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Research Article

EVALUATOIN OF GENOTOXICITY OF *RICINUS COMMUNIS* (LEAVES AND SEEDS) BY THEIR SILVER NANO-PARTICLES USING COMET ASSAY

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ABSTRACT

Ricinus communis commonly known as castor oil plant is used in Indian indigenous medicinal system of *Ayurveda* to treat various diseases like sleeping disorders (insomnia), gall bladder pain, headache, backache, expulsion of placenta and arthritis. The present study deals with the genotoxic effects of silver nanoparticles of *Ricinus communis* leaves and seeds aqueous extracts in human lymphocytes and in *Escherichia coli* by the comet assay. Using aqueous extracts of seeds and leaves powder of *Ricinus communis,* silver nano particles are prepared. The results showed increase in tail length when the *E. coli* samples were treated with four different concentrations of leaves silver nanoparticles, as the concentration increases tail length also increases denoting DNA damage at high concentrations. Mild genotoxicity was observed when human lymphocytes were used against different concentrations of leaves silver nanoparticles. When seed nanoparticles of *Ricinus communis* (four different concentrations) treated with *E. coli,* mild genotoxicity is observed but when seed nanoparticles were treated with human lymphocytes, results showed higher level of genomic damage (nature of genotoxicity is increasing with the increasing concentration).

Keywords: *Ricinus communis*, Comet assay, Genotoxicity, Nanoparticles.

1. INTRODUCTION

Ricinus communis is a perennial flowering plant which belongs to Family of *Euphorbiaceae* and subfamily of *Acalyphoideae*. Castor is native of East African, India but is widely located across the tropical regions. *Ricinus* name was used by Carl Linnaeus which stand for ticks in Latin. The plant is monoecious (unisexual flowers), inflorescence is terminal spike like and the fruit is capsule containing shiny oval seeds [1]. Plant is mainly cultivated for its oil which is used for various purposes since 2000BC. Castor oil is used in treating wounds and cure ailments (due its wound healing property it is also called Palm of Christ), it is also given to children suffering from parasitic worm infections. Ayurveda uses castor in anti-inflammatory herbal medicines [2]. Methanolic leaf extracts of *Ricinus communis* are known to have anti-microbial, liver protective and antinociceptive activities [3]. Various extracts of *Ricinus communis*are used to treat more than 15 types of disorders.

Ricinus plant is very poisonous due to the presence of a compound called Ricin (globular glycosylated heterodimer protein) which is found in seeds of the plant. It triggers respiratory, cardiac issues like asthma, tachycardia and is also responsible for causing hypotension, diarrhea [4-6]. Leaves of the castor plant are also toxic which on consumption leads to muscle tremors, pyrexia depression, hypovolemic shock and abdominal distention [7].

Toxicology is the modern branch of science that explores the areas of genetics, biochemical, molecular and cellular biology. It basically deals with the study of chemical and physical characterization of poisons and their mode of action as they tend to damage genetic content of organism [8]. Genetic toxicology involves the study of poisonous agents that reacts directly with the nucleic acids altering the genes expressions at molecular and cellular levels [9, 10].

Among the various other techniques, comet assay is widely used in the area of toxicology. Comet assay is also known as single-cell gel electrophoresis. Ostling and Johansson came up with this technique for the first time in 1984 and Singh et al modified it in 1988. This assay is used for studying DNA damage and repair in clinical and dietary studies [11]. There are two types of comet assays,

one is alkaline comet assay and another is neutral comet assay. Due to high reproducibility and cost effectiveness, comet assay is comparatively favoured compared to other techniques. As DNA is negatively charged, it migrates towards the positive electrode, when the electricity is supplied (electrophoresis). Under the influence of electric current damage, DNA starts to move from the undamaged part which looks like head and tail (head denoting undamaged part and tail indicating damaged part) that is why the name comet is given. Increase in tail length indicates high genotoxicity [12]. Comet assay is used in clinical oncology to study the response of tumor cells to various drugs and radiotherapy [13].

Nowadays, we all are searching for traditional medicines which are cheap and have less side-effects, so wide range of research is going on with different plant species and their therapeutic principles. The main objective of this study was to assess the genotoxicity of *Ricinus communis* leaves and seeds using comet assay.

