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## Effects of *Aegle marmelos* (L.) Leaf Extract and Green Nanoparticles on Lipid Profile

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**Abstract.** Green silver nanoparticles (GNPs) synthesized mediated by *A. marmelos* leaf extract has been characterized and *A. marmelos* leaf extract (LE) and GNPs were evaluated for the regulation of hyperlipidaemia in rat model. Colour change of solution (1mg/mL LE, 1mMol AgNO<sub>3</sub> and 99mL distilled water) from pale yellow to dark brown, highest absorption of spectrum at 200nm and a broad spectrum at 474nm of UV-visible spectroscopy and Scanning electron microscopy (SEM) provides confirmation of synthesis of GNPs with diameter of 60nm to 120nm in size and spherical in shape. Fourier transform infrared spectroscopy (FTIR) analysis shows transmission peak at 3275 cm<sup>-1</sup> corresponding to O-H and H- stretch, 1604 cm<sup>-1</sup> corresponding to C=C stretch, 1384 cm<sup>-1</sup> corresponding to N=O bend, 1072 cm<sup>-1</sup> corresponding to C=N stretch which provides the confirmation about the presence of alcohols and phenols, represents alkenes, aliphatic nitro compound, aliphatic amines in the GNPs. The results show that both LE and GNPs significantly (p<0.001) reduced the total cholesterol, low density lipoprotein (LDL) and triglycerides concentration in the serum while LE at LD and HD and GNPs at LD had no significant effect on serum high density lipoprotein (HDL) but HDL concentration significantly increases at HD of LE when compared with controls. However, the GNPs shows significantly (p<0.001) higher efficacy in the reduction of total cholesterol, LDL and triglyceride concentration of serum when compare to LE. The study suggests that, both LE and GNPs may have beneficial effect on hyperlipidemia and associated diseases.

**Keywords:** *Aegle marmelos*, nanoparticle, hyperlipidemia, disorder, cholesterol

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### 1. Introduction

Medicinal plants have been used as the major source of treatment and disease management almost in all developing countries of the world because herbal medicines are inexpensive, easily available and no side effect [1, 2]. Which play an important role to improve the immunological

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response against various pathologies due to the presence of different phytochemicals, which are associated with therapeutic efficacy against various diseases and disorders [3, 4].

Cholesterol is an important lipid found in human blood which is essential for the normal functioning of the body. However, when it is in excess, it may cause health problems such as arteriosclerosis, hypertension, resulting in myocardial infarction, renal failure, cerebral haemorrhage, diabetes mellitus etc [5, 6] Cholesterol is carried in the blood by molecules called lipoproteins and among them three important are HDL, LDL and Triglycerides [7].

Rudenko *et al.* [8] reported that experimental and clinical studies have been shown that, in the body cholesterol transported in the form of chylomicrons, VLDL, IDL and LDL classes of lipoproteins, which are known as pro-atherogenic cholesterol, because of association with cardiovascular disease and are called as bad cholesterol [9]. HDL is called as good lipid because, it balance the effects of the hypercoagulable state in type 2 diabetics and decreases the high risk of cardiovascular complications and there is a significant negative correlation between HDL and activated partial thromboplastin time [10].

In recent decades, the applications of nanotechnology have been extensively explored as a broad area in the field of pharmacology. Nanotechnology concerns the size of matters in the range between 1nm to 100 nm of drug, natural or synthetic polymer loaded material acting as a carrier which have unique physicochemical properties such as ultra-small size, large surface to volume ratio, high reactivity and unique interactions with structural components such as core, emulsion to works as carrier and functional groups includes the therapeutic molecules and ligands for targeting location of biological systems, which are significantly improved the pharmacological efficacy of drug in contrast to the free drug counterparts [11, 13].

Delivery and efficacy of synthetic and many herbal drugs is often limited because of site or target specific action of therapeutic molecules and they require few modifications such as changing the molecular structure of the drug or their proper distribution by incorporation in carrier system [14]. Mathur and Govind [15] reported that when the materials are incorporated into nano carriers, they require lesser quantity to exert the action in target area, and this is useful, when dealing with effective phytomolecules.

