antioxidant enzymes and the free radicals scavengers normally protect a cell from the toxic effects of free radicals. When the balance between free radical production and antioxidant defenses is lost, ‘oxidative stress’ results which through a series of events deregulates the cellular functions leading to various
pathological conditions including diabetes mellitus, atherosclerosis, ageing and inflammatory diseases.\[6\] Antioxidant based drugs and formulations for the prevention and treatment of complex diseases like Alzheimer’s disease and cancer have appeared during last three decades.\[7\] Increased consumption of whole grains, fruits and vegetables reduce the risk of chronic diseases like cancer and heart diseases.\[8\] Diabetes mellitus is characterized by elevated blood glucose levels. The disease cannot be cured totally and progresses with time to more complications like cardiovascular, eye, and kidney disorders. Existing allopathic drugs like metformin or glibenclamide (both administered orally) as well as insulin injections can lower blood glucose but are costly and not properly available to the rural people of Bangladesh. The disease is reaching endemic proportions in Bangladesh and the average cost for treatment of merely diabetes without added complications is beyond the capability of most people.\[9,10\] Herbal drugs can therefore be considered as a better alternative to synthetic anti-inflammatory drugs. The qualitative phytochemical analysis of extracts from the roots and leaves of Cissus quadrangularis showed the presence of glycosides, alkaloids, flavonoids, phenolic compounds, saponins, steroids and tannins.\[11\] Plants are a reliable source for the treatment of diseases. According to WHO, approximately eighty percentages of the people depend on traditional medicines for their primary healthcare. The drug industries also depend on plants for new drugs because synthetic medicines produce side effects .\[12\] The most “green” eco-environmentally technologies and chemicals are becoming increasingly popular.\[13\] Silver nanoparticles are reported to possess anti-viral , anti-bacterial.\[14,16,17,18\] Obesity is a serious health problem. Among the multiple factors contributing to its etiology, the sedentary life styles, white collar jobs, lack of exercise, psychological factors, and the consumption of energy rich diets are the major ones.\[19\] Developing countries like India are also struggling to manage the impact of myocardial infarction along with the growing burden of obesity, Type II diabetes and hypertension .\[20\] In recent years, an increasing number of young Indians are succumbing to myocardial infarction due to unusual risk factors characterized by high triglycerides, low High Density Lipoproteins (HDL), glucose intolerance, insulin resistance, abdominal obesity and increased lipoprotein (a) levels.\[21\] Natural products have been the starting point for the discovery of many important modern drugs. This fact has led to chemical and pharmacological investigations and general biological screening programs for natural products all over the world.\[22\] It is mandatory to study plant mediated biological synthesis of nanoparticles is growing importance due to its simplicity and eco-friendly. Hence in the present investigation, much focus of attention were given by extracting Cissus quadrangularis to validate its medicinal importance by synthesizing nanoparticles of Cissus quadrangularis against inflammation, antimicrobial activity, obesity studies in mice model was carried out.

Materials and methods
The present project was carried out in the department of PG Biochemistry at V.V.Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India. The preliminary work (AgNPs synthesis) was done in V.V.Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India. The pharmacological activity of mice study was carried out in the Venture Institute of Biotechnology and Bioinformatics Research, Madurai.

Collection of samples
The plant Cissus quadrangularis was collected from Madurai District, Tamil Nadu, India. The plant was identified and authenticated by a botanist Assistant Professor Dr.B.Karunaiselvi Department of Botany, V.V.Vanniaperumal College for women, Virudhunagar, Tamilnadu,India. The stem part of the plant was washed several times with distilled water and kept for drying under shade. After drying, it was powdered using a domestic grinder. The plant powdered was then stored in an airtight container.

Preparation of the extract
10g of plant powder were weighed and was extracted with 80 ml of petroleum ether and 20 ml of chloroform using soxhlet apparatus with temperature maintained for extraction was 55°C. The duration of soxhlet extraction was 3 to 4 hours. The extracts were concentrated by pouring them into clean round bottom flask and were allowed for evaporation of solvents by using distillation apparatus. Then the concentrated crude extract was stored at 4°C in airtight bottle until further use.

