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HEPATOPROTECTIVE ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING AQUEOUS EXTRACT OF *CUSCUTA REFLEXA* AGAINST CCL4 INDUCED TOXICITY IN RATS

Ranjan Rakesh[1]*, Kumar Amar[2], Kumar Manoj[1], and Sinha Manoranjan Prasad[1]

[1]"Department of Zoology, Ranchi University", Ranchi, India.
[2]"Department of Zoology, Jamshedpur Cooperative College", Jamshedpur, India.

***Corresponding Author: Ranjan Rakesh**

"Department of Zoology, Ranchi University", Ranchi, India.

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ABSTRACT

In the present study the impact of nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* were investigated on rats intoxicated with carbon tetrachloride (CCl_4). CCl_4 is known to intoxicate the liver of rats which can be easily observed by examining the total protein, total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase ALT). *Cuscuta reflexa* have been used in traditional medicine culture from time immemorial. In this study the nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* was found to have curative impact on liver profile of CCl_4 intoxicated rats.

KEYWORDS: *Cuscuta reflexa, CCl_4, hepatoprotective, liver profile, aqueous extract, Silver nanoparticles*.

1. INTRODUCTION

Herbal medicines are in great demand in the developing world for primary health care needs.[1] But slowly the herbal medicines are also gaining grounds in the developed countries because herbal medicines have been reported to be as effective as their conventional counterparts.[2,3] The plant drugs constitute about 25% of total drugs in United States, while in developing countries such as China and India, where the medical facilities are still out of reach for a major portions of the populations, the contribution is as much as 80%. The plants have contributed remarkably to diversified industries such as fine chemicals, cosmetics, pharmaceuticals and drugs and industrial raw materials etc. Medicinal plants have been playing sole role in coping with a number of deadly diseases including cancer and diseases associated with viral onslaught.[4] There are innumerables of medicinal plants which are being used since time immemorial as effective medicines; one of them is *Cuscuta reflexa*. *Cuscuta reflexa* is a parasitic plant belonging to family Convolvulaceae, it is commonly known as amarbel, akashbel and by many other names in different traditions. Traditionally it is referred to as miracle plant because of its diverse medicinal properties. The plant is rootless, perennial, leafless climbing parasitic, twining herb, it obtains food from varieties of host plants using specialized organ called haustorium.[5] *Cuscuta reflexa* does not have any references in Vedic and Samhita kala, but it is well defined in Nighantus such as Bhavprakash Nighantu, Raj Nighantu, Adarsh Nighantu, Shankar Nighantu and Madanpal Nighantu. The extract of *Cuscuta reflexa* is traditionally used to cure jaundice, gout, body aches, constipation, flatulence, liver disorders.[5]

Owing to the above information about *Cuscuta reflexa*, the present work was undertaken to establish firsthand knowledge regarding the use of silver nanoparticles synthesized using aqueou extracts of *Cuscuta reflexa* as hepatoprotective agent. There are information in traditional contexts describing the use of *Cuscuta reflexa* extracts and whole plant in case of jaundice and liver related ailments, but no scientific validation has been established. This work will provide a firsthand knowledge about the use of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*.

2. MATERIALS AND METHODS

2.1. Plant Materials

Cuscuta reflexa stems were collected from bougainvillea (host). The stems were washed with distilled water and disinfected with 0.1% $HgCl_2$ solution for 5 min and dried in shade away from direct light for 20 days and ground to fine powder using electrical grinder. The powder obtained was sieved and stored in air-tight containers for future use.[6]

2.2. Preparation of Extracts

The fine powder of *Cuscuta reflexa* was made into thimble for loading in Soxhlet apparatus and extraction was done using distilled water. The extraction was

continuously done for 72 hours. The extract thus obtained was concentrated using vacuum rotary evaporator and extracts were kept in dessicator until used.[7]

2.3. Phytochemical Screening

Preliminary phytochemical screenings were conducted on *Cuscuta reflexa* aqueous extract in accordance to previously published standards.[8, 9]

2.4. Synthesis of Silver nanoparticles

The silver nanoparticles were synthesized using $AgNo_3$ solution. A change in the colour of solution confirmed the formation of silver nanoparticles.[6] The nano size, shape, capping and stability of synthesized nanoparticles was characterized using scanning electron microscope, fourier transform infrared spectroscopy, dynamic light scattering and zeta potential analysis. The synthesis and characterization of nanoparticles have already been reported by us, and the findings are already published.[10]

2.5. Animals

Albino rats weighing about 175-200 g were used in the study. They were maintained under standard laboratory conditions at ambient temperature of 25 ± 2 °C and relative humidity at 50 ± 15% with dark-light cycle of 12h. Animals were fed with a commercial pellet diet and water *ad libitum*. The experiment was performed after prior approval of Animal Ethics committee of Ranchi University, Ranchi (Proceeding no. 46, Page no. 137).