*Aegle marmelos* belonging to the family Rutaceae and different parts of the plant has been used as antibacterial, antiviral, radioprotective, anticancer, antidiabetic, antihyperlipidaemic, cardioprotective agent [9,16]. *A. marmelos* leaf contains different types of phytochemicals such as flavonoids, tannins, phenols, and polysaccharides etc. which possess strong reducing activity and are associated with above therapeutic potentiality [17, 18].

Therefore, the present study have been carried out to synthesis and characterization of Green nanoparticles mediated by *A. marmelos* leaf extract and comparative effect of *A. marmelos* leaf extract and Green nanoparticles on lipid profile of male Wister albino rat.

## 2. Materials and Methods

### 2.1 Collection of plant material

The fresh and tender leaves were collected, dried in a shade under room temperature for six to seven days and then crushed into coarse powdery substance by using electric grinder. The coarse powdery substance was dried again and was then sieved to get fine powder using the fine plastic sieve, which was then stored in an air tight bottle in the laboratory until required [19].

## 2.2 Extract preparation

50g of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature using 350mL methanol and distilled water separately. The extract obtained was filtered, concentrated after dryness in rotary flash evaporator maintained at 45°C, percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies [20].

## 2.3 Synthesis of GNPs

The reaction mixture was prepared by adding 1mL of the fruiting body extract to 99 mL of 1 mM AgNO<sub>3</sub> (169.87 mg) solution in a 250mL round-bottom flask, which was mounted with a cooling condenser and magnetic stir bar. The mixture was allowed to stir for 2h at 90°C (immediate color change was observed from light yellow to dark brown, and thereafter no further color change was observed even after 2h). After 2h, the mixture was allowed to cool down before being centrifuged. The centrifugation was performed at room temperature and a speed of 9000 rpm. After washing three times with distilled water, a black powder was obtained that was dried overnight in an oven at 80°C [21, 22].

## 2.4 Characterisation of silver nanoparticles

### 2.4.1 UV-Vis spectra analysis

The reduction of pure Ag<sup>+</sup> ions was monitored by measuring the UV-visible spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into Milli-Q water. UV-Vis spectral analysis was done by using Parkin Elmer lambda 25 UV-Visible spectrophotometer.

### 2.4.2 FTIR analysis

FTIR analysis was carried out on IPResting-21 (Shimadzu) in the diffuse reflectance mode operated at a resolution of 4 cm<sup>-1</sup> in the range of 400 to 4 000 cm<sup>-1</sup> to evaluate the functional groups that might be involved in nanoparticle formation.

### 2.4.3 SEM (Scanning electron microscopy) analysis of silver nanoparticles

SEM analysis was done using JEOL JSM-6390 LV (Japan) SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min and was coated with gold using ion sputter.

## 2.5 Animals

Albino rats (175-200 g) were used in the study. They were maintained under standard laboratory conditions at ambient temperature of 25 ± 2°C and 50 ± 15% relative humidity with a 12h light-dark cycle. Animals were fed with commercial pellet diet and water *ad libitum*. The experiments were performed after prior approval of the study protocol by the institutional animal ethics committee of Ranchi University, Ranchi.

## 2.6 Acute toxicity studies

The acute toxicity studies were performed in accordance with the Organization for Economic Co-operation and Development [23] guidelines no. 425 (up and down procedure). No death was

observed till the end of the study. The test samples were found safe up to the dose of 2000 mg/kg and from the results 500 mg/kg was chosen as the maximum dose for further experimentation.

### 2.7 Experimental design

Group A: Rats in this group served as control and were neither treated with *A. marmelos* leaf extract nor GNPs and were fed with normal commercial rat chows and received 1mL of distilled water orally throughout the entire period of the experiment

Group B: Rats in this group received with 250 mg/kg body weight of *A. marmelos* leaf extract and were fed with normal commercial rat chows.

Group C: Rats in this group received with 500 mg/kg body weight of *A. marmelos* leaf extract and were fed with normal commercial rat chows.

Group D: Rats in this group received with 250 mg/kg body weight of GNPs and were fed with normal commercial rat chows.

Group E: Rats in this group received with 500 mg/kg body weight of GNPs and were fed with normal commercial rat chows.

### 2.8 Sample Collection

By the end of each experimental period, the rats were reweighed, starved for 24 hours and sacrificed under chloroform anesthesia. 5mL of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and allowed to clot for 30 minutes before centrifuging at 800g (Wisperfuge 1384 Samson, Holland) for 5 minutes. The supernatant was used for the lipid analysis [24].

