CHEMOPREVENTIVE PROPERTIES OF LEAF EXTRACTS OF *SARACA ASOCA IN SALMONELLA TYPHYMURIUM STRAIN TA-100*

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Abstract: In India *Saraca asoca* (Family: Fabaceae; Subfamily: Caesalpinaceae), commonly known as 'Sita Ashoka' has been widely used in traditional medicine for many ailments such leucorrhoea, bleeding hemorrhoids, dysfunctional uterine bleeding etc. The aerial parts of the tree are chiefly used in medicines and it has been reported to contain chemicals such as glycoside, flavonoids, tannins, saponins, alkanes, esters and primary alcohols. The aim of this study was to investigate the mutagenicity and antimutagenicity of leaf extracts of *S. asoca* by the Salmonella microsome assay (Ames test) using *Salmonella typhimurium* tester strains TA100 without (S9) metabolization, by the preincubation method. *S. asoca* leaves were found to be strongly antimutagenic against sodium azide. Considering the excellent antimutagenic activities showed by the leaf extracts of *S. asoca*, we conclude that it is a good source for chemo preventive agents. But further study has to be done to characterize the phyto chemical or combination of phytochemicals that impart chemo preventive properties to *S. asoca* leaf extracts.

Index Terms **– Mutagenicity, medicinal plants, *Saraca asoca*, chemopreventive**

I. INTRODUCTION

India has one of the oldest and diverse cultural traditions associated with the use of medicinal plants. Herbs being easily available to human beings have been explored to the maximum for their medicinal purposes, as is evident from the raising number of the herbal formulations which are now used worldwide for therapeutic uses. However, the use of these herbs without assessing their mutagenicity can pose a potential risk to the patients. These herbal formulations are complex mixtures of the different phytochemicals that can be potent mutagens. Espanga et al., (2014) investigated the mutagenicity and antimutagenicity of hydro alcoholic leaf extract of six species of *Byrsonima* which is widely used in Brazil as a medicinal herb using the salmonella microsome assay and found that two species *B. coccolobifolia* and *B. ligustrifolia* showed mutagenic activity. However, the extracts of *B. verbascifolia*, *B. correifolia*, *B. fagifolia* and *B. intermedia* were found to be strongly antimutagenic. Other studies have also shown that some popular medicinal plant species are mutagenic (Razak et al, 2007).

There is an increasing awareness that certain naturally occurring substances in plants and other sources have protective effects against environmental mutagens or carcinogens and endogenous mutagens. Hence research work related to the discovery, characterization and use of the natural antimutagenic agents is receiving considerable attention. Antimutagenic effect of green tea against smoke-induced mutations in humans were investigated by Lee et al., (1997) and it was found that green tea can block the cigarette smoking induced increase in sister chromatid exchange frequency.

Saraca asoca is a wild evergreen tree of the family Fabaceae. It has long been used in traditional medicine as an excellent source of gynecological problems. Different parts of the plant have been used in the Ayurvedic system of medicine. Besides being an important tree in traditional medicine, the bark of Ashoka plant is also used to prepare cosmetics that help to improve skin complexion. It helps to prevent the condition of scanty and difficult urination, and even acts as an antidote to scorpion bite. The dried flowers are advantageous for diabetic patients. Previous researches have revealed antimicrobial, anti-inflammatory, antimenorronic, analgesic, antidiabetic antihelmintic and antiulcer properties (Bhalerao et al., 2014). Nag, et al., (2013) have confirmed the antioxidant, antimutagenic and genoprotective property of *Saraca asoca* bark extract. The present study was under taken to evaluate the chemo preventive effects of *Saraca asoca* leaf extract in the absence of metabolic activation system.

II MATERIALS AND METHODS

2.1 Plant material collection and Extraction

Saraca asoca leaves were collected from Puthayam, Kollam Kerala (GPS coordinates – 8.9050579,76.9254937). The plant material was properly washed with tap water, rinsed with distilled water and dried in oven at 40ºC. The dried leaves were ground into fine powder. The powdered sample were extracted with water using Soxhlet apparatus. 10gm of the dry plant powder was subjected to Soxhlet extraction with 300ml of water. The extraction was carried out until the solution become colorless and temperature was maintained at 90ºC. The color of the extract was dark brown. The concentrated extract was collected in a beaker and kept on hot plate, heated at 30-40ºC till all the solvent got evaporated. The dried extract obtained was in the form of dark brown crystals and it was kept in small air tight containers at room temperature for their further use.

