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Detoxification of *Abrus precatorius L.* seeds by Ayurvedic *Shodhana* process and anti-inflammatory potential of the detoxified extract

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ABSTRACT

Background: *Abrus precatorius* seeds traditionally used for the treatment of sciatica and alopecia contains the toxic protein, abrin, a Type II Ribosome Inactivating Protein. *Ayurveda* recommends the use of *Abrus* seeds after the *Shodhana* process (detoxification). **Objective:** The current study was aimed at performing the *Shodhana* process, *swedana* (boiling) of *Abrus precatorius* seeds using water as a medium and to evaluate the anti-inflammatory potential of seed extract post detoxification. **Materials and Methods:** Non-detoxified and detoxified extracts were prepared and subsequently subjected to various *in vitro* and *in vivo* assays. In hemagglutination assay, the non-detoxified extract shows higher agglutination of RBCs than detoxified extract indicating riddance of toxic hemagglutinating proteins by *Shodhana*. This was confirmed by the SDSPAGE analysis of detoxified extract revealing the absence of abrin band in detoxified extract when compared to non-detoxified extract. **Results:** The cytotoxicity assay in HeLa cell line expresses a higher reduction in growth percentage of the cells with non-detoxified extract as compared to detoxified extract indicating successful detoxification. Brine shrimp lethality test indicated the reduction in toxicity index of detoxified extract as compared to non-detoxified extract. Further, the whole body apoptosis assay in zebrafish revealed that percentage of viable cells were greater for detoxified extract than non-detoxified extract. The anti-inflammatory studies using carrageenan induced paw edema model in rats was carried out on the extracts with doses of 100 mg/kg and 200 mg/kg, *per oral,* where the detoxified extract exhibited significant inhibition of rat paw edema at both the doses comparable to that of Diclofenac sodium. **Conclusion:** Absence of toxicity and the retention of the anti-inflammatory activity of detoxified *Abrus* seed extract confirmed that the *Swedana* process is effective in carrying out the detoxification without affecting its therapeutic potential.

Key Words: Abrin, *Abrus precatorius*, anti-inflammatory, detoxification, *Gunja* seeds, *shodhana*

INTRODUCTION

Ayurveda, the science of life, is a comprehensive medical system that has been the traditional system of health care in India for more than 5000 years, and is one of the world's oldest medical systems. The term '*Ayurveda*' combines the Sanskrit words '*ayur*' (life) and '*veda*' (science or knowledge), meaning the science of life. A chief aim of *Ayurvedic* practices is to cleanse the body of substances that can cause disease, thus helping to reestablish harmony and balance.[1,2] There are many drugs used in *Ayurveda* originated from sources like plants, animals, and minerals. There are many herbs with toxicity liabilities, but have been useful in some disease conditions.[3] *Ayurveda* describes that these toxic herbs should be used medicinally after proper detoxification processes. The process of detoxification without harming its medicinal properties (*gunas*) is referred to as the process of '*Shodhana*' or '*Shodhana Prakriya*' in *Ayurveda*.[4] The concept of *shodhana* treatment was highly accepted by the pioneers of *Rasashastra* (Eighth Century A.D.) especially for the purification of herbomineral drugs. The process of detoxification are said to bring about favorable changes that modifies the therapeutic effect and also renders the drug free from poisonous effects. Some of the *shodhana* processes mentioned in *Ayurvedi*c texts are *Nirvapana* (cooling), *Dhalana* (melting and dipping into

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liquid medium), *Bharjana* (roasting), *Bhavana* (trichuration), *Swedana* (steaming), *Shoshana* (drying) etc., These *shodhana* treatments make use of different *shodhana* media like medium of acidic (e.g., lemon, buttermilk, *kanji*) or alkaline (e.g., C*hurnodaka* – lime water) or of neutral nature (e.g., water). These treatments are performed with or without the help of heat given for a specified period of time.[5,6]

Gunja or Jequirity seeds (*Abrus precatorius*), (family: Fabaceae), from a well known toxic plant of *Ayurveda* under *Upavisha Vargas* (semi poisonous/toxic family) is suggested to be used only after proper *shodhana* process. The seeds are ovoid, scarlet, and red with a black notch at the hilum.[4] In the indigenous system of medicine, extracts of seeds have been used externally for the treatment of ulcers and skin affections.[3] The seeds have also been used for the affections of nervous system, and their paste is applied locally in sciatica, stiffness of shoulder joint, and paralysis.[7,8] *Gunja* seeds contains a number of constituents like alkaloids, flavonoids, tannins, triterpenoids, proteins etc., amongst which a toxalbumin, abrin, is considered primarily responsible for the toxic effects of the seeds.[9-11]