### 2.9 Analytical procedure

Estimation of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides was done by cholesterol oxidase - phenol aminoantipyrine method [25].

All results were expressed as mean  $\pm$  standard error of mean (S. E. M.). Data was analysed using one-way ANOVA followed by Student's t-test,  $p < 0.05$  was considered as statistically significant.

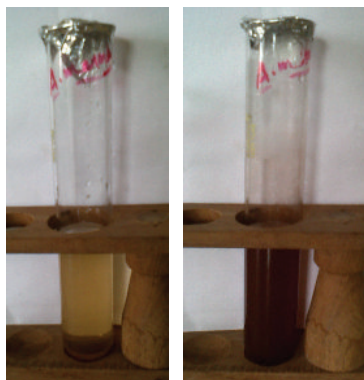
## 3. Results and Discussion

### 3.1 Synthesis of GNPs

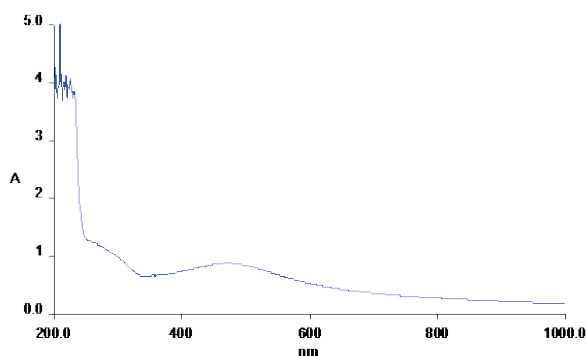
Synthesis of green nanoparticles mediated by aqueous *A. marmelos* leaf extract and  $\text{AgNO}_3$  is presented in Fig. 1. The figure shows change in the initial colour of mixture (leaf extract and  $\text{AgNO}_3$ ) from light yellow (Fig. 1A) to dark brown (Fig. 1B) after 2hours incubation indicating the formation of green nanoparticles [21, 22]. Mohan *et al.* [26] reported that colour changes from light to dark brown increases with increase in temperature and incubation period during synthesis of silver nanoparticle mediated by *Terminalia chebula* extract. A similar phenomenon was observed in the present study which provided confirmation of GNPs synthesis.

### 3.2 UV-visible spectra analysis

The result of UV-Visible spectroscopy is presented in Fig. 2. The spectra analysis provides confirmation of formation and stability of nanoparticles[22]. The absorption spectrum obtained from UV-Visible absorption spectroscopy showed a broad peak at 474 nm and highest



**Figure 1.** (A) Photograph of Plant extract and (B) AgNO<sub>3</sub> and Plant extract mediated silver nano particle solution after 2h of heating at 80°C.



**Figure 2.** UV-Visible spectrum of *A. marmelos* and AgNO<sub>3</sub> mediated GNPs.

**Table 1.** Lipid profile for 7 days along with their percent increase (+) or decrease (-) in relation to the control values in male rats treated with *A. marmelos* leaf extract (M ± SD, n = 6)

Group	Total cholesterol	% increase or decrease	HDL	% increase or decrease	LDL	% increase or decrease	Triglyceride	% increase or decrease
Control	77.29 ± 0.14	-----	44.29 ± 0.20	-----	36.44 ± 0.20	-----	119.45 ± 0.13	-----
LD (250mg/kg b.w.)	74.26 ± 0.29*	-3.92%	44.16 ± 0.28ns	-0.29%	33.12 ± 0.32*	-9.11%	111.57 ± 0.31*	-6.59%
HD (500mg/kg b.w.)	71.53 ± 0.42*	-7.45%	45.83 ± 0.42**	3.47%	31.15 ± 0.47*	-14.51	71.43 ± 0.59*	-40.9

\* =  $p < 0.001$ ; \*\* =  $p < 0.05$ ; ns = non significant

absorption of spectra represent peak at 200nm corresponds to the plasmon resonance (Fig. 2). Kumar *et al.* [22] reported similar results. They found highest absorption spectrum at 200nm of alion mediated silver nanoparticle solution by UV- Visible absorption spectroscopy. Khan *et*

