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Hepatoprotective potential of *Pterocarpus santalinus* mediated biogenic silver nanoparticles against induced hepatitis in male albino rats

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Abstract

Hepatitis infection burdens have been increasing and alarming around the globe in recent years. The mainstream therapeutics of hepatitis infections are non-specific with numerous side effects. To combat viral hepatitis, a novel therapeutic platform is needed. In the present study, we explored a novel biogenic approach of nanotechnology as an alternative therapeutic platform for treating hepatitis. Biogenic silver nanoparticles (PTNPs) synthesised from *Pterocarpus santalinus* bark extract were used in the treatment against induced hepatitis. D-Galactosamine was given to male Wistar strain rats to induce hepatitis similar to viral hepatitis inflammations. The hepatic serum markers, Alanine aminotransferase and Aspartate aminotransferase were examined during the hepatitis conditions. The results obtained indicated that the Alanine aminotransferase and Aspartate aminotransferase enzyme levels were significantly reduced after the treatment with PTNPs. The hepatoprotective activity demonstrated from PTNPs would find application in alternate therapeutics in the treatment of hepatitis

Keywords: Hepatitis, Biogenic silver nanoparticles (PTNPs), D-Galactosamine, Alanine aminotransferase, Aspartate aminotransferase.

1. Introduction

Liver diseases are alarming in the present world with their mortality and morbidity. These diseases are increasing day by day with the changes in peoples' lifestyles. The liver, a major metabolic organ, is often damaged permanently due to the ill effects of diseases. According to WHO reports, the mortality of hepatitis reaches more than 600,000 people each year, and 325 million people live with hepatitis infection. Despite effective cure, people living with hepatitis virus have been increasing[1]. Hepatitis is one of the ailments of liver diseases caused by viral infections, drug intoxication and alcohol consumption[2,3]. It is a

significant health concern in developed, undeveloped and developing countries. Although several therapies have been available for the treatment of hepatitis for decades, there is no proper cure, and it has numerous limitations and side effects[4]. As many synthetic drugs are available with constraints, there is a need for an alternative approach for novel therapeutic agents to treat hepatic injuries.

D- Galactosamine (D-GalN) is one of the hepatotoxicants and closely corresponds to human viral hepatitis[5]. The effect of D-GalN has been documented in hepatitis conditions of rat liver. Elevated reactive oxygen species (ROS) levels have been identified in D-GalN induced rat hepatocytes for the damage[6]. Previous studies also reported a relationship between oxidative stress and hepatocellular injury. Few medicinal herbs have also been displaying hepatoprotective activities[7,8].

Transaminases like Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) are two liver enzymes involved in the metabolism of gluconeogenesis and amino acid conversions[9]. These enzymes perform the intermediary reactions of glucose and protein metabolisms. In these reactions, ALT transforms alanine into pyruvate, converted to oxaloacetate or acetyl coenzyme A by pyruvate decarboxylase or pyruvate dehydrogenase, respectively. Aspartate metabolism produces oxaloacetate and glutamate, which are accompanied by AST. In the liver pathophysiology of viral hepatitis or induced hepatitis, the serum levels of these enzymes have a significant role in ascertaining the disease conditions.

With the recent advancements of nanotechnology, therapeutics have been transformed with biogenic silver nanoparticles[10,11]. The use of plants in traditional medicinal practices lacks drug specificity to cure the disease properly. The biogenic nanoparticles approach has become a novel therapy in treating diseases in recent times when the existing methods fail. The use of plant extract along with transitions metals paved the way for a greener method to synthesise biogenic metallic nanoparticles for treating chronic ailments. The phytochemicals of the plant extracts are natural and nontoxic and act as capping agents to stabilise the nanoparticles[12]. The surface engineer of the metallic nanoparticles with biomolecules makes them biocompatible and bioavailable with high pharmacological effects in treatments of nanomedicine[13].

Pterocarpus santalinus is an endemic plant of Southern Andhra Pradesh, India, with several medicinal properties. It belongs to the family *Fabaceae*, is widely grown in the Seshachalam hills of Eastern Ghats, and is commonly known as Red sander or Rakthachandan[14]. It has been medicated as a remedy in the treatments of fever, bronchitis, chronic cystitis, ulcer, cancer and mental aberrations. As the biological activities of the *Pterocarpus santalinus* manifested on different diseases are promising, the effect of *P. santalinus* mediated silver nanoparticles (PTNPs) against hepatitis was not known yet. In this study, we aimed to explore the hepatoprotective potential of PTNPs against induced hepatitis.