The *Ayurvedic* texts recommend the *shodhana* process of *Abrus* seeds. Earlier studies have reported *shodhana* of *Gunja* seed with *Nimbu Swarasa* obtaining *Nimbu Swarasa Sodhita Gunja seed*, with *Kanji* and water obtaining *Kanji Sodhita Gunja* seed, with water *Sodhita Gunja* seed, and using cow's milk to obtain *Godugdha Sodhita Gunja* seed.[12] While others have reported the process of soaking *Abrus* seeds in cow urine for seven days and roasting in cow ghee till get swollen to make it *shodhit* (detoxified).[13]

Thus, there was a need to confirm the success of detoxification by *shodhana* process on *Abrus precatorius* seeds. Further, it was imperative to decipher the impact of *shodhana* on the therapeutic potential of *Abrus precatorius* seeds. The current research work was aimed at carrying out the detoxification of seeds of *Abrus precatorius* by traditional *shodhana* process, *swedana*, mentioned in *Ayurvedic* texts and to demonstrate the efficacy of detoxification by SDS Polyacrylamide gel electrophoresis (SDS-PAGE) for abrin determination, *in vitro* methods like hemagglutination assay, cytotoxicity study using HeLa cell line, *in vivo* assays with brine shrimp lethality assay, and whole body apoptosis study using zebrafish. Further, to reaffirm the anti-inflammatory potential of the detoxified and non-detoxified extract in an acute rodent model of inflammation and to confirm that the activity of *Abrus precatorius* seed extract is retained after the *shodhana* process.

MATERIALS AND METHODS

Collection and authentication of seeds of *Abrus precatorius*

Seeds of *Abrus precatorius* were purchased from local market (APMC Market), Vashi, Navi Mumbai. The seeds were authenticated by Dr. Ganesh Iyer, Head of Department, Department of Life Sciences, Ramnarain Ruia College, Matunga, Dadar (E), Mumbai 400 019. Then the seeds were washed with purified water and dried at room temperature. Dried seeds were used for the *shodhana* and extraction.

Detoxification (*Shodhana* process) and extraction of *Abrus precatorius* seeds

Seeds were decorticated, defatted, and ground to get dry powder. 50 g of this powder was kept for cold maceration for 24 h in distilled water (powder to water ratio was 1:5). After cold maceration; the macerate was minced in a mixer along with the medium to obtain slurry. The slurry was then subjected to centrifugation at 6000 rpm for 15 min. The pellets were discarded and supernatant was collected. The process of centrifugation was repeated on the supernatant which was collected from the first centrifugation. Again, the pellets were discarded and supernatant collected. Final supernatant obtained was subjected to lyophilization and stored in a vacuum desiccator at room temperature. The extract obtained is the non-detoxified extract. In *shodhana* process, seeds were tied in muslin cloth and boiled in distilled water for 3 h continuously. Seeds were removed from the medium and decorticated, defatted and ground to get wet powder. Then the dried powder was treated in a similar manner as that of the non-detoxified seeds. Final supernatant obtained was subjected to lyophilization.[4] The extract obtained is the detoxified extract.

Hemagglutination assay

Hemagglutination assay was performed in V-microtiter plates by serially diluting a 100 μL of freeze dried extract solution (5 mg/mL) of non-detoxified (rows A and B) and detoxified (rows E and F) extract into 100 μL of phosphate buffered saline (PBS). Added to each well was 20 μL of 5% Human RBC suspension and hemagglutination was determined after one hour incubation at 37° C as the reciprocal of the greatest dilution that gave visible aggregation. Well no 12, 24, 60, and 72 served as control.[4,14,15]

SDS Polyacrylamide gel electrophoresis

The abrin rich fraction, non detoxified extract and detoxified extracts along with bovine serum albumin (BSA) (65.5 kDa) as standard were submitted to SDS-PAGE. Stacking gel 5% and resolving gel 12%, sample loading buffer (2X)

and electrode buffer (5X) were used. Electrophoresis was carried out until the bromophenol dye reached the bottom of the gel. The stacking gel was performed at 70 V and resolving gel at 120 V. After electrophoresis, gel was removed from the plate and kept in developing solution for one hour and then overnight in staining solution of Coomassie Brilliant Blue-R 250. After staining, gel was kept for 30 min in destaining solution. The gel documentation was performed using ImageLab software in Geldoc XR+ (BIORAD Laboratories) and the bands are visualized for toxic protein Abrin (66 kD).[16,17] Abrin rich fraction is a purified dialyzed fraction of lectins obtained from the non-detoxified seeds.