**Table 2.** Lipid profile for 7 days along with their percent increase (+) or decrease (-) in relation to the control values in male rats treated with GNPs ( $M \pm SD$ ,  $n = 6$ )

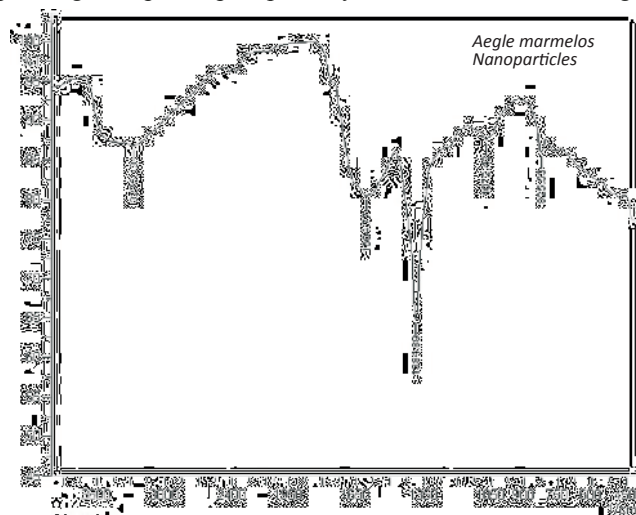
Group	Total cholesterol	% increase or decrease	HDL	% increase or decrease	LDL	% increase or decrease	Triglyceride	% increase or decrease
Control	77.29 ± 0.14	-----	44.29 ± 0.20	-----	36.44 ± 0.20	-----	119.45 ± 0.13	-----
LD (250mg/kg b.w.)	71.34 ± 0.55*	7.69%	44.09 ± 0.31ns	0.45%	32.07 ± 0.41*	11.99%	105.61 ± 0.80*	11.58%
HD (500mg/kg b.w.)	65.63 ± 0.33*	15.08%	43.56 ± 0.28ns	1.64%	27.25 ± 0.13*	25.21%	61.25 ± 0.40*	48.72%

\* =  $p < 0.001$ ; ns = non significant

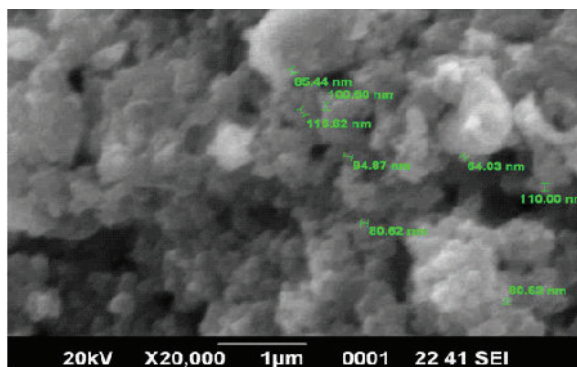
al. [21] reported in UV-Visible broad peak at higher wavelength which indicates increases in the particle size and narrow line at shorter wavelength represents smaller particle size.

### 3.3 FTIR analysis

Result of FTIR analysis is presented in Fig. 3. FTIR analysis was carried out to analyse the dual role of plant extract as capping agent and as a bioreductant [21] and to analyse presence of therapeutic molecules by the analysis of bonds and functional groups in [27, 28] FT-IR absorption spectra of green nanoparticle mediated *A. marmelos* leaf extract and AgNO<sub>3</sub> is presented in Fig. 4. The spectra showed broad transmission peak at 3275 cm<sup>-1</sup> corresponding to hydrogen bonded hydroxyl group (O-H and H- stretch) of alcohols and phenols, 1604 cm<sup>-1</sup> corresponding to C=C stretch represents alkenes, 1384 cm<sup>-1</sup> corresponding to N=O bend as aliphatic nitro compound, 1072 cm<sup>-1</sup> corresponding to C=N stretch represents aliphatic amines, 825 cm<sup>-1</sup> corresponding to aliphatic phosphate symmetric P-O-C stretching [29, 30].



