ABSTRACT

Semecarpus anacardium (SA) is known as bhallatak in India and ‘marking nut’ by Europeans. This plant is widely available in sub-Himalayan province, tropical and central part of our country India. It is a deciduous tree; medium in size. The colour of fruit is black when ripe as well as smooth and shiny in texture. The fruit is generally categorized as toxic and the integral part of the fruit i.e. nut is about 1 inch long in size.

Bhallataka (Semecarpus anacardium Linn; Anacardiaceae) fruit is one of the upavishadravya (semi poisonous drugs). Its importance and utility is increasing day by day because of its therapeutic significance in many diseases including cancer. Though the fruits of Bhallatakahas many therapeutic values, pharmacies are scared to use this drug because of its irritant vesicating nature. The fruit contains tarry oil which causes contact dermatitis. Medically it is very well recognized as Urushiol induced contact dermatitis because the chemical Urushiol is responsible for dermatitis. If this vesicant nature is removed, the drug could be a good source for pharmaceutical industries in manufacturing many formulations containing Bhallataka as an ingredient.

Shodhana is the purificatory measure used in Ayurveda to purify toxic medicinal plants (upavishadravyas), by various pharmaceutical procedures like soaking, rubbing and washing etc. with specific medias like gomutra (cow’s urine), godugdha (cow’s milk) etc. Ayurveda advocates bhallataka after shodhana(purificatory procedures). Though there are different shodhana methods mentioned in Ayurveda, the shodhana method mentioned in the text Rasamrutam was adopted and quoted in API (Ayurvedic Pharmacopeia of India) and Ayurvedic formulary of India (AFI). The procedure is soaking the fruits in cow’s urine, cow’s milk and rubbing it in brick powder.

In the past, researchers have identified SA nuts extracts for potent anticarcinogenic potential. SA also possesses analgesic, hypoglycaemic, hepatoprotective, anthelmintic, anticancer, neuroprotective, anti-inflammatory, antioxidant, antimicrobial, antispermatogenic, anti-atherogenic, hypolipidemic, hypocholesterolemic, memory enhancing, cardioprotective, aphrodisiac and antituberculosis activity. However, thorough literature survey does not reveal the effect of Shodhana on different activities shown by SA. The present study was an attempt to evaluate the effect of shodhana on different pharmacological activities of SA. We have selected two potential activities like anticancer and nootropic activity. The method recommended in API/AFI was used for shodhana purpose. The effect of shodhana on toxic principle present in
SA was also evaluated. Thus, the present study has been undertaken to perform shodhana of nuts of *Semecarpus anacardium* Linn. by following established Ayurvedic Pharmacopoeia procedure and also to study the effect of shodhana on toxicity profile, phytochemical profile, anticancer profile and nootropic activity of *Semecarpus anacardium* nuts.

The nuts both pre shodhit and shodhit were subjected to maceration by methanol. Phytochemical as well as pharmacological parameters have been studied. Phytochemical analysis including % yield, TLC, HPTLC, GC-MS, flavonoid content and phenolic content have been determined to showcase the impact of shodhana. The toxicity study was also done using standard protocol. Selective pharmacological screening like nootropic and anticancer activity has been performed to know the effect of shodhana.

Shodhana of nuts improved the yield of methanolic extract of *Semecarpus anacardium* from 12%w/w to 15%w/w. Again there is a decrease in flavonoid and phenolic content in shodhit extracts which are in agreement with earlier studies. It was noticed that number of spots in TLC does not change. In addition, spot 1 $R_f$ value matches with HPTLC studies, $R_f$ values. But maximum height and areas were found to be decreased in both PSM as well as SM which were noticed on observing the concentrations values. These interpretation put forth that shodhana does not have qualitative effect but has a quantitative effect on the phytoconstituents.

Our studies on GC-MS have elucidated with n-hexadecanoic acid, heptafluorobutyricacid and 4-methoxyphenylester as major components in pre shodhit (PSM) and shodhitmethanolic extract (SM) of SA nuts. There were three compounds present in pre shodhit extract. One of them is urushiol like structure (1, 2-Benzenediol, 3-(8, 11, 14-pentadecatrieny)-, (Z, Z), retention time 56.270). Five compounds were found in shodhitmethanolic extract. One of them is anacardol derivative (Anacardol, tetrahydro-; retention time 51.538). Earlier reports have revealed that shodhana helps in conversion of toxic urushiol into non toxicanacardol. So the presence of anacardol derivative in shodhit extract and urushiol derivative in pre shodhit extract further confirms that shodhana helps in removal of toxic principle urushiol.

To study the toxic and allergic effects of *Semecarpus anacardium* three toxicity tests were conducted. They were acute toxicity, subacute toxicity and skin irritation test. Acute toxicity study was done as per OECD (Organisation for Economic Co-operation and Development) guideline showed no toxicity up to a single dose of 2000mg/kg for both shodhit and pre shodhit drug. Thus these drugs can be considered as Unclassified/Category-5 drug.
In the subacute toxicity study, daily administration of Pre shodhit and Shodhit Drugs by oral route once daily at the dose level of 100, 200 and 500 mg/kg/day for 28 days did not show any toxicity in Swiss albino mice, therefore no-observed-adverse-effect-level (NOAEL) of pre shodhit and Shodhit Drugs is considered to be more than 500 mg/kg/kg/day in both the sexes under this study condition.

Skin irritation assay was carried out by using both pre shodhit and shodhit methanolic extract on the ear lobe of the Guinea pig for 14 days and allowed for observation. After 14 days it was found that the irritation effect of shodhit drug was less as compared to pre shodhit drug. Thus we found that may be due to shodhana of *Semecarpus anacardium* the toxic chemicals responsible for skin irritations were removed. This supports our phytochemical analysis results.