In vitro cytotoxicity evaluation using HeLa cell line

The cell lines were grown in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96-well microtiter plates in 100 µL at plating densities, depending on the doubling time of cell line. After cell inoculation, the microtiter plates were incubated at 37° C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, one 96-well plate containing 5 * 10^3 cells/well was fixed *in situ* with tricarboxylic acid (TCA), to represent a measurement of the cell population at the time of drug addition (Tz). Extracts or Adriamycin was initially solubilized in dimethyl sulfoxide (DMSO) at 100mg/mL and diluted to 1mg/mL using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1mg/mL) was thawed and diluted to 100 µg/mL, 200 µg/mL, 400 µg/mL, and 800 µg/mL with complete medium containing test compound. Aliquots of 10 µL of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µL of medium, resulting in the required final drug concentrations of 10 µg/mL, 20 µg/mL, 40 µg/mL, and 80 µg/mL. The study was performed in triplicates.

Endpoint measurement

After compound addition, plates were incubated at standard conditions for 48 h and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µL of cold 30% (w/v) TCA (final concentration, 10%TCA) and incubated for 60 min at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µL) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 min at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.[18,19]

Brine Shrimp Lethality Assay

Brine shrimp (*Artemia salina*) were hatched using brine shrimp eggs in a conical vessel (1 L), filled with sterile artificial sea water (prepared using sea salt 38 g/L adjusted to pH 8.5) under constant aeration for 48 h in the dark. After hatching, 10 nauplii were drawn from the vessel through a glass capillary from brighter portion of vessel and placed in each test tube containing 4.5 mL of brine solution. In each experiment, 0.5 mL of detoxified and non-detoxified extracts were added to 4.5 mL of brine solution and maintained at room temperature under the light. Experiments were conducted along with the control (vehicle treated), different concentrations (10, 20, 40, 80 µg/mL) of detoxified and non detoxified extract and dead nauplii were counted after 24 h.[20-22]

% mortality was calculated using the following formula:

$$\% \text{ mortality} = \frac{\text{No of deed nauplii}}{\text{Initial of nauplii}} \times 100$$

Whole body apoptosis study using zebrafish

Healthy zebrafish (*Danio rerio*) were reared in a 25 L aquarium containing dechlorinated tap water and were fed with commercial food. Fish were maintained with a 12 h light-12 h dark photoperiod with continuous aeration. For LD_{50} determination of non-detoxified extract, zebrafish were exposed to solutions of different concentrations of non-detoxified extract (0.1, 0.2, 0.3, 0.4, 0.5 mg/mL) for 2 days in a test system containing extract dissolved in dechlorinated water. Each zebrafish was kept in 500 mL water in a glass test system covered by a glass plate. Water was continuously aerated, and temperature was maintained at 27 ± 2° C, pH ranged from 7.2-8.4. The concentration of 0.5 mg/mL was found to be the LD_{50} of non-detoxified extract at which no lethality was found in detoxified extract. This concentration was then selected as the working concentration for the toxicity study.

For experimental purpose, zebrafish were divided into four groups (n = 4) as control, Standard Adriamycin (0.5mg/mL), Non-detoxified Extract (0.5 mg/mL) and Detoxified Extract (0.5 mg/mL). In the experimental groups, each zebrafish was kept in 500 mL water in a glass test system covered by a glass plate. Water was continuously aerated and temperature was maintained at 27 ± 2° C, pH ranged from 7.2-8.4. Fish were fed freeze dried commercial fish food once a day. During exposure, fish were monitored for abnormal behavior and mortality. After the study period, fish were euthanized and their tails and fins were removed. Each fish was washed with water twice and stored in -20° C till further study. Mitochondrial

fractions were obtained from the whole zebrafish body by differential centrifugations. Whole body was homogenized in sucrose solution by homogenizer (50 mL capacity). Homogenate was centrifuged at 3000 rpm for 10 min; supernatant was collected, and then re-centrifuged at 8000 rpm for 20 min. The resultant pellets were collected and resuspended in 10 mL HEPES buffer. These mitochondrial fractions were then preserved at -70°C until the experiments were performed. Mitochondrial viability was evaluated by the MTT assay which is a colorimetric assay for assessing cell viability. This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent (e.g., isopropanol) and the released, solubilized formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.[23-25]