2.2 Chemicals

Nutrient broth, 70% ethanol, magnesium sulphate heptahydrate (MgSO4·7H2O), potassium phosphate, dibasic (K2HPO4) (anhydrous), sodium ammonium phosphate tetrahydrate (NaNH4HPO4·4H2O), D-biotin, L-histidine, dextrose, potassium chloride (KCl), magnesium chloride hexahydrate (MgCl2·6H2O), agar, sodium chloride (NaCl), sodium azide(NaN_3), crystal violet, ampicillin, Hydrochloric acid (HCl), sodium dihydrogen phosphate monohydrate (NaH2PO4·H2O) and disodium hydrogen phosphate.

2.3 Mutagenicity assay

Mutagenicity of the leaf extract was determined using Ames test, according to the standard protocol described by Maron and Ames (1983) using histidine mutant *S. typhimurium* TA100 in the absence of S9 activation. TA100 has a mutation in gene for histidine synthesis. 10ml of nutrient broth was inoculated with a single isolated colony of TA100 and incubated for 5-8 h at 37 °C in a shaker incubator at 120 rpm to ensure sufficient aeration for 1 x 10^{-9} bacterial cells in Erlenmeyer flasks (10 ml). Sodium azide was used as a positive control and autoclaved distilled water was used as a negative control in the experiment. Five different concentrations of both freshly prepared positive control and leaf extract in distilled water were prepared (0.1µg/100µl, 0.2µg/100µl, 0.3µg/100µl, 0.4µg /100µl and 0.5µg/100µl). The positive, negative control and leaf extract were mixed in the top agar and spread on to the corresponding minimal glucose plates. After incubation for 24 h at 37 °C, spontaneous revertant colonies were clearly visible and counted manually.

Mutagenicity calculation:

$$MR = \frac{SR + IR}{SR \times NEG\ control}$$

Where, MR= Mutagenicity Ratio, SR= Spontaneous Reversion rate and IR= Induced Reversion

2.4 Anti-mutagenicity assay

For antimutagenicity assay 5 plates of glucose-minimal salts agar medium were prepared. Before performing the experiment, inoculated a single fresh colony of *S. typhimurium* TA 100 in nutrient broth and incubated for 5-8 h at 37 °C in an incubator shaker at 120 rpm to ensure sufficient aeration for 1 x 10^{-9} bacterial cells in Erlenmeyer flask (10 ml). Five different concentrations (0.1µg/100µl, 0.2µg/100µl, 0.3µg/100µl 0.4µg/100µl 0.5µg/100µl) of positive controls (sodium azide) and *S. asoca* leaf extract were prepared. To assess the antimutagenicity, 100µl of each concentration of positive control was mixed with 100µl of the corresponding concentration of leaf extract and added to the top agar, after that pure bacterial culture was mixed to the top agar and was poured onto petri plates and uniformly spread the bacteria on the surface of minimal glucose agar plates. After incubation for 24 h at 37°C, spontaneous revertant colonies were counted manually. The antimutagenicity results were expressed as percent inhibition (the ability of the compounds to inhibit the action of the known mutagen), calculated as described by Tachino et al., (1994).

Inhibition % = 100 - [(T/M) X 100]

where T is the number of revertant colonies in a plate containing mutagen and compounds and M is the number of revertant colonies in a plate containing the mutagen alone. Results were interpreted as no antimutagenic effect when the inhibition was lower than 25%, a moderate effect for a value between 25% and 40% and strong antimutagenicity for values greater than 40% (Resende et al., 2012; Negi et al., 2003).

III. RESULTS AND DISCUSSION

The dried leaves of *S. asoca* were extracted with water using soxhlet, a brown colored crystalline powder was obtained. The phase contrast image of the fine crystals of the leaf extracts of *S. asoca* is shown in Figure.1, this crystalline powder was used to evaluate the mutagenicity and antimutagenicity profile.

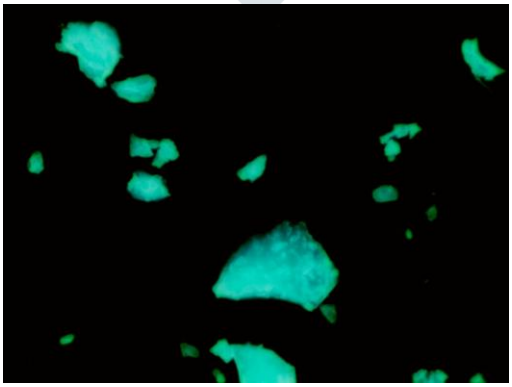

Figure. 1 Phase contrast image of crystalline extract of the *S. asoca* water extracts

3.1 Results of Mutagenicity test

For mutagenicity assay, five different concentration of *S. asoca* were used. Mutagenicity ratios of the *S. asoca* extract were found in the range of 0.012-0.016. Table.1 shows the number of revertants/plate and the mutagenic ratio (MR) of the positive control, negative control and *S. asoca* leaf extracts observed in S. *typhimurium strain* TA100 in the absence (−S9) of metabolic activation.