There are some reports of nootropic activity of *Semecarpus anacardium*. The present study was undertaken to evaluate the effect of shodhana on nootropic activity of SA. Spatial learning and working memory was considered for evaluation. The parameters used were spontaneous alternation behavior (Y-maze), number of correct responses (Radial maze) and transfer latency (elevated plus maze) in day 1. Scopolamine, an anticholinergic drug, was used to induce cognitive deficit. Scopolamine can completely abolish spontaneous alternation behavior (SAB).

Y – Maze is popularly used for assessment of SAB. Many parts of the brain including hippocampus are involved in this task. Hippocampus is crucial for the formation and use of spatial memory. Hippocampal lesions in rodents impair spatial memory on radial arm maze task. The eight arm radial maze is recognized as an excellent model of spatial memory. Both pre shodhit and shodhit drug reversed the scopolamine induced decrease in percentage spontaneous alternation behaviour in Y-maze and number of correct responses in radial maze. This justifies the nootropic activity of methanolic extract of *Semecarpus anacardium*. However, shodhana decreases the nootropic activity in both the models as there is a significant decrease in % spontaneous alteration (Y maze) and number of correct responses (Radial maze).

Elevated plus maze is a widely accepted model for study of learning and memory in rodents where decrease in transfer latency (the time elapsed between the movements of an animal from an open area to a closed arm) is related with increased memory function. Transfer latency is used as a parameter to assess consolidation or retrieval mechanisms of learning and memory. However, treatment of drugs 30 min prior to first day exposure may be used for acquisition related action of drugs. Scopolamine produces acquisition deficits. The prolongation of transfer latency by Scopolamine can be reversed by pretreatment with cholinergic drugs like...
Physostigmine, tacrine etc. In the present study, Scopolamine induced increase in transfer latency in elevated plus maze was significantly decreased by pre shodhit drug only. Shodhit drug has no significant effect on acquisition deficit caused by Scopolamine. However, the improvement in acquisition deficit was significantly decreased by shodhana.

Acetylcholine plays a main role in cholinergic transmission in brain especially hippocampus and involved in spatial memory performance. ACh is degraded by acetylcholinesterase (AChE) enzyme. So a drug which inhibits AChE enzyme can increase cholinergic function and improve memory. In the present study, both pre shodhit and shodhit drug showed dose dependent inhibition of AChE activity in whole brain. Again, there is a decrease in % inhibition of AChE activity by shodhit drug which suggests a decrease in cholinergic function. So decrease in nootropic activity of shodhit SA may be attributed to decreased cholinergic function. In the present study, shodhana might have removed some anticholinesterase principles.

HeLa cells have been reported to be sensitive to a broad range of anticancer drugs and this characteristic makes it suitable for our studies. In the mitochondria of living cells MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide, a tetrazole) is reduced to formazan. MTT is yellow in color whereas formazan is purple in color. The absorbance of formazan is measured at 490 nm in a spectrophotometer. Since reduction takes place when mitochondrial reductase enzymes are active, the conversion of MTT to formazan can be directly related to the number of viable cells.

From the data, it is evident that 50% inhibition is seen between 200 to 300μL concentration and inhibition of 73.9% could be seen at highest concentration of 500ug/ml of pre shodhit extract. Whereas the toxicity is significantly (p<0.05) reduced in shodhit extract bringing down the 50% inhibition even in the highest concentration (500µg/ml) thus clearly reflecting that shodhana process indeed reduces the cytotoxicity potential of SA. This result is supported by another finding from our study that shodhana also increases the number of viable cells.

To know the mechanism of cytotoxicity role of oxidative stress and caspase 3 pathway were studied. Thiobarbituric acid reactive substances (TBARS), protein carbonyl, super oxide dismutase (SOD) and glutathione-SH assay were conducted as markers of oxidative stress. TBARS indices in our study revealed 6.7 nmoles/1000 cells and 5.57 nmoles/1000 cells with PSM and SM respectively, suggesting significantly reduced (p<0.05) stress levels and hence reduced cytotoxicity. The process of shodhana significantly (p<0.05) elevated the superoxide dismutase activity of cells exposed by shodhit extract as compared to pre shodhit extract. Cells
exposed to shodhit extract revealed significantly (p<0.05) lower values of protein carbonyl in nm moles/mg of protein, although statistically non-significant as compared to control and pre shodhit extract. Glutathione-SH values were significantly (p<0.05) elevated in shodhit extracts as compared to pre shodhit extract.

Methanolic extracts of *Semecarpus anacardium* showed cytotoxicity. There is a linear correlation between concentration and cytotoxicity. It follows Caspase 3 pathway to induce apoptosis. Caspase 3 is a cysteine protease that plays a significant role in the process of apoptotic cell death. Shodhana reduced the cytotoxicity of *Semecarpus anacardium*. The reduction in cytotoxicity may be attributed to reduction in oxidative stress as evident from decrease in TBARS, Protein carbonyl and increase in SOD, Glutathione SH.

So shodhana method referred in Ayurvedic Pharmacopoeia of India can be used for shodhana of *Semecarpus anacardium* nuts. It not only reduces toxicity but also alters its pharmacological activities. It reduced cytotoxicity without affecting anticancer activity significantly. It also attenuated nootropic activity. The effect of Shodhana on other pharmacological activities of *Semecarpus anacardium* can be conducted in the line of present work. This work can also be extended to other poisonous and semi poisonous plants for which shodhana method is described in Ayurvedic Pharmacopoeia of India.