Evaluation of anti-inflammatory activity of extracts on carrageenan induced rat paw edema

The anti-inflammatory effect of non-detoxified and detoxified extract of *Abrus precatorius* seeds were assessed in acute inflammation model with, carrageenan induced rat paw edema.[26-28] Male Wistar rats (weight range: 200 ± 20 g) were used for the study (Total no. 36; n = 6 per group). The animals were placed in the experimental room maintained under standard conditions of temperature (25°C ± 2°C) and relative humidity (55 ± 10%), fed with commercially available rodent food pellets and water *ad libitum*. The experiments were performed after approval of the Institutional Animal Ethics Committee (IAEC no 242). Initially normal paw volume of each rat was noted. Paw edema was induced by injecting 0.1 mL of 1% carrageenan in physiological saline into the subplantar tissue of the left hind paw of each rat. The doses of both extracts for the anti-inflammatory study were decided from the previously reported studies and LD_{50}.[29] The non-detoxified and detoxified extracts of the *Abrus precatorius* seeds were dissolved in vehicle and administered (100 and 200 mg/kg) orally 30 min prior to carrageenan administration. The paw volume was measured at 0, 30, 60, 120, 180 and 360 min by the plethysmometer. Control rats received distilled water, while Diclofenac sodium (10 mg/kg, *per oral*) was used as standard drug. Change in paw volume was noted, and percent inhibition of paw edema was calculated by the formula.

$$\% \text{ Inhibition} = (V_C - V_T) \times 100/V_C$$

V_C = Change in paw volume in control group

V_T = Change in paw volume in treated group

Statistical analysis

The anti-inflammatory activity was analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's test where $P < 0.05$ was considered significant.

RESULTS

The detoxification and extraction of *Abrus precatorius* seeds and *in vitro* confirmation of effective detoxification

The main aim of any *shodhana* process is to reduce the toxic constituents to some extent or by potentiating their chemical transformation to non-toxic or relatively less toxic substances.[30-32] We employed *swedana* as the *shodhana* process for the detoxification of the *Abrus precatorius* seeds. When *Abrus* seeds were subjected to *shodhana*, the toxin protein is expected to be deactivated by the mechanism of denaturation.[33] The data obtained by the battery of *in vitro* tests and *in vivo* assays conducted by us supports our hypothesis that *shodhana* of *Abrus* seeds reduces its toxicity while retaining its therapeutic potential.

The detoxification of *Abrus precatorius* seeds had been carried out by traditional *Ayurvedic shodhana* process known as *Swedana* which involved boiling of seeds in water for 3 hrs followed by extraction of seeds by cold maceration. The percent yield of lyophilized non-detoxified extract was found to be 2.78% w/w while that of lyophilized detoxified extract was found to be 0.86% w/w.

The hemagglutination assay showed, the hemagglutination titer for the detoxified seeds was much lower, that is 2^4, than the non-detoxified seeds at 2^{11}. The *shodhana* process destroys the *Abrus* agglutinin and reduces its property to act as an antigen and binding to RBCs there by reducing the hemagglutination [Figure 1].

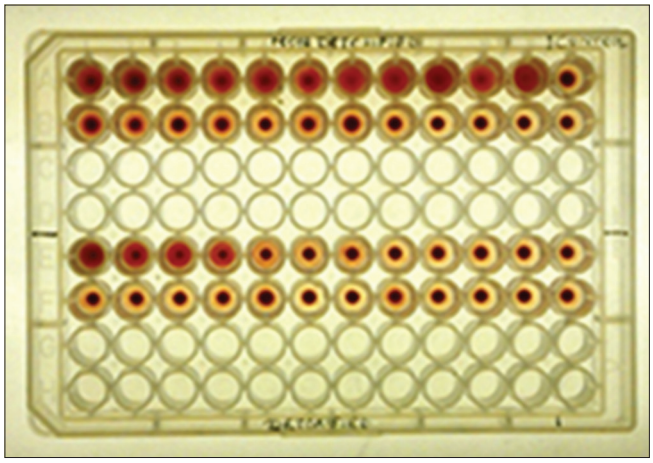

Figure 1: Hemagglutination assay of non-detoxified and detoxified extract of Abrus precatorius seeds Rows A and B: Non-detoxified extract Rows E and F: Detoxified extract

Non-detoxified and detoxified extract, both were subjected to SDS-PAGE (12%) along with Bovine serum albumin (BSA) (65.5 kD) as a standard under non-reducing conditions where non-detoxified extract showed the band at 66 kD indicating the presence of toxic protein abrin, while the band was absent in detoxified extract, indicating that the *shodhana* process we employed was successful in carrying out the detoxification *of Abrus precatorius* seeds [Figure 2].