At all concentration the numbers of revertants in *S. asoca* extract were nearly same or lower than the number of the revertants scored in the negative plate (0.01). Mutagenicity ratio of positive (mutagen-sodium azide) was found in the ratio of 0.052. The results showed that there was no increase in the number of revertant colonies in the plant extract relative to the negative control (Figure.2), indicating that *S. asoca* extracts are nonmutagenic. According to Maron & Ames (1983), plant extract tested with Ames test have mutagenic effect when there is more than twofold increase in the number of revertant colonies over negative control. But in the present study the number of revertants colonies produced by plant extract was below negative control. Potential mutagens also show a concentration dose response relationship in which there is an increase in the number of the revertants with increasing concentration of the mutagen. Sodium azide showed a dose response relationship as its mutagenicity ratio of sodium azide increased with the increasing concentration. But *S. asoca* leaf extract did not showed a similar response and the mutagenicity ratio was approximately 0.01 at all concentrations. This shows that *S. asoca* leaf extracts are nonmutagenic without metabolic activation.

Table 1. Mutagenicity activity expressed as number of revertants per plate and the mutagenicity ratio of Extract- Water extract of *S. asoca* leaves, Positive control- Sodium azide and Negative control- Sterile Distilled water.

S.l. no	Plate	Concentration used µg/plate	No: of colonies per plate	Mutagenicity Ratio (MR)
1	Positive I	0.1µg	732	0.0487
2	Positive II	0.2µg	552	0.0611
3	Positive III	0.3µg	1216	0.0761
4	Positive IV	0.4µg	1584	0.0969
5	Positive V	0.5µg	1480	0.0910
6	Extract I	0.1µg	75	0.012
7	Extract II	0.2µg	139	0.015
8	Extract III	0.3µg	144	0.016
9	Extract IV	0.4µg	138	0.015
10	Extract V	0.5µg	116	0.014
11	Negative	0.1µl	136	0.01

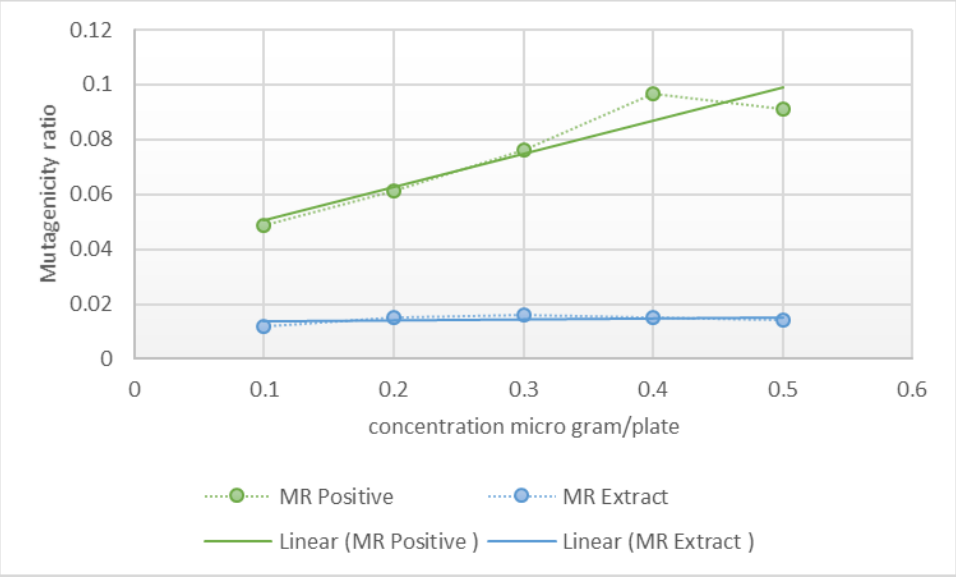

Figure 2. The mutagenicity ratio of water extract of *S. asoca* leaves and Positive control- Sodium azide at different concentrations and their corresponding dose response patterns.

3.2 Results for antimutagenicity test

The antimutagenic effect of *S. asoca* extract was assessed from the number of revertants/plate, and the percent inhibition (% I) of the mutagenic activity of sodium azide. The effect of the leaf extracts on the mutagenicity of sodium azide are shown in Table 2. All five concentrations tested in the present study showed more than 40% inhibition. *Saraca asoca* leaf extracts when mixed with equal concentrations of sodium azide in the range of 0.01-0.05 g/plate were able to achieve an inhibition of 89.6-97.4%. There was a considerable decrease in the number of revertants scored at each concentration when the mutagen was mixed with *S. asoca* leaf extract Figure 3. The results indicate that *S. asoca* leaf extract can be considered a strong antimutagen against sodium azide as it showed more than 40% reduction in the number of revertants formed by sodium azide at all concentrations. Resende et al.,

(2012) stated that compound which can reduce the number of revertants colonies more than 40% can be considered strongly antimutagenic. It was also confirmed that the reduction in the number of the revertants is not because these extracts effect the viability of the cells. It was found that the number of viable bacterial cells was not less than 60% of that observed in the negative control, which confirms that *S. asoca* does not affect the viability of the bacterial cells (Lira et al., 2008).