Preparation of stock solution of plant extract
400mg of extract was taken and dissolved in 40 ml of double distilled water and filtered using Whatmann filter paper No 1. It was stored at room temperature for further experiments as stock solution.
Synthesis of silver nanoparticles (AgNps)

In a typical synthesis of silver (Ag) nanoparticles, the stock solution (40 ml) was added to 10 ml of 10mM silver nitrate aqueous solution and kept at room temperature. The experiment was done in triplicate for reproducibility. After 1 hour the colour of the solution changed from colourless to honey brown indicating the formation of silver nanoparticles this is confirmed by UV-visible spectroscopy.

Evaluation of pharmacologic studies using nanoparticles

Anti-inflammatory study

Experimental design

Albino mice of either sex weighing between 130-170 g were procured from animal house Venture Institute of Biotechnology and Bioinformatics Research, Madurai used for the present study. They were maintained under standard conditions (24 - 28°C) and fed a standard diet for mice and given water. The care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India.

Formalin-induced mice paw oedema

Albino rats of both sexes weighing 150-200g were divided into 3 groups. Overnight fasted adult rats were randomized into 3 groups and treated as follows. First group was given saline orally- 5ml/kg (control), second group was injected paracetamol 10mg/kg subcutaneously and the next groups were given 100 mg/kg concentration of nanoparticles orally. After 30minutes, formalin 0.1 ml of 1% formaldehyde was injected in the planter region of the right paw of all the rats. The right paw (non-inflamed) served as reference for comparison. The circumference of all rats was measured at 1, 2, 3 and 4 hours after changes. The volume was measured before and after 3 hours of formalin treatment.[23]

\[
\text{Percentage of Anti-Inflammatory Activity} = \frac{V_c - V_t}{V_c} \times 100
\]

\(V_c = \text{control}\)
\(V_t = \text{test sample}\)
\(V_t = \text{Mean paw volume in the drug treated group}\)
\(V_c = \text{Mean paw volume in control group}\)

Immunomodulation

Albino mice weighing 100-200g were divided into 3 groups. Each group comprise 3 animals. A volume of 1% hydrocortisone was injected intradermally into animal’s abdomen. Group I animals served as control. Group II animals injected with hydrocortisone and provided egg feed only. Group III animals injected with hydrocortisone and provided egg feed and with nanoparticles extract treated mice for 10 days. At the end of the study all the mice were decapitated for collection of blood samples to carry out biochemical assays.

Obesity

The animals were made obese by administration of high fat diet (2:1 ratios of ghee and animal feed). Then they were grouped into 4 groups, 3 animals in each group. The animals were identified by picric acid marking. Group I animals served as control. Group II animals induced with obesity. Group III were induced with obesity and treated with silver nanoparticale. Group IV were silver nanoparticles alone treated mice for 10 days. At the end of the study, the blood was collected from all the rats to carry out decapitated for collection of blood samples to carry out biochemical assays. Serum Lipid profiles was determined by standard procedure. Body weight of the mice at regular interval recorded after 10 days treatment.[24]
In the present investigation, plant mediated AgNPs was synthesized. The biosynthesis of NPs was initially detected by the colour change of the solution from light yellow to honey brown. The synthesized NPs were pharmacologically examined.

### Anti-inflammation

Anti-inflammation activity of the *Cissus quadrangularis* nanoparticles in mice model was studied using formalin induced paw oedema method. The relative percentage of oedema induced inhibition by the test compound (plant extract) was recorded after 1.2 and 3 hours causing oedema with 7.0 %, 18.0 %, 39.0 % and 50.0 % at 4 hours of exposure respectively. Then the percentage of inhibition was progressively increased and reached the maximum inhibition with 50 % at 4 hours.

<table>
<thead>
<tr>
<th>S.N</th>
<th>Treatment of animal</th>
<th>Before formalin induced paw size (mm)</th>
<th>After formalin induced paw size (mm)</th>
<th>Percentage of inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1.2</td>
<td>2.3</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Standard (paracetamol) 100mg/kg</td>
<td>1.5</td>
<td>3.6</td>
<td>7.00 18.00 39.00 50.0</td>
</tr>
<tr>
<td>3</td>
<td>Test (<em>Cissus quadrangularis</em> nanoparticles) 100mg/ kg</td>
<td>1.0</td>
<td>2.8</td>
<td>7.00 18.00 39.00 50.0</td>
</tr>
</tbody>
</table>