In vitro cytotoxicity screening of non-detoxified and detoxified extracts was conducted to determine the reduced cytotoxicity potential of the detoxified extract compared to non-detoxified extract. The results were obtained as percent cell growth curve which expresses the reduction in the cytotoxicity of detoxified extract in HeLa cells as compared to the non-detoxified extract. The related parameters Total Growth Inhibition (TGI), Growth Inhibition of 50% cells (GI_{50}) were also obtained [Tables 1 and 2].

Effect of extracts in brine shrimp lethality assay and whole body apoptosis study in zebra fish

The toxicity of the non-detoxified and detoxified extracts was evaluated at different concentrations using brine shrimp lethality assay. The non-detoxified extract induced higher mortality than the detoxified extract indicating that the cytotoxic principle in the *Abrus* seeds had been removed by the *shodhana* process. The standard Adriamycin has produced the maximum overall mortality and 100% mortality at 80 µg/mL [Table 3].

Whole body apoptosis study was carried out on zebrafish (*Danio rerio*) to compare the toxicity of extracts on cells as *Abrus precatorius* contains abrin, a toxic protein, which causes cell death via an apoptotic pathway. The percent cell viability was measured in response to both the extracts where non-detoxified extract was found to reduce cell viability similar to the cytotoxic drug Adriamycin. Detoxified extract was found to retain the cell viability indicating reduction in the toxicity propensity of the detoxified extract [Table 4].

Anti-inflammatory activity of extracts in carrageenan induced rat paw edema

The detoxified and non-detoxified extracts both reduced the carrageenan induced rat paw edema significantly in a dose dependent manner. The different doses of the

Table 1: Effect of extracts on % growth in HeLa cell line

Sample	% Growth- human cervix cancer cell line (HeLa)			
	Drug concentrations (µg/mL)			
	10	20	40	80
ND	37.70±2.91	7.49±1.64	6.33±1.61	−4.13
D	100.00	90.79±2.58	87.18±1.03	72.44±5.58
ADR	No growth	No growth	No growth	No growth

Values are expressed as Mean±SEM, *n*=3, ND: Non-detoxified Extract (100µg/mL), D: Detoxified extract (100 µg/mL), ADR: Adriamycin (100 µg/mL), HeLa: Human cervix cancer cell line

Table 2: Effect of extracts on TGI and GI_{50} values in HeLa cell line

Sample	Drug concentrations (µg/mL) calculated from graph	
	TGI	GI_{50}
ND	60.7	10.3
D	>80	>80

ND: Non-detoxified Extract (100µg/mL), D: Detoxified extract (100µg/mL), TGI: Total growth inhibition, GI50: Growth inhibition 50%, HeLa: Human cervix cancer cell line

Table 3: Effect of extracts on % mortality in brine shrimp lethality assay

Concentration µg/mL	% mortality		
	Non-detoxified extract	Detoxified extract	Adriamycin
10	66.67±0.37	36.67±0.48	93.33±0.18
20	73.33±0.18	40±0.32	93.33±0.18
40	80±0.32	46.67±0.37	96.67±0.18
80	93.33±0.18	60±0.32	100

Values are expressed as mean±SEM, *n*=10

Table 4: Effect of extracts on % cell viability in whole body apoptosis study using zebrafish

Groups	% cell viability
Adriamycin	21.28±0.005
Non-detoxified extract	28.36±0.009
Detoxified extract	47.66±0.003