2. Materials and methods

2.1. Plant sample collection and silver nanoparticles synthesis

Fresh bark material of *Pterocarpus santalinus* was collected from Sri Venkateswara Ayurvedic pharmacy Srinivasamangapuram, Tirupati. The bark was washed with sterile distilled water to remove any dirt material and shade dried for three weeks. The dried bark was chopped and used as an extracted sourceto synthesise biogenic silver nanoparticles. The biogenic silver nanoparticles weresynthesised using the method from Saxena reports[15].

2.2. Chemicals

D-Galactoseamine hydrochloride was purchased from Sigma Aldrich (St. Louise, Missouri, USA). Commercial kits were obtained to determine Alanine aminotransferase(ALT) and Alanine aminotransferase(AST). The assays were quantified using a ChemWell-T auto analyser following the manufacturer's protocol.

2.3. Animals

Male adult Wister strain albino rats (190 ± 20g) were purchased from M/s Raghavendra Enterprises, Bangalore, India. The rats were lodged in polypropylene cages with room temperature 27± 2^0C under a 12 h light-dark cycle photoperiod providing free access to rodent pellet diet (Lipton animal feed, Pune) water ad libitum in the whole experimental period. The studies performed were carried out following the guidelines approved by the Institutional Animal Ethical Committee.

2.4 Experimental design and drug treatment

Thirty male Wistar strain rats were randomly divided into five groups (n=6 in each group).

Group I Normal control (NC): The rats in this group were given saline daily for 21 days.**Group II Biogenic AgNPs treatment(PTNP):** The rats from this group received biogenic silver nanoparticles (PTNPs) 30mg/kg body weight.

Group III Hepatitis Control (Hpt): The rats in this group were given a small dose of D-Galactosamine (800mg/kgb/w) intraperitoneally to induce hepatitis 48h before sacrifice.

Group IV Hepatitis + Biogenic AgNPs (PTNP) treatment (Hpt+PTNP): Pre-treatment of biogenic silver nanoparticles, PTNPs were administered to this group orally for 21 days, and a single dose of D-Galactosamine (800mg/kgb/w)was given intraperitoneally to induce hepatitis 48h before sacrifice.

Group V Hepatitis + Silymarin standard treatment (Hpt+Std): Pre-treatment of the standard drug silymarin (100 mg/kg b/w) was administered orally to the rats of this group for 21 days. Rats were given a small dose of D-Galactosamine (800mg/kgb/w) intraperitoneally to induce hepatitis 48h before sacrifice.

2.5 Collection of blood and separation of serum

Upon the completion of the experimental period, the rat blood was collected from the retro-orbital plexus into micro centrifuges tubes under anaesthetic conditions. The serum was separated by centrifugation at 3000rpm for 10 min at 4^0C. The separated serum was stored at -20^0C for estimation of serum biomarkers like AST and ALP

2.6. Measurement of serum levels of ALT and AST

Serum activity of ALT and AST were determined by previous protocols (Yajima et al., 2004) and expressed as international units per litre (U/L).

2.7. Statistical analysis

Quantitative results were expressed as ± standard deviation and compared using Duncan's multiple comparison tests. The data analysis was carried out using SPSS (Version

21; SPSS Inc, Chicago, IL, USA) and MS Office Excel software. $P < 0.05$ was considered statistically significant with the obtained data.

3. Results

Effect of *P.santalinus* mediated biogenic silver nanoparticles(PTNPs) in D-Galactosamine (D-GalN) induced male Wistar rats

3.1. Effect of PTNPs on hepatic serum marker, Alanine aminotransferase (ALT):

Hepatitis was induced intraperitoneally with a single dose of D-GalN in Group-II rats before 48 h of sacrifice. Elevated levels of ALT activity was observed in hepatitis control rats when compared to normal, and PTNPs treated control groups (Fig-1). Conversely, a significant reduction of ALT activity (30mg/kg/bw) was pronounced in PTNPs pre-treated and hepatitis induced Group-III rats ($p < 0.05$).

3.2. Effect of PTNPs on hepatic serum marker, Aspartate aminotransferase (AST):

Intraperitoneal injection of D-GalN was given to induce hepatitis in Group-II rats before 48 h of sacrifice. Elevated levels of AST activity was found after 48 h of hepatitis induction (Fig-2). However, the AST activity was significantly reduced in PTNPs pre-treated and hepatitis induced Group-III rats ($p < 0.05$).

