Haryana Vet. (March, 2024) **63(SI),** 76-79 ISSN 0033-4359 (P) **Research Article**

SUB ACUTE ORAL TOXICITY OF *JATROPHA CURCAS* LINN. LEAF EXTRACT COATED SILVER NANOPARTICALS IN WISTAR RATS

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ABSTRACT

The synthesis of silver nanoparticles (SNPs) was carried out using biological reduction method with extract prepared from leaves of *Azadirachta indica* (neem-5%). The characterisation was done by coating with *Jatropha curcas* leaf extract (JcLE) by TEM. The 50 wistar rats of either sex were randomly assigned in five groups. The control group was dosed with only vehicle, whereas, four treatment groups were dosed at the rate of 100, 200, 400 and 800 mg/kg of *J. curcas* leaf extract orally in combination of 0.17 mg/kg SNPs for 28 days. The last fifth group was considered as satellite group. In exposed rats, the aqueous leaf extract of *J. curcas* coated SNPs had no effect on general behaviour, body weight, or feed intake rations. There were no changes in haemoglobin, TEC, or TLC. Only a rise in monocyte count was seen in DLC. The blood clotting time was increased significantly with increasing dose and duration of exposure. A gradual increase in AST and ALT values was noticed in biochemical tests. BUN, creatinine, and total protein levels all increased significantly. Grossly, focal congestion and necrosis recorded in liver. Histopathological analysis revealed dose depended alteration in liver followed by kidney and heart were observed in rats treated with JcLE coated SNPs as compared to control groups.

Keywords: *Jatropha curcas*, Silver nanoparticles, Oral toxicity

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Nanoparticles has various potential biomedical applications including target drug delivery, imaging and biosensors, diagnosis and therapy of diseases (Wang and Wang, 2014). A unique physical and chemical properties of silver nanoparticles such as optical, electrical, thermal, strong electrical conductivity and biological qualities intended their use in medical, food, health care and industrial purposes (Mukherjee *et al.*, 2001., Li *et al.*, 2010 and Gurunathan *et al.,* 2015). A variety of techniques are available for the synthesis of silver nanoparticles (Jain *et al.*, 2009) and biological synthesis is considered as a cost-effective and environmentally friendly alternative to chemical and physical methods (Varghese *et al.*, 2015).

The *Jatropha curcas* belongs to Euphorbiaceae family. Africa, North America, and the Caribbean are all native to these plants. *Jatropha* has been used as a therapeutic herb in many ancient medical systems around the world. This plant is prevalent in the Indian folklores with tremendous ethnobotanical significance (Thomas *et al.*, 2008). The *J. curcas* are traditionally used in the treatment of scaticia, dropsy, paralysis, rheumatism, dysentery, diarrhoea and various skin diseases. The seeds of *Jatropha* spp. are commonly used in the treatment of arthritis, jaundice and as a contractive and water extract of branches in human immunodeficiency virus infection and tumor. While, extracts prepared from whole plants have medicinal values in the treatment of allergies, burns, cuts, wound inflammation, leprosy, leucoderma and smallpox (Prasad *et al.*, 2012) and wound healing (Sharma *et al.*, 2012). The plant also possesses many pharmacological properties like disinfectant, anti-inflammatory activity, antioxidant activity, antiparasitic including anti insect activity, wound healing, antidiarrheal, antimicrobial, anticancer, antiviral, antidiabetic, analgesic, hepatoprotective, wound healing, anticoagulant and procoagulant, antifertility and abortifacient activities (Abdelgadir and Van Staden 2013, Laxane *et al.*, 2013). The *J. curcas* leaves exert anti-arthritic activity (Baroroh *et al.*, 2014. Globally, around 383 different products are estimated based on silver nano particles which represents around 24% of all nano-products in use. As a result, scientists are attempting to understand the negative effects on living organisms. Considering the toxicity of silver nanoparticles, the present research was conducted to evaluate the sub-acute oral toxicity of *J. curcas* Linn. leaf extract coated silver nanoparticles in wistar rats according to OECD- Guidelines 407.

MATERIALS AND METHODS

Experimental Animals

A total of 50 wistar rats having age of 8-10 weeks and 160-220 gm body weight of either sex were used. Rats were selected in accordance with their health monitoring report and behavioural examination. The wistar rats were maintained as per the standard laboratory conditions by

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implementing the CPCSEA guidelines.