### Immunomodulation

The present study was undertaken to assess the immune system which is one of the main indication for the therapy with the herbal immunomodulators. Since *Cissus quadrangularis* can able to restore the decreased immune response in immuno suppressed animals like mice, the present study the effect of herbal extract on the antibody response against SRBC in immuno suppressed mice with hydrocortisone. Table 2 depicts a significant increase in the spleen weight and the total cell number in spleen of controlled treated mice. Whereas, treatment of mice with hydrocortisone lead to a significant immuno suppression expressed in decreasing spleen weight of 0.14±0.03 over the control which is raised to 0.19±0.04. The number of spleen cells was found to decrease in hydrocortisone treated mice 3.9± 1.6×10^7 whereas, it was found to increase in control treated mice. The effect of hydrocortisone induced in mice were compensated by the treatment with extract of *Cissus quadrangularis* exhibited and cell yield of 5.7 ±0.8×10^7. The role of serum enzymes SGOT and SGPT of 184 U/ml and 149 U/ml with hydrocortisone treated mice whereas, a decreased level of enzyme serum biomarkers of SGOT and SGPT revealed 168U/ml and 130.2 U/ml which was found to be decreased. However, the control treated mice exhibited 163 U/ml and 136 U/ml for SGOT and SGPT respectively.

<table>
<thead>
<tr>
<th>S.N</th>
<th>Groups</th>
<th>Spleen weight (g)</th>
<th>Cell yield/Spleen</th>
<th>Level of serum SGOT</th>
<th>Level of serum SGPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.19 ± 0.04</td>
<td>6.5 ± 0.3 × 10^7</td>
<td>163.8 U/ml</td>
<td>136.5 U/ml</td>
</tr>
<tr>
<td>2</td>
<td>Hydrocortisone</td>
<td>0.14 ± 0.03</td>
<td>3.9 ± 1.6 × 10^7</td>
<td>184.0 U/ml</td>
<td>149.0 U/ml</td>
</tr>
<tr>
<td>3</td>
<td>Hydrocortisone and plant extract (AgNPs of C.Q)</td>
<td>0.18 ± 0.02</td>
<td>5.7 ± 0.8 × 10^7</td>
<td>168.5 U/ml</td>
<td>130.2 U/ml</td>
</tr>
</tbody>
</table>
Obesity

High cholesterol diet such as egg feed diet is also associated with dyslipidemia as well as hypertension. In the present study, the lipid profile in serum control and experimental treated groups to assess the obesity with and without the nanoparticles, Cissus quadrangularis were performed. The results revealed and increased cholesterol in group II that is mice feed with egg diet showed 178.30 ± 2.00 mg/dl over the control (normal) mice which exhibited 107.50 mg/dl.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (Induced obesity fed with egg)</th>
<th>Group III (Induced obesity and treated with nanoparticles)</th>
<th>Group IV (silver nanoparticle alone treated mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cholesterol (mg/dl)</td>
<td>107.50 ±0.58</td>
<td>178.30±2.00</td>
<td>172.5±0.82</td>
<td>128±4.1</td>
</tr>
<tr>
<td>2</td>
<td>Triglycerides (mg/dl)</td>
<td>74.28±0.23</td>
<td>169±3.0</td>
<td>110.43±0.46</td>
<td>74.01±0.088</td>
</tr>
<tr>
<td>3</td>
<td>HDL</td>
<td>33.1±0.06</td>
<td>40.0±0.09</td>
<td>37.9±0.07</td>
<td>32.5±0.06</td>
</tr>
<tr>
<td>4</td>
<td>LDL</td>
<td>62.5±0.06</td>
<td>133.08±0.09</td>
<td>72.9±0.08</td>
<td>69.75±0.95</td>
</tr>
</tbody>
</table>

Similarly the nanoparticles synthesized plant extract Cissus quadrangularis along with egg feed induced mice revealed a significant reduction in total cholesterol with 112.5 ± 0.82 followed by group IV which exhibited 128 ± 4.1 mg/dl that is nanoparticle synthesized plant extract alone treated mice from the above results. Obesity can be reduced with the plant extract Cissus quadrangularis. To analyze the triglycerides where in a significant increase in triglyceride level was observed with egg feed diet when compared to the control which exhibited 74.28 ± 0.23 mg/dl. Studies pertaining to HDL revealed group II mice showed 40.0 ± 0.09 mg/dl over the normal control treated mice showed 33.2 mg/dl. It is further interesting to note that group III mice induced with obesity and treated with plant extract found to express in minimum decrease of 37.9 over the plant extract alone treated mice (group IV).