Values are expressed as mean±SEM, *n*=4

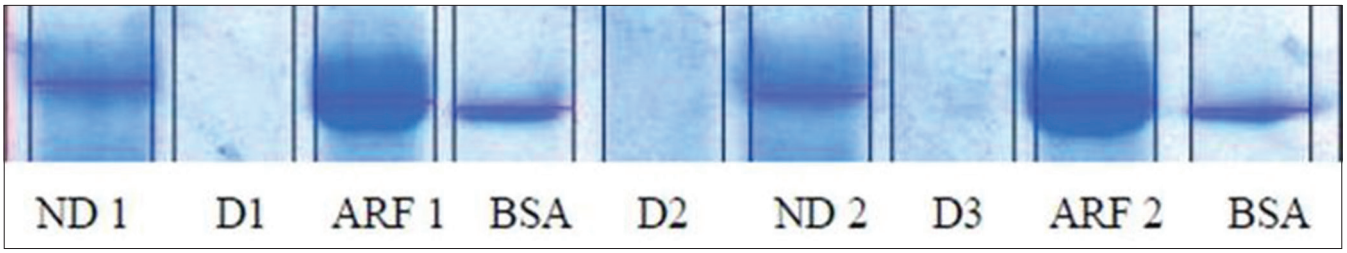

Figure 2: SDS Polyacrylamide gel electrophoresis for Abrin determination ARF: Abrin Rich Fraction (10 mg/mL) ND 1: Nondetoxified Extract 1 (10 mg/mL) ND 2: Nondetoxified Extract 2 (20 mg/mL) D 1: Detoxified Extract 1 (10 mg/mL) D 2: Detoxified Extract 2 (20 mg/mL) D 3: Detoxified Extract 2 (40 mg/mL) BSA: Bovine Serum Albumin (5 mg/mL)

extracts (100 and 200 mg/kg, *po*) significantly inhibited the inflammation to the extent of standard Diclofenac sodium (10 mg/kg, *po*) as compared to the control group ($P < 0.05$). The reduction in inflammation by detoxified extract suggests that the detoxification process employed did not lead to the loss of anti-inflammatory potential of *Abrus precatorius* seeds [Table 5 and 6].

DISCUSSION

Gunja (*Abrus precatorius* Linn.), a well-known plant of *Ayurveda* under *Upavisha* group (sub/semi poisonous group), is being used extensively in different formulations with great therapeutic significance and is being advocated to use, in various diseases like *Indralupta* (alopecia), *Shotha* (edema), *Krimi* (helminthes), *Kustha* (skin diseases), *Kandu* (itching), *Prameha* (urinary disorders) etc., after proper processing known as *shodhana* (detoxification procedure).[30,31] The toxin reported to be present in *Abrus* seeds include a toxic lectin, Abrin, is a type II ribosome inactivating protein (RIP), consisting of an A-chain, which is a single polypeptide chain and the toxic principle binding to cytosol and a B-chain, which is a lectin linked through a disulfide bond. A chain is non toxic to intact cells and requires B-chain for its action.[32] It causes cell death via inhibition of protein biosynthesis through depurination of a single adenine residue (A4 and 324) of the 28S ribosomal subunit.[33] Many Plant lectins are resistant to heat and proteolytic cleavage but sugar binding activities of lectins gets destroyed by heating at higher temperature. The heat denatured lectins do not show any biological and cellular activity.[34,35]

The *Shodhana* process employed leads to the denaturation and removal of the toxic protein Abrin from the seeds. Its effectiveness was demonstrated by the outcomes of hemagglutination assay and SDSPAGE abrin band analysis. This was substantiated by the *in vitro* cytotoxicity studies using HeLa cell lines where percentage of control growth for the detoxified extract was much higher than that of the non-detoxified extract. At higher concentration that is at 80 μg/mL the non-detoxified extract has shown complete inhibition of cell growth as compared to detoxified extract. The TGI parameter also reveals that the non-detoxified extract expresses higher inhibition of cell growth as compared to the detoxified extract. These results indicate that the *shodhana* process that was employed leads to the reduction of the cytotoxic principle abrin which reduces its cell toxicity.

The brine shrimp lethality assay revealed that the detoxification process employed was effective in removing the toxic principle from the seeds wherein the percentage of mortality induced by the detoxified abstract was much lower than the non-detoxified extract and standard Adriamycin. The detoxification process lowered the mortality induction by 50% *vis-a-vis* the non-detoxified extract.

The whole body apoptosis study using zebrafish was carried for non-detoxified extract and detoxified extract. The percent cell viability on exposure to detoxified extract was found to be much higher than the non-detoxified extract whereas the standard, Adriamycin has shown the maximum reduction in cell viability. This indicates the effectiveness of the detoxification process in removing the cytotoxic components present in *Abrus precatorius* seeds.