Table 2. Antimutagenic activity of water extract of *S. asoca* leaves on positive control- Sodium azide expressed as no of revertants per plate and % inhibition.

Sample	Concentrations used µg/plate	No. of revertants/plate (positive)	Inhibition % (I)
1	0.1 E + 0.1 M	76	89.6
2	0.2 E + 0.2 M	50	90.6
3	0.3 E + 0.3 M	41	96.7
4	0.4 E + 0.4 M	50	96.9
5	0.5 E + 0.5 M	39	97.4

Note- E- *S. asoca* leaf extract; M- sodium azide

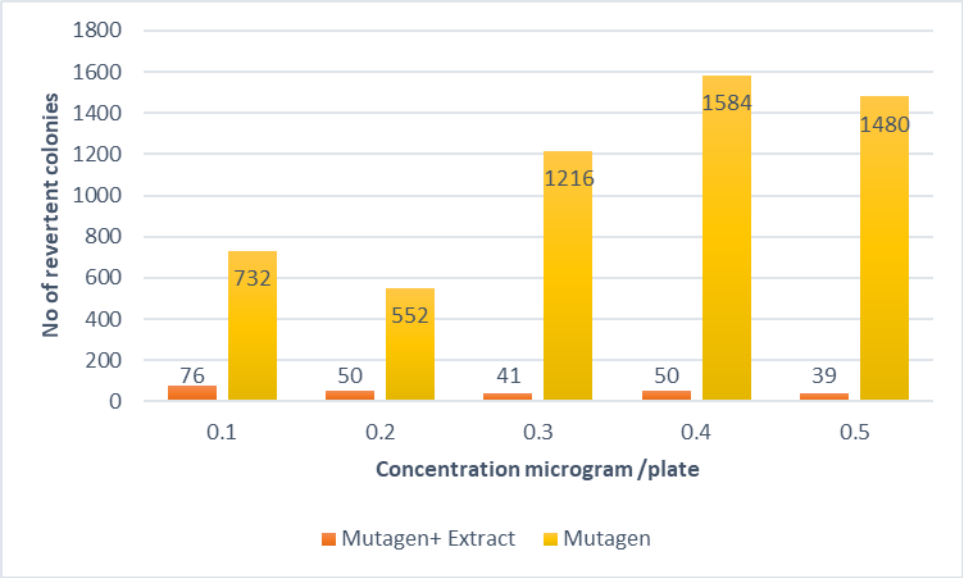

Figure 3. Effect of water extract of *S. asoca* leaves on positive control- Sodium azide: graph shows the no of revertants per plate with mutagen alone and mutagen-extract mixture.

Cancer is one of the major concerns to human health in the present world, as the exposure to genotoxic chemicals have increased with the industrial development. The damage to the genetic material, changes in DNA sequence etc. can lead to mutations in genes which can lead to carcinogenesis. There is considerable awareness regarding the chemicals used as pesticides and other toxic industrial chemicals, which are now a days used with caution. Traditional herbal formulations have been used effectively for the management of disorders that are now being accepted worldwide. But many plants used to treat day to day ailments are not assessed for the mutagenicity. These herbs contain complexes of phytochemicals which can be mutagenic. Such herbs if used in medicinal formulations can be mutagenic and cause genotoxic effects to the user.

Earlier studies have shown that many plants which are commonly used are mutagenic. Razak et al, (2007) studied mutagenic and carcinogenic properties of plants used in African traditional medicine and the results showed that at least 64 plant species used in African traditional medicine are potentially mutagenic based on their ability to induce genetic changes. People are unknowingly being exposed to potential mutagens. It is important to assess the mutagenicity of the herbal plants which are more commonly used in traditional medicine and in medicinal formulations. Plants having mutagenic ability must be used with caution. In India, plants have been extensively used in folk medicine for treatment of various diseases. *S. asoca* traditionally known as 'Sita Asoca is of great value in Indian traditional medicine "Ayurveda".

Saraca asoca is a herbal plant with many beneficial properties. Varma, (2010) studied another species *Saraca indica* and found that it possesses CNS depressant activity. Saha et al, (2012) investigated the qualitative phytochemical constituents from extract of the flower *Saraca asoca* and obtained active constituent gallic acid. The present study was under taken to assess the mutagenicity and the antimutagenic properties of *S. asoca