4. Discussion

The study evaluated the hepatoprotective response of biogenic silver nanoparticles (PYNPs) synthesised from bark extracts of *Pterocarpus santalinus*, an endemic plant of southern Andhra Pradesh, India. D-Galactosamine (D-GalN) was used in the present study for experimental production of liver damage similar to viral hepatitis[5]. A symbolic symptom of liver damage has been exhibited by increasing enzyme activities in serum. These enzymes act as markers for hepatic impairment[16]. This study evaluated the enzyme activities of transaminases in serum through a non-invasive method. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are transaminase enzymes with a higher importance in the amino acid metabolism of the liver[17]. ALT and AST activity levels were evaluated in the serum samples of all experimental groups like Normal control, PTNPs control, Hepatitis control, Hepatitis rats treated with PTNPs, and Hepatitis rats treated with standard drug silymarin. During the hepatitis induction, there was a significant increase was observed in the ALT and AST enzyme activities. Despite the elevation of hepatitis, the hepatic serum

markers activity was lowered upon treatment with biogenic silver nanoparticles, PTNPS (Group -IV) and silymarin standard (Group-V). The reduced ability of the liver to metabolise or excrete substances was surfaced through the steady increase of ALT and AST enzyme activities after 48 hours of a single dose of D-GalN. The decrease in the synthesis of hepatic serum markers by liver cells can be assumed from the deleterious effects of hepatic cells in hepatitis disease[18,19]. Furthermore, the activities are in agreement with the established reports of silver nanoparticles of biological origin[20]**.** The results in this study inhibited the elevated levels of ALT and AST upon treatment with *Pterocarpus santalinus* silver nanoparticles (PTNPs). The potent inhibitory effect of transaminases may be attributed to the nanoformulations of PTNPs. The biogenic silver nanoparticles were stabilised from the exudates of *P. santalinus,* and the active functional entities of the bark extracts such as alkynes, amines and alkyl halides have performed the therapeutic role in protecting the liver cells by inhibiting the elevated enzymes[21,22].

Liver hepatocytes consist of high levels of transaminases such as ALT and AST that can leak into the serum when a liver injury occurs. ALT participates in the glutamate cycle in both degradative and biosynthetic ways, along with substrates delivered from muscle tissue. In addition, ALT engages in cellular nitrogen metabolism and also liver gluconeogenesis. Reversible transamination is catalysed by this enzyme between alanine and oxoglutarate to produce pyruvate and glutamate and vice versa[23]. Besides, the AST also performs reversible transamination between aspartate and oxoglutarate to form oxaloacetate and glutamate. Transamination reactions mediate the production of aspartate, glutamate, and glutamine from ammonia, a glycolysis intermediate, and allow the carbon atoms from these four amino acids to be used for glucose synthesis under fasting conditions[24].

The silver nanoparticles synthesised from *P.santalinus*has significant implications on the fate and effect against the liver hepatitis conditions[25]. The PTNPs reactions with amino acids in the liver, particularly alanine and aspartate, may strongly affect this study. The physiochemical properties of the PTNPs, which differ in properties from the bulk components of silver metal and bark extracts, are easily affected by the alanine and aspartate amino acids with higher binding energies towards the extract exudates of *P.santalinus,* which are surface engineered oh the silver ions[26].

Enhanced levels of ALT and AST enzyme levels in the serum have a decisive role in the pathogenesis of liver diseases of glutamate metabolism[27,28]. As the pathways of glutamate metabolism are linked to aminotransferase reactions, the idea concerning the function of aberrant glutamate metabolism in the pathophysiology of hepatitis is unclear. It shows that the liver plays a crucial role in the metabolic control of the individual. The glutamine metabolism enzymes in the liver play a vital role in determining the amount of glutamine released into circulation. Furthermore, glutamate promotes the conversion of pyruvate to alanine. Aminotransferases start the metabolism of practically all amino acids, and the transfer of the amino group creates glutamate, which can subsequently be used as a substrate for aspartate aminotransferase. ALT and AST serum concentrations are commonly used as a biomarker for liver impairment caused by drugs, viruses, alcohol addiction, and fatty liver[29].

The present experimental investigation from the treatment of biogenic silver nanoparticles, PTNPs, presented a protective role in suppressing induced hepatitis. The PTNPs synthesised through a novel method of greener nanotechnology can enhance the protective role even at low concentrations (30mg/kg/bw) against hepatitis. Furthermore, the capping agents from the bark extracts of *P.santalinus* mediated a protective role and biodistribution of PYNPs to enhance the protective role against hepatitis.

5. Conclusion

In conclusion, the present investigation of biogenic silver nanoparticles (PTNPs) against induced hepatitis suggests that the PTNPs have a remarkable protective role that inhibits the elevated serum ALT and AST enzyme activities. Hepatitis is one of the global outbreak epidemic threats and has been a burden to cure completely. Moreover, the existing drug molecules evict many side effects after prolonged exposure. Therefore, the biogenic silver nanoparticles, PTNPs, examined in this study at a lower concentration potentially decreased hepatitis conditions and can be used as nanotherapeutic molecules in treating viral hepatitis. However, more research is required to explore molecular mechanisms and target receptors for the PTNPs.