The anti-inflammatory activity of both the extracts was evaluated by using carrageenan induced rat paw edema as a model. The main objective of the study was to demonstrate that the activity of the detoxified extract remains unchanged after the employed *shodhana* process. In the carrageenan administered animals the swelling reached severe proportion in the third-hour and the swelling was maintained until fourth–hour. In the Diclofenac treated group, paw edema was decreased significantly throughout the period of study with maximum inhibition of 89.64%. The swelling of paw had abated by the sixth-hour in Diclofenac administered rats. Further, the animals treated with non-detoxified and detoxified extract (100 mg/kg) showed considerable reduction in swelling (maximum percentage of inhibition was 52.49 and 52.76, respectively) as compared to positive control group. The animals treated with non-detoxified and detoxified extracts (200 mg/kg) showed marked reduction in the paw edema (maximum percentage of inhibition was 80.83 and 88.60, respectively).

Table 5: Effect of extracts on rat paw volume

Groups	Dose (mg/kg)	Paw volume (mL) (min)				
		30	60	120	180	360
Positive control		0.46±0.04	0.5±0.06	0.63±0.04	0.64±0.08	0.32±0.03
Diclofenac Sodium	10	0.32±0.03*	0.29±0.02**	0.14±0.03***	0.07±0.02***	0.03±0.01***
Non-detoxified extract	100	0.37±0.04	0.44±0.04	0.33±0.04***	0.3±0.05***	0.2±0.07
Non-detoxified extract	200	0.24±0.03***	0.28±0.04**	0.19±0.03***	0.17±0.04***	0.06±0.01***
Detoxified extract	100	0.31±0.03*	0.35±0.04	0.34±0.05***	0.3±0.05***	0.17±0.06*
Detoxified extract	200	0.24±0.03***	0.28±0.01**	0.24±0.05***	0.12±0.02***	0.04±0.01***

Values are expressed as mean±SEM (n=6). One Way ANOVA followed by Dunnett's test, significance at P<0.05*, P<0.001**, P<0.0001*** as compared to positive control

Table 6: Effect of extracts on% inhibition of rat paw edema

Groups	Dose (mg/kg)	% Inhibiton of paw edema (min)				
		30	60	120	180	360
Positive control		-	-	-	-	-
Diclofenac Sodium	10	31.79	40.61	78.13	89.50	89.64
Non-detoxified extract	100	22.14	10.92	47.47	52.49	36.79
Non-detoxified extract	200	48.57	42.66	69.87	74.02	80.83
Detoxified extract	100	32.86	27.99	45.87	52.76	48.70
Detoxified extract	200	48.21	41.98	61.07	80.84	88.60

Hence, the different doses of non-detoxified and detoxified extract inhibited the paw edema significantly ($P < 0.05$) when compared to the control group. Development of carrageenan induced edema is biphasic; the first phase is attributed to the release of histamine, 5-HT, and kinins, whereas the second phase is related to the release of prostaglandins.[36] Thus, the present investigations has demonstrated that the non-detoxified and detoxified extract of *Abrus precatorius* seeds showed significant reduction in the biphasic response of carrageenan induced paw edema in a dose dependent manner. The maximum percentage reduction in paw edema was shown by the detoxified extract at 200 mg/kg (88.60%) indicating that the other components of the *Abrus precatorius* seed extract apart from abrin and abrus agglutinin which includes glycosides of abranin, pelargonidin, cyanidin, and delphinidin, flavonoids, isoflavoquinone like abruquinone[8,10,11] are instrumental in mediating its therapeutic actions after the *shodhana* process. Corroborating on similar aspects Gotecha *et al.* have recently revealed that the methanolic extract of *Shodhit* (detoxified) *Abrus precatorius* seeds significantly decreased the paw edema induced by carrageenan in rats at the dose of 500 mg/kg as compared to *Ashodhit* (non-detoxified) seeds. The process of *Shodhana* utilized by them encompassed soaking the seeds in cow urine for seven days and roasting in cow ghee till swollen to make it *shodhit*. *Shodhit* (detoxified) and *Ashodhit* (non-detoxified) seeds were then powdered and methanolic extract was prepared.[13]

CONCLUSION

The current investigations unravels that the *shodhana* process used in the study is successful in carrying out the detoxification of *Abrus precatorius* seeds without affecting its anti-inflammatory properties. Further, studies should be directed to confirm the retention of other reported medicinal properties of *Abrus precatorius* seeds.

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