Discussion

The green chemistry approach towards the synthesis of silver nanoparticle was studied and it has many advantages such as, eco-friendly rapid approach and economic viability application of this eco-friendly nanoparticle antibacterial, obesity, anti-inflammatory, and immunomodulation were performed. The anti-inflammatory performance of the nanoparticles was confirmed for the structural relative activity. Nanoparticles were tested for anti-inflammatory study with paw oedema test. The result showed that the compound possessed anti-inflammatory activity. The formalin induced paw oedema is one of the most important commonly employed techniques for evaluation of anti-inflammatory drugs. It is based upon the ability of drug to inhibit oedema induced hind paw of mice in the planter tissue of paw. In general, nanoparticles exhibited promising anti-inflammatory activity by oral administration at a dose of 100 mg/kg compared to the reference drug paracetamol and relative percentage inhibition of oedema which recorded the values after 1 hour was 7% and after 39%. It was noteworthy to emphasize a maximum inhibition with 50% after 4 hours recorded revealed an excellent anti-inflammatory activity which may be attributed due to substitution of phenyl ring at fourth position which give rise to an increase anti-inflammatory activity. The methanolic extracts of the Cissus quadrangularis are proved to possess pharmacological activities such as antioxidant, antulcer, analgesic and anti-inflammatory. In the present study, the immune response suppressed through treatment with hydrocortisone was normalized or modulated after administration of Cissus quadrangularis nanoparticles. Since the hydrocortisone induced immunosuppression is associated with decrease in number of T-lymphocytes and it lead to decrease in interleukin production also. Besides the plant extract possessing immunological potential for the activation of phagocytic cells and the presence of polysaccharides also stimulate cytokine production. Our results where in total agreement with Bodinet et al., 2002. The influence of herbal extract triggered the production of interleukin. The influence of herbal extract on antibody response was examined by plant forming cell assay. It is interesting to note that administration of nanoparticles may cause a significant enhancement of antibody response against SRBC leading to increase in the number of spleen cells and spleen weight but the immnosuppressed mice by treatment with hydrocortisone. The plant extract played on important role in normalizing antibody response against red blood cells (RBC).
The present study was aimed to assess anti-obesity effect of nanoparticle synthesized plant extract *Cissus quadrangularis* with fat rich diet of egg feed induced in albino mice. Initially the body weight of mice in all groups was in the range of 71 to 110 g. After 10 days the high fat diet consumed mice (group II) increased in their body weight. Then it was treated with *Cissus quadrangularis* a medicinally important plant drugs. A gradual reduction in body weight was observed with the plant extract alone treated mice the body weight more or less same. Our results are in agreement with Keisu et al., 2004 [28] has been reported that after induction with hemogranate seed oil the body weight of mice were significantly decreased. The present study findings suggested that *Cissus quadrangularis* treatment has positive effect on maintaining body weight of obese mice. Studies made on lipid profile between serum control and experimental treated groups exhibited a drastic differences observed in lipid profile especially the cholesterol found to increase significantly in egg feed diet. Whereas after treatment with nanoparticle synthesized plant extract showed a significant reduction in cholesterol. Our results were in total conformity with jiji et al., 2009.[24] Similarly the lipid profile of triglycerides clearly envisaged reduction in plant extract and egg feed mice where as group III indicated significant increased triglyceride content. Similar study was made presently with the activity of triglycerides emphasis that mice supplemented with egg lead to increasing cholesterol and triglycerides leading to increased hypertension in mice. [29] High cholesterol diet such as egg feed diet is associated with dyslipidemia. Similarly the other lipid profile parameters which include HDL and LDL exhibited similar results. LDL was found to be high in experimentally treated group II compared to group I. The above results coincides with the findings of Laurance et al., 2001 and Vijaya, 2009[30,31] reported the lipid lowering effect of 50% ethanolic extract of *Aegle marmelos* triton and diet induced hyperlipidemic model of albino mice. In tune with above discussion the level of HDL was assessed. HDL level has found to be increased moderately in *Cissus quadrangularis* treated groups of II group and IV group on the basis of the above results in the present study. It can be concluded that *Cissus quadrangularis* revealed high antilipidemic activity than the control.

**Conclusion**

The nanoparticles obtained from *Cissus quadrangularis* exhibited significant anti-inflammatory, antimicrobial, immunomodulatory and hypolipidemic activities without any serious side effects. These findings clearly demonstrated that the bioactive metabolites present in *Cissus quadrangularis* can be used for the treatment of disease.

**Acknowledgement**

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