2.6. Acute Toxicity Study

The acute oral toxicity of nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* was determined following OECD guidelines 423.[11] It is the principle of the test to reduce and use minimum possible number of animals. The substance (extracts) was administered orally to a group of animals at one of the defined doses. The substance was tested using a step wise procedure, each step using three animals of single sex (normally females). The absence of substance-related mortality of the animals dosed at one step determined the next step. A total of 15 healthy adult albino rats weighing about 175 – 200 g and 8-12 weeks old were used in the study. Females were nulliparous and non-pregnant. The rats were fasted overnight prior to dosing. After dosing, food was withheld for 3-4 hours. Three animals were used in each step, since nothing was known about the toxic level of the substance in rats, the starting dose was selected as minimum (5 mg/kg body weight). The substances were administered in single dose by using oral gavage. Animals were observed individually after dosing, at least once for first 30 minutes, periodically during first hour, with special attention during first 4 hours and daily thereafter, for a total of 14 days. However, the duration of observation was not fixed rigidly. Dosed animals were caged separately post 14 days' observation and observed for next 14 days. No mortality was observed up to a dose of 2000 mg/kg of body weight of rats.[12]

2.7. Research Design

The animals were divided into 6 groups. The group 1 consisted of 3 rats, this group served as control and the rats were treated with distilled water orally for 7 days. Post 7 days, the blood samples were collected following the orbital sinus blood sample collection method.[13] Group 2 consisted of 6 rats, the rats in this group were treated with solution of CCl_4 orally for seven days. after 7 days blood samples were collected from the rats to see the impact of CCl_4 administration in rats. The data was recorded and then the rats were divided into 2 groups (Group 3, Group 4) consisting of 3 rats each which respectively received low doses and high doses of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* for 14 days. Post 14 days of administration of the rats with synthesized nanoparticles, the blood samples were collected again and analyzed to study the impact of synthesized nanoparticle on the hepatic profile of CCl_4 intoxicated rats. The distribution of groups were as follows –

Group 1 (control) – received distilled water orally (3 rats).

Group 2 (test group) – received CCl_4 solution orally (6 rats).

Group 3 (NpLD) derived from group 2 – received low dose of aqueous extract orally (3 rats).

Group 4 (NpHD) derived from group 2 – received high dose of aqueous extract orally (3 rats).

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening

The preliminary phytochemical screening showed the presence of phytochemicals such as flavonoids, carbohydrates, glycosides, phytosterols, phenolics and tannins. The phytochemicals are naturally occurring compounds in plant foods, have been reported to provide various biological functions in humans.[14] Raj *et al.* (2010).[15] reported that the alkaloid fractions derived from *Hygrophila auriculata* leaves posed hepatoprotective activity against CCl_4 induced toxicity in rats. Sawi and Sleem (2010)[16] concluded that the hepatoprotective activity of extracts of *Senna surattensis* may be due to the free radical scavenging activity posed due to the antioxidant activity of flavonoids present in the extract. The terpenoids,[17] saponin,[18] phenolics,[19] tannins[20] have also been reported to possess antioxidant and hepatoprotective activity in plant extracts possessing them. In our work of synthesis and characterization of silver nanoparticles using aqueous extract of *Cuscuta reflexa*, we found that the phytochemicals played important role in formation of silver nanoparticles, which was confirmed by the results of FTIR analysis.[10]

Table 1: results of preliminary phytochemical screening of aqueous extract of *Cuscuta reflexa*.

Phytochemicals	Presence/absence in aqueous extract of *Cuscuta reflexa*
Alkaloids	-
Flavonoids	+
Terpenoids	+
Saponins	-
Phenolics	+
Tannins	+

3.2. Total bilirubin

Liver is a large organ and plays important role in different important physiological activities, thus any impairment in hepatic functioning can result into serious health consequences. The result of impact of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* is graphically presented as figure 1. The bilirubin is an endogenous anion derived from haemoglobin degradation from the RBC in liver. Lower than normal bilirubin is usually not a concern, but elevated levels may indicate liver damage or disease.[7] The higher level of total bilirubin in the blood indicates that the liver is not clearing bilirubin properly. The results show that the treatment of CCl_4 showed a significant increase in the total bilirubin level, the increased level of total bilirubin level indicates liver damage or dysfunction induced by treatment of CCl_4. The rats treated with CCl_4 were then treated with low dose and high dose of nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*. The total bilirubin level in control rats were 0.52 ± 0.005 mg/dl, after treatment with CCl_4 the total bilirubin was elevated to 0.61 ± 0.02 mg/dl due to hepatic damage induced by CCl_4. The CCl_4 intoxicated rats were then treated with silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*, the treatment showed a decrease in the elevated total bilirubin levels which reduced to 0.51 ± 0.033 mg/dl, 0.512 ± 0.27 mg/dl respectively in low dose and high dose groups.