Drugs and chemicals

The silver nanoparticles (SNPs) were synthesized using biological reduction method. *Azadirachta indica* (Neem) leaf extract and silver nitrate was used to produce the silver nano particles.

Preparation of *Jatropha curcas* linn. Leaf extract:

A 20% cold aqueous leaf extract of *J. curcas* was prepared using 200 gm powder in 1 lit. of distilled water. The flask was stored in the refrigerator for 48 hours for complete maceration, with periodic shaking with an electrically operated flask shaker to properly mix the powder and distilled water. After 48 hours, the resultant solutions were filtered and the filtrate was poured into the evaporating dishes, the extracts were weighed and kept in a screw cap vial and used as and when required.

Synthesis of silver nanoparticle:

The silver nanoparticles were synthesized by the biological method by using the *A. indica* leaves. The *A. indica* leaf extract was separated into conical flasks and filtered using Whatmans No. 42 filter paper. Then, prepared solution of silver nitrate (10^{-3} M) was stored in an amber coloured bottle. In a Biochemical oxygen demand (BOD), 5 mL of leaf extract (*Azadirachta indica*) was added to 95 mL of 10^{-3} M $AgNo_3$ solution and bottle was incubated at room temperature for 28 hours. The changes in the colour of solution i.e., pale green to dark brown which indicates synthesis of AgNPs from the leaves (*A. indica*) was observed periodically.

Coating of silver nanoparticles with *Jatropha curcas* extract:

To coat synthesised silver nanoparticles (SNPs) with *J. curcas* leaf extract, the synthesised silver nanoparticles solution (0.17 mg/kg b.wt.) was mixed with various doses of aqueous leaf extract of *J. curcas* (100, 200, 400 and 800 mg). The prepared coated concentrations were kept at room temperature with intermittent stirring (for 1 hour) to covalently modify the surface of SNPs with the phytochemicals presents in *J. curcas* leaf extract.

Characterization of silver nanoparticles

Transmission Electron Microscopy (TEM) was used for characterization of the silver nano particles synthesis. Floatation method used for sample preparation-TEM {Model-JEM-1400 (HR). The staining of samples was done at Shri Sai histopathology centre, Shivaram pally, Rajendranagar, Hyderabad, India. The visualization of images was taken from the National Institute of Animal Biotechnology (NAIB), Hyderabad.

RESULTS AND DISCUSSION

Extract preparation

In the cold aqueous extract preparation, from the 3 kg of powder average 140 gm of aqueous extract was obtained. Per cent extractability was calculated and it was found 4.66% for 100 gm of powder of *J. curcas* leaves.

Synthesis of silver nanoparticles

The synthesis of silver nanoparticles was confirmed by change in colour from pale green to dark brown. Similar result was observed by (Namratha and Monica 2013; Kumar *et al.*, 2014). The synthesis of silver nanoparticles of various shapes, primarily spherical, was demonstrated by transmission electron microscopy (TEM) pictures. Average particles size of silver nanoparticles was 22.47 nm. Similar observation was reported (Okafor *et al.*, 2013). The TEM images *J. curcas* leaf extract shown their presence specifically around the silver nanoparticles. The average diameter of coated silver nanoparticles was found 31.92 nm. Similar observation was reported (Rafique *et al.*, 2017), the TEM image of Ag, colloid particles were observed as spherical and average particle was in the range of 8-52 nm.

Haematological and Biochemical parameters

In this research, *J. curcas* coated silver nanoparticles not shown any sign of toxicity on Hb, TEC, and TLC values at any of the concentrations used for 28 days. Similar result was observed by Kim *et al.*, 2010), the haemoglobin values were 16.76, 16.47 and 16.93g/dl, respectively. No significant difference was observed in haemoglobin values. In TEC and TLC values Igbinosa *et al*. (2013) found similar result in rats treated with methanolic extract of *J. curcas*. Whereas, a significant increase in monocyte count was observed compared to normal range from II to V group of treatment. In collaboration with our findings, Kim *et al.* (2010) also depicted the similar observations. In the present study difference in blood clotting time was observed in all the groups with different time factors. However, Osoniyi and Onajobi, (2003) reported that the latex of *J. curcas* significantly reduced the clotting time in human blood and it was varied between 4 and 8 minutes with a mean of 5.83±1.25 minutes.