2. MATERIAL AND METHODS

Fresh leaves and ripened fruits of *Ricinus communis* were collected from Pragathi Enclaves community park, Miyapur, Hyderabad, state of Telangana. Collected leaves are washed under running tap water and then with distilled water several times. Leaves and fruits (capsule) were sun dried, up on drying capsules pop-opens releasing seeds which were collected. The dried samples were blended into fine powder and stored in air tight containers.

2.1. Isolation of human lymphocytes

HUMAN blood (5ml) was obtained from a healthy donor, transferred the blood into a falcon tube and centrifuged for 15 minutes at room temperature. Following the centrifugation, peripheral blood mononuclear cells were separated into a buffy top layer and the blood cells concentrated at the bottom of the tube as they are denser. Gently pipetted out the cloudy layer having PBMC into tubes and added 0.5 ml of RIPA media to each tube [14].

2.2. Preparation of silver nanoparticles of Ricinus communis leaf and seeds

To prepare leaf silver nanoparticles, around 10 gm of leaf powder was weighed and added in a conical flask containing 100ml of distilled water. Kept the flask in a heater for 10 minutes at 65°C (color changed to dark greenish brown). The flask was taken out and shaken well and if required heated again for 5minutes at 60°C, and allowed cool down. 50ml of 2Mm of silver nitrate was added, covered the flask with foil and heated again for 10 minutes at 70°C, after heating incubated the flask for 24 hours in dark at room temperature. After 24 hours, collected the pellet part by discarding the liquid supernatant. The pellet was taken into a falcon tube, centrifuged it for 10 minutes, discarded the supernatant and collected the pellet, repeated the process if required, and added 2ml of methanol, centrifuged for 5 minutes, removed the supernatant and left the pellet for some days at room temperature for air dry. The pH was maintained to 8 using KOH [15].

To prepare seeds silver nanoparticles, around 7.5 gm of seed powder was weighed in a conical flask having 100ml of distilled water. The flask was kept in a heater and heated for 5-7 minutes at 55°C, taken it out and allowed to cool down. Filtered the solution and added 50ml of 2Mm of silver nitrate and heated again for 15 minutes at 75°C (color changed from milky white to creamish). The flask was taken out, allowed to cool and kept aside for 24 hours in dark at room temperature. Rest of the procedure was same as mentioned above. From the solid extract was made using DMSO.

2.3. Preparation of test solutions

Human lymphocytes and *E. coli* are tested against the four different concentrations (25µl, 50 µl 75 µl and 100 µl) of silver nanoparticles of leaves and seeds of *Ricinus communis*. Eight eppendorfs were taken, 4 for seed and 4 for leaf testing samples, to each added abovementioned concentrations of testing samples (nanoparticles of leaves and seeds) and inoculated them with 1ml of *E. coli* culture. Following inoculation incubated them for 1 hour at room temperature. Testing with human lymphocytes follows the same procedure but here we used 400µl plasma.

2.4. Preparation of chemicals

The chemicals needed for the comet assay included 1% NMA (normal melting agar), 0.8% LMA (low melting agar), Lysis buffer, Neutralizing buffer, Electrophoretic buffer, Staining solutions, PBS and distilled water.

2.5. Comet assay

Dust free, plain slides were covered by first layer of 1% of NMA, allowed the slides to dry in Hot air oven for 2 minutes. For 2nd layer, 50 µl of sample test of each concentration was mixed with 280 µl of LMA and immediately poured on slides, spreaded evenly; secured the slides with coverslips (make sure to prepare one slide