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Figures

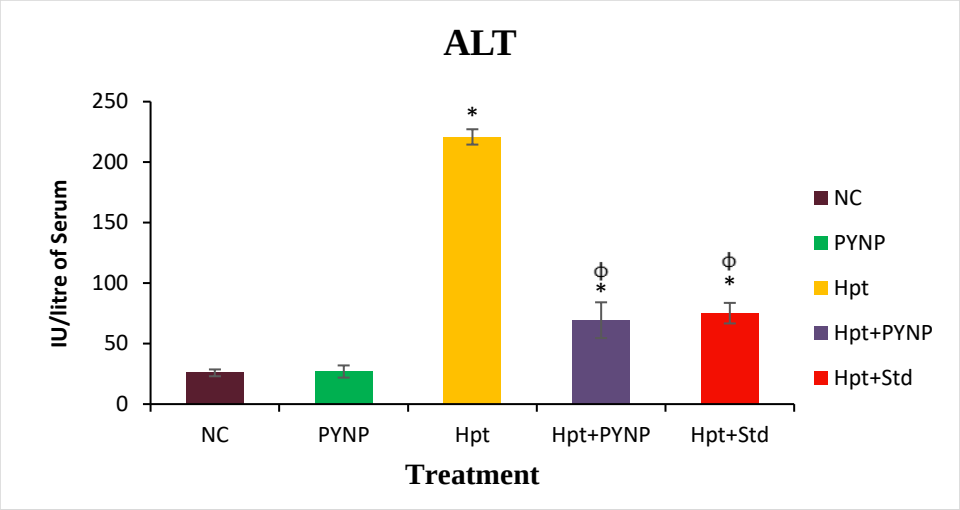

Figure-1: Effect of B-AgNPs (PTNP) on Alanine aminotransferase (ALT) activity in the Serum of Normal control(NC), B-AgNPs control (PTNP), Hepatitis induced (Hpt), Experimental (Hpt+PTNP) and Reference drug standard (Hpt+Std) groups of male albino rats

*significant at $p < 0.05$ compared with normal control; **ϕ** significant atp < 0.05 compared with hepatitis control

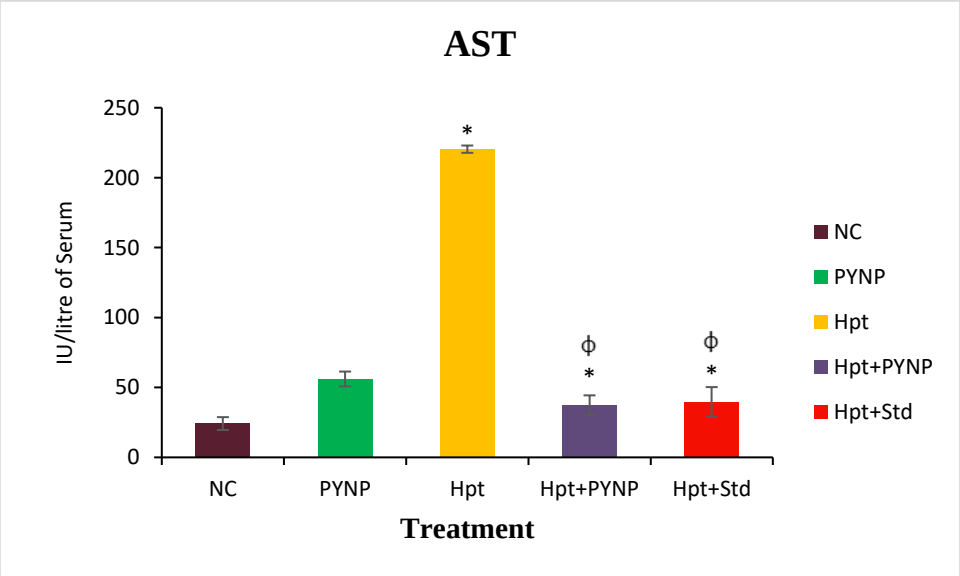

Figure -2: Effect of B-AgNPs (PTNP) on Aspartate aminotransferase (AST) activity in the Serum of Normal control(NC), B-AgNPs control (PTNP), Hepatitis induced (Hpt), Experimental (Hpt+PTNP) and Reference drug standard (Hpt+Std) groups of male albino rats

*significant at $p < 0.05$ compared with normal control; **ϕ** significant atp < 0.05 compared with hepatitis control