In the present study, biochemical parameters revealed the gradual increases in AST and ALT values from group II to group V, which confirms the dose dependent toxicity in AST values as compared with control group. Igbinosa *et al.* (2013) reported the similar result in wistar rats treated with methanolic leaf extract of *J. curcas* at 500 mg/kg, 1000 mg/kg and 2000 mg/kg body weight. In the present study

Table 1. Haematological values (Blood clotting time, Lymphocytes, Monocyte and Neutrophil) of experimental groups at different intervals of oral administration of JC coated SNPS in rats for 28-day

(Mean ± SE)

Groups	Blood clotting time (Second)				LYMPHOCYTE %				MONOCYTE%				NEUTROPHIL %			
Days	0 day	7th day	14th day	28th day	0 day	7th day	14th day	28th day	0 day	7th day	14th day	28th day	0 day	7th day	14th day	28th day
Control	50.06± 0.12	51.60± 0.15	50.02± 0.08	52.20± 0.10	73.93± 0.78	73.66± 1.06	71.78± 2.52	72.91± 1.90	5.62± 0.49	5.83± 0.48	5.69± 0.38	5.97± 0.48	17.95± 1.25	18.45± 0.640	20.32± 2.23	18.65± 2.95
100 mg/kg dose	51.41± 0.09	53.40± 0.11	59.63± 0.14	54.32± 0.13	73.24± 1.19	72.41± 0.92	69.25± 1.27	72.13± 2.18	5.95± 0.36	7.02± 0.18	6.93± 0.20	6.91± 0.12	17.77± 1.25	18.42± 1.057	21.40± 1.12	18.58± 2.13
200 mg/kg dose	51.64± 0.11	59.63± 0.29	62.12± 0.18	63.83± 0.12	72.25± 0.57	72.02± 1.10	72.25± 1.11	73.09± 1.48	5.97± 0.38	6.81± 0.24	7.53 ± 0.22	7.92± 0.25	19.41± 1.068	18.91± 1.13	17.56± 0.84	16.55± 2.17
400 mg/kg dose	50.24± 0.08	61.02± 0.16	62.52± 0.27	69.61± 0.22	72.63± 0.91	71.38± 1.73	68.09± 1.30	70.95± 0.87	5.90± 0.39	7.06± 0.27	7.68± 0.28	7.60± 0.25	19.18± 1.21	19.15± 1.86	21.85± 0.76	18.97± 0.96
800 mg/kg dose	51.85± 0.14	65.88± 0.14	77.11± 0.20	85.27± 0.18	73.09± 0.42	69.69± 1.85	69.58± 1.54	69.38± 2.92	5.86± 0.43	7.01± 0.13	7.57± 0.20	7.90± 0.28	18.93± 1.39	21.02± 1.81	20.65± 1.86	20.25± 2.71

Table 2. Biochemical values of experimental groups at different intervals of oral administration of JC coated SNPS in rats for 28-day

(Mean ± SE)

Group	AST (IU/L)				ALT (IU/L)				BUN (mg/dl)				Creatinine (mg/dl)				Total protein (g/dl)			
Days	0 day	7th day	14th day	28th day	0 day	7th day	14th day	28th day	0 day	7th day	14th day	28th day	0 day	7th day	14th day	28th day	0 day	7th day	14th day	28th day
Control	130.81± 2.20	129.98± 1.89	130.56± 1.98	131.23± 1.61	70.60± 2.57	70.28± 2.33	69.22± 1.97	70.76± 2.98	20.54± 0.48	20.83± 0.72	19.98± 0.46	20.94± 0.52	0.66± 0.27	0.67± 0.02	0.68± 0.02	0.65± 0.02	6.87± 0.14	7.02± 0.22	7.11± 0.11	7.28± 0.14
100 mg/kg dose	130.36± 2.40	145.10± 2.33	152.01± 2.22	154.70± 3.02	70.98± 2.70	71.06± 2.10	70.72± 0.91	71.13± 1.58	20.73± 0.59	22.97± 0.35	23.80± 0.66	24.51± 0.30	0.68± 0.01	0.68± 0.02	0.74± 0.02	0.75± 0.02	6.99± 0.11	7.17± 0.22	7.49± 0.31	7.63± 0.10
200 mg/kg dose	129.46± 1.68	156.8± 3.66	159.93± 2.06	162.81± 3.20	70.80± 4.37	71.70± 2.64	72.87± 2.91	73.22± 3.19	20.99± 0.61	23.60± 0.88	23.99± 0.30	25.93± 0.63	0.67± 0.02	0.70± 0.01	0.74± 0.03	0.76± 0.02	7.15± 0.18	7.75± 0.23	7.78± 0.14	8.51± 0.31
400 mg/kg dose	130.35± 1.52	163.69± 2.26	164.08± 1.89	170.01± 1.62	70.45± 2.06	73.29± 3.08	73.10± 1.13	73.37± 2.92	20.90± 0.54	23.76± 0.87	24.70± 0.70	26.03± 0.26	0.68± 0.02	0.74± 0.03	0.72± 0.02	0.75± 0.02	7.19± 0.23	7.70± 0.22	7.71± 0.17	8.10± 0.19
800 mg/kg dose	131.85± 1.37	169.13± 1.23	169.74± 3.19	171.94± 1.66	71.93± 2.65	74.47± 3.62	75.52± 0.93	81.35± 4.47	20.55± 0.26	24.62± 1.32	23.19± 0.38	24.68± 0.52	0.67± 0.027	0.72± 0.01	0.76± 0.03	0.76± 0.01	7.07± 0.14	7.89± 0.22	8.17± 0.21	8.25± 0.15

Note- AST- Aspartate aminotransferase, ALT- Alanine Transaminase, BUN- Blood Urea Nitrogen

there was significantly increase in BUN and creatinine values. Similar result was recorded by Mahe *et al.* (2017). Chibuogwu *et al.* (2021) also observed similar finding in mice treated with leaves extract of *J. tanjorensis* in creatinine values. So based on the results and literature it was concluded that there was toxic effect of *J. curas* coated silver nanoparticle.

Gross and histopathological findings

Grossly, focal congestion and necrosis recoded in liver whereas, no gross alteration were observed in heart and kidney. In organ weights, non-significant variation were observed in mean relative organ weights of liver, heart and kidney in all the treated groups. The histopathological observation of liver in high dose treated group were showed severe congestion, haemorrhages, dilatation of central vein with necrobiotic changes, dilatation of sinusoidal tissue space, congestion of blood vessels, fatty degeneration with marked infiltration of mononuclear cell and cellular degeneration. Heart was showed infiltration of mononuclear cell, hyperemic blood vessel, extravasation of blood, mild inflammation and cellular degeneration, hyaline degeneration, haemorrhage and presence of necrotic myocardial fibres. Kidney revealed severe cellular swelling, necrosis, congestion, hydropic degeneration, marked infiltration of mononuclear cells in intertubular tissue space, mild to moderate focal and multifocal hyaline cast located in the lumen of exposed renal tubules and hydropic degeneration with haemorrhages in high dose treated group as compared with the rest of the treatment group except control.

There was toxicity sign in aqueous extract of *J. curas* coated silver nanoparticles in this study was observed in the higher doses. Even, Heydrnejad *et al.* (2015) received the similar pattern of toxicity in BALB/c mice treated with silver nanoparticles at 20 and 50 ppm for 14t^{h} days (orally). In the present study histopathological observation revealed vast damage to liver. Mahe *et al.* (2017) reported that minor interstitial haemorrhages and vascular congestion in kidney was observed in the rats treated with *J. curcas* leaf extract @ 250 mg/kg and Sulaiman *et al.* (2015) observed hemorrhage in heart and presence of necrotic myocardial fibers with mild lymphocytic infiltration in rats treated with silver nanoparticles at 10, 50 and 100 mg/kg body weight orally for 30 days.

CONCLUSIONS

In the present study, the aqueous extract of *J. curcas* leaf coated silver naopaticles did not showed any sign of toxicity on general behaviour, body weight and feed intake

ratio in exposed rats. On hematological parameters, monocyte count and blood clotting time values was increased significantly shown sign of toxicity. The biochemical parameters shown gradual increases in AST, ALT, BUN, Creatinine values as compared with control groups, confirms the dose dependent toxicity. Dose dependent mild changes in liver were noted. Dose dependent severe changes in histoarchitecture of liver, kidney and heart were observed in treatment groups as compared with the control group.

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