as blank). The slides were kept in fridge for 20 to 25 minutes. 2 coupling jars were taken and added 30ml of lysis buffer, 1ml of 10% DMSO and 1x triton-x to each of them, kept the jars in fridge for 20 minutes. Following the 20 minutes of incubation, the slides were takenout and made the 3rd layer with 150 µl of NMA, covered with cover slip and again kept them in fridge for 20-25 minutes. Taken out the slides from fridge and put them in coupling jars containing chilled lysis solution, kept the jars in fridge for overnight incubation. After24 hours, slides were removed from lysis solution, drained, and placed them in electrophoretic tank side by side without disturbing agarose layer, make sure that sides are directed towards anode. The tank was filled with electrophoretic buffer (PH>13) till all the slides get submerged. The slides were left there for 30 minutes to allow unwinding of DNA. The gel electrophoresis was run for 30 minutes at 100mA, 20 V [16, 17]. After electrophoresis, the slides were washed with chilled neutralizing buffer for 5 minutes 3 times, lastly washed with distilled water. Slides were air dried thoroughly and gently introduced in coupling jars having fixing solution for 20 minutes, washed them gently with distilled water once. The slides were kept aside for air drying. For staining, the slides mix staining solution A and staining solution B (freshly prepared) in 1:2 ratio and poured it all over the dried slides (as staining solution is light sensitive, work is preferably carried out in dark rooms). The slides were kept in staining solution for 15-20 minutes, during the staining period a gentle agitation was done until greyish to blackish color developed [18, 19]. The slides were taken out, washed once with distilled water and air dried. Slides can be studied under microscope (traditional methods) or using high-tech software [20], hundred cells were selected per slide for quantified analyzation of tail length [21].

2.6. Statistics

A simple statistical formula is used to calculate the percentage rate in respective test samples, with each concentration the value varies. Given below is the formula to use.

Percentage of inhibition = a-b/a-c x 100.

Where, a is tail length of positive control, b is tail length in the presence of testing solution and c is tail length of negative control.

3. RESULTS AND DISCUSSION

Comet assay is an efficient tool in measuring the nucleic acid damage (DNA). Main objective of this study is to evaluate genotoxicity of *Ricinus communis*. When 4 different concentrations (25 µl , 50 µl , 75 µl , 100 µl) of leaf and seed silver nanoparticles were treated against human lymphocytes, two different results were obtained. Leaf nanoparticles exhibits mild genotoxicity (as the concentration increases mild genotoxicity is observed), with first two concentrations no tails were observed but with 75 µl concentration tail lengths of 1.8, 2.5, 2.6, 2.8, 3.4, 4.5, µm, p value of 0.0149were observed and with 100 µl concentration tail lengths of 3, 4, 5, 6, 8, 10, 15 µm, p value of 0.0218 were observed. Whereas treatment with seed nanoparticles showed high DNA damage. As the concentration increases, genomic damage also increases, 100µl concentration showed necrosis where p value becomes insignificant. This indicates that leaves are mildly toxic whereas seeds are potent genotoxic to humans.

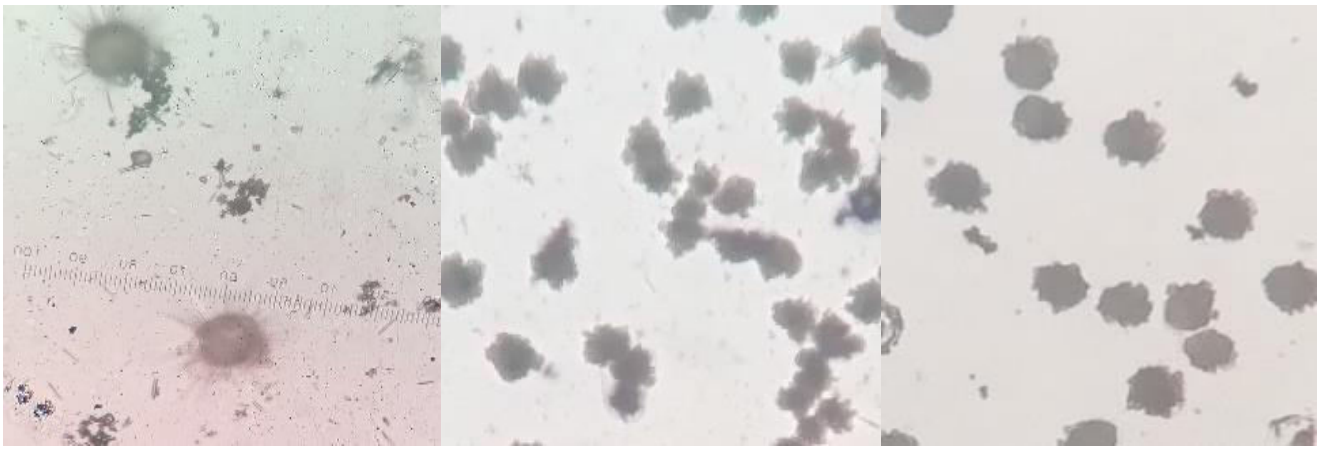
Table 1: Genotoxic activity of leaves nano-particles of *Ricinus communis* on human lymphocytes