**Figure 3.** FTIR Spectrum of *A. marmelos* and AgNO<sub>3</sub> mediated GNPs.



**Figure 4.** SEM image of *A. marmelos* and  $\text{AgNO}_3$  mediated green nano particles

### 3.4 SEM analysis of GNPs

Result of SEM of nanoparticles is shown in Fig. 4. Scanning electron microscopy provided the final confirmation about the morphology of synthesized green nanoparticles. The green nanoparticles were of spherical and cubical shapes and were within the diameter of 60nm to 120nm. The average diameters of the particles were of 70nm. [22] reported similar results, where the size of green nanoparticles synthesized from alion of *Aloe vera* and  $\text{AgNO}_3$  was in the range of 287-293nm and average size of nanopiclrs were 70nm. Firdhouse *et al.* [31] reported the size and shape of green nanoparticle mediated by ethanolic leaf extract of *Pisonia grandis* were of 20nm to 150nm in diameter and spherical shaped. In the present study GNPs mediated by *A. marmelos* leaf extract were smaller than above studied nanoparticles.

### 3.5 Effect of *A. marmelos* leaf extract (LE) and GNPs on lipid profile:

Impact of *A. marmelos* leaf extract (LE) and GNPs on lipid profile of male Wister albino rat is presented in Table 1 and 2 respectively. LE at low dose significantly ( $p < 0.001$ ) decreases by 3.92%, 9.11% and 6.59% total cholesterol, LDL and triglycerides respectively. High dose of LE is more effective and significantly ( $p < 0.001$ ) reduces the total cholesterol, LDL and triglycerides. However the HDL level significantly ( $p < 0.05$ ) increases (7.99%) at HD of LE but there are no significant changes occurred at LD (Table 1). Owolabi *et al.* [32] reported aqueous and ethanolic leaf extracts of *Blighia sapida* upon daily administration significantly ( $p < 0.05$ ) reduced the levels of total cholesterol, triglycerides and LDL-cholesterol and significantly ( $p < 0.05$ ) elevates HDL-cholesterol in normal albino rats. Oyewole *et al.* [33] reported the similar results in Wistar rats when treated with *Ficus exasperate* leaf extract.

Kucukkurt and Dundar [34] reported phytochemicals (phenols, tannins, saponins etc.) of *Platycodi radix* leaf extract are associated with decrease in serum cholesterol level and there is significant correlation between increases in HDL concentration and decrease in total cholesterol, triglyceride and LDL level in rat when they were treated. HDL are the smallest lipoproteins contain apo A-I and apo A-II as major components which resemble cholesterol-free flattened spherical lipoprotein and these complexes are capable of picking up cholesterol from blood, carried internally, from cells by interaction with the ATP-binding cassette transporter A1 (ABCA1) and increases the HDL level [35]. Kwiterovich [36] reported HDL remove cholesterol from antheroma within arteries and transport it back to the liver for its excretion or reutilization,



thus high level of HDL-cholesterol protect against cardiovascular disease.

However in the present study GNPs at both LD and HD significantly ( $p < 0.001$ ) decreases the total cholesterol, LDL and triglycerides level and there is no significant changes observed in HDL concentration when compare with control (Table 2) but in the present study GNPs shows significantly ( $p < 0.001$ ) higher efficacy than LE to decrease the total cholesterol, LDL and triglycerides level when compared with control (Table 1 and 2). Shinde and More [37] reported Simvastatin mediated nanoparticles significantly ( $p < 0.001$ ) decreases the plasma cholesterol and triglyceride level but HDL level was significantly increases ( $p < 0.001$ ) compare to standard antihyperlipidemic agent Simvastatin in albion rats.

It is reported that higher HDL level have fewer risk of cardiovascular problems and are correlated with better cardiovascular health but there is no significant effect in cardiovascular system further increase in HDL level after gaining its optimum level [38, 39]. Landmark Framingham Heart Study reported that, as HDL varies from high to low the risk of heart disease increases 10-fold for a fixed level of LDL and for a fixed level of HDL, the risk increases 3-fold as LDL varies from low to high [40, 41].

In the present study GNPs showed much significant efficacy to reduce the blood total cholesterol, LDL, triglycerides and does not significantly reduce the HDL level compare to LE, however the *A. marmelos* leaf extract showed good antihyperlipidemic activity and both GNPs and LE does not show mortality at above mentioned doses. Therefore, GNPs and LE are used in the patients suffering from hypelipidaemia and can be used to prevent hypelipidaemia associated diseases.

#### 4. Acknowledgement

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