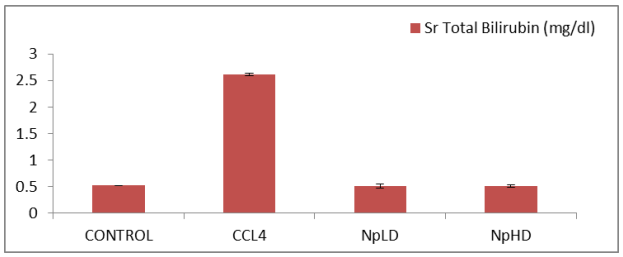

Figure 1: Impacts of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* on total bilirubin level of albino rats.

3.3. Total Protein

The total protein test measures the total amount of albumin and globulin in the body. It is used as part of routine health and is also included in liver function tests (LFT). The liver is major source of most of serum proteins. The parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors. The total protein test measures the total amount of proteins found in the fluid portion of blood. This test is often done to diagnose liver diseases.[21] The result of total protein analysis has been presented as figure 2.

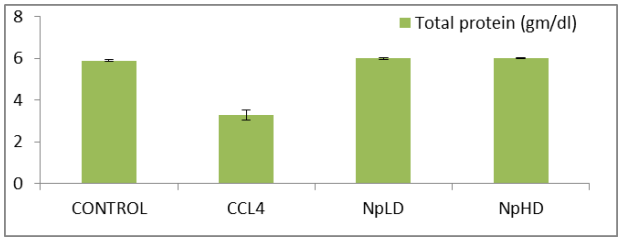

Figure 2: Impacts of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* on total protein level of albino rats.

The total protein level in control rats were 5.9 ± 0.038 gm/dl. The total protein level in CCl_4 treated rats decreased to 3.28 ± 0.23 gm/dl. The CCl_4 intoxicated rats were treated with silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*. Post treatment the total protein level recovered and rose to normal level which was 6.00 ± 0.041 gm/dl and 6.01 ± 0.027 gm/dl in case of low dose and high dose groups of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*.

3.4. Alkaline Phosphatase

The alkaline phosphatases (intracellular enzyme) are a family of zinc metaloenzymes, with a serine at the active centre, the highest concentration of alkaline phosphatase (ALP) are present in the cells of liver. Elevated levels of ALP in the blood are most commonly caused by liver diseases involving hepatic cellular injury.[21] Thus the elevated levels of ALP in blood shows the probable damage to hepatic cells. In the present study post treatment with CCl_4, the ALP level raised to 508.2 ± 5.23 IU/L from 176.24 ± 6.2 IU/L (control). This increase is a clear indication of liver damage induced by CCl_4 toxicity in rats. When the CCl_4 intoxicated rats were treated with silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*, the ALP levels recovered (figure 3) i.e. 166.34 ± 2.1 IU/L, 163.32 ± 1.6 IU/L in low dose and high dose concentration groups respectively. The data clearly shows the hepatoprotective activity of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*.

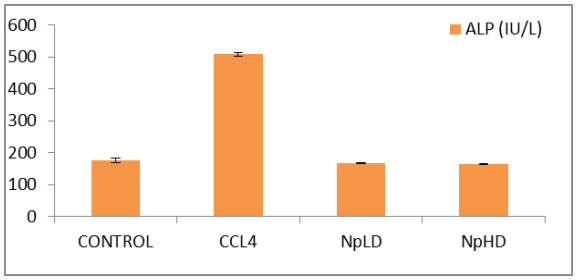

Figure 3: Impacts of silver nanoparticles synthesized using aqueous extracts of *Cuscuta reflexa* on Alkaline Phosphatase activity of albino rats.

3.5. Aspartate aminotransferase

Aspartate aminotransferase (AST) exists in two isoenzymes namely mitochondrial and cytoplasmic form. It is found in highest concentration in the liver followed by heart, muscle, kidney, brain, pancreas and lungs. When the liver is damaged, it releases AST in the blood stream. Increase AST in blood may be an indication of liver damage.[21] There was a significant elevation in AST levels in rats treated with CCl_4 (107.2 ± 4.25 IU/L) as compared to the control (52.3 ± 2.36 IU/L). The elevated levels of AST decreased on treatment of intoxicated rats with silver nanoparticle synthesized using aqueous extract of *Cuscuta reflexa* (45.6 ± 2.1 IU/L, 44.44 ± 2.01 IU/L) The results are graphically presented in figure 4.

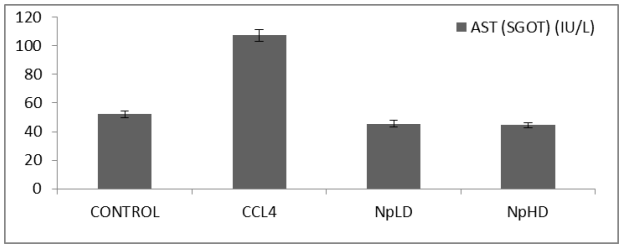

Figure 4: Impacts of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* on Aspartate animotransferase level of albino rats.

3.6. Alanine aminotransferase

The alanine aminotransferase (ALT) is an enzyme mainly found in liver, which makes its specific to assess liver damage. The body uses ALT to breakdown food into energy. Normally, ALT levels in the blood are low, if the liver is damaged, it will release more ALT in the blood. In the present study the ALT level increased from 146.63 ± 1.2 IU/L (control) to 206.2 ± 8.63 IU/L in CCl_4 intoxicated rats. The ALT levels of CCl_4 intoxicated rats recovered post treatment with silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*. The results are graphically represented in figure 5.

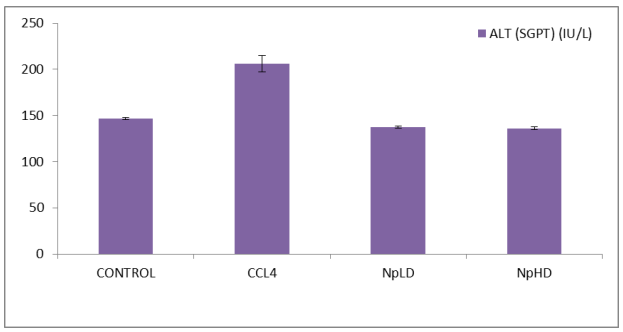

Figure 5: Impacts of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* on total Alanine aminotransferase level of albino rats.

The results (figure 1 – 5) clearly reveal the hepatoprotective activity of nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*. The phytochemical screening of aqueous extract of *Cuscuta reflexa* showed the presence of flavonoids, terpenoids, phenolics and tannins. The CCl_4 treatment induced hepatotoxicity in rats, and the results clearly show the recovery of the rats from the induced hepatotoxicity. All the phytochemicals found in the preliminary screening have been known to have antioxidant properties. The phytochemicals are naturally occurring compounds in plant foods, and have been reported to provide various biological functions in humans.[14] Raj *et al.* (2010)[15] reported that the flavonoids and tannins fractions derived from *Hygrophila auriculata* leaves posed hepatoprotective activity against CCl_4 induced toxicity in rats. Sawi and Sleem (2010)[16] concluded that the hepatoprotective activity of extracts of *Senna surattensis* may be due to the free radical scavenging activity posed due to the antioxidant activity of flavonoids present in the extract. The terpenoids,[17] phenolics,[18,19] tannins[20] have also been reported to possess antioxidant and hepatoprotective activity in plant extracts possessing them.

The silver nanoparticles synthesized using aqueous extract extract of *Cuscuta reflexa* significantly raised the the total bilirubin, total protein to normal levels, which were lowered post treatment of CCl_4. The ALP, AST and ALT levels were significantly raised after treatment with CCl_4. But after treatment of the intoxicated rats with silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*, significant lowering in ALP, AST and ALT levels were observed. This clearly indicates the hepatoprotective impact of silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa*. In an extensive study Kumar *et al.*, (2020)[21] have reported presence of flavonoids, phenols and tannins in the aqueous extract of *Punica granatum*. They synthesized silver nanoparticles and found that the phytochemicals played key role in formation of silver nanoparticles. They further reported that the silver nanoparticles showed enhanced hepatoprotective activity as compared to the aqueous extract of *Punica granatum* alone, this enhanced property was attributed to the nanoscale of the nanoparticles which were capped by the phytochemicals. In present study we report the presence of alkaloids, flavonoids, terpenoids, saponins, phenolics and tannins. During formation of silver nanoparticles the phytochemicals were loaded on the surface of nanoparticles, which makes it easy for them to cross the cell membrane.[21] Probably this facilitated drug delivery across the membrane is responsible for the enhanced hepatoprotective activity of the silver nanoparticle synthesized using aqueous extract of *Cuscuta reflexa*.

Thus on the basis of result of this study it is clear that the silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* shows clear hepatoprotective activity against CCl_4 induced toxicity in rats. This further indicates that the silver nanoparticles synthesized using aqueous extract of *Cuscuta reflexa* and/or its derivatives may be used as potent hepatoprotective agent against liver related ailments.

4. CONFLICT OF INTERESTS

The authors declare that there is not conflict of interest.

5. ACKNOWLEDGEMENTS

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