Leaves nano-particles	Cell scored	Tail length ±SE	P value
untreated	100	0.31±0.02	NA
25 µl (1.25mg)	100	0.44±0.09	0.0029
50 µl (2.5mg)	100	0.65±0.12	0.0014
75 µl (3.7mg)	100	3.1±1.25	0.0142
100 µl (5mg)	100	6.5±3.04	0.0218

Table 2: Genotoxic activity of seeds nano-particles of *Ricinus communis* on human lymphocytes

Seeds nano-particles	Cell scored	Tail length ±SE	P value
Untreated	100	0.31±0.02	NA
25 µl (1.25mg)	100	0.41±0.09	0.0041
50 µl (2.5mg)	100	0.61±1.0	0.4270
75 µl (3.7mg)	100	necrosis	NA
100 µl (5mg)	100	necrosis	NA

Analysis of genomic damage of leaf nanoparticles (*Ricinus communis*) against *E. coli* showed a significant increase in tail length, highest concentration i.e. 100 µl of leaf nanoparticles exhibits necrosis indicating anti-microbial activity. Investigation of seed nanoparticles with *E. coli* culture revealed moderate genotoxicity (no tails were observed when cells are treated with 25 and 50 µl concentrations but the treatment with last two concentrations showed tails of 4 µm and 6 µm hence less amount of DNA was damaged).



1 2 3

Image 1; lymphocyte necrosis (seed nanoparticles), Image 2; E. coli cells showing DNA damage (leaf nanoparticles) long tails can be observed, Image 3; E. cells small tails are observed (seed nanoparticles).

Fig. 1: Human lymphocytes and *E. coli* after treating with leaf and seed nanoparticles of *Ricinus communis*

Table 3: Genotoxic activity of leaves nano-particles of *Ricinus communis* on *E. coli*

Leaves nano-particles	Cell scored	Tail length ±SE	P value
Untreated cells	100	0.10±0.01	NA
25 µl (1.25mg)	100	0.21±0.09	0.1032
50 µl (2.5mg)	100	2.6±0.1	0.0001
75 µl (3.7mg)	100	11.5±0.1	0.0001
100 µl (5mg)	100	necrosis	NA

Table 4: Genotoxic activity of seeds nano-particles of *Ricinus communis* on *E. coli*

Seeds nano-particles	Cell scored	Tail length ±SE	P value
Untreated cells	100	0.10±0.01	NA
25 µl (1.25mg)	100	0.24±0.02	0.0004
50 µl (2.5mg)	100	0.28±0.04	0.0016
75 µl (3.7mg)	100	4.1±0.12	0.0001
100 µl (5mg)	100	6.5±0.24	0.0001

NA = not applicable*

Research studies of Funatsu G and G Balin can be related to present research outcomes. Their studies evaluated DNA damage properties of Ricinus communis in the field of toxicology [22, 23]. With respect to all concentrations of seed and leaf silver nanoparticles, we got high genomic damage at 75 and 100 µl in human lymphocytes with seed silver nanoparticles, in case of E. coli severe genotoxicity is observed at 75 and 100 µl with leaf silver nanoparticles.

4. CONCLUSION

The present study is the assessment of genotoxicity of leaves and seed of *Ricinus communis* in human lymphocytes and in *E. coli* using their silver nanoparticles by comet assay. From our studies, it was concluded that leaves and seeds of *Ricinus communis* possess genotoxic properties, our results demonstrated that as the concentration increases more damage was observed, reported majorly in human lymphocytes and in *E. coli* cells.

Conflict of interest

There is no conflict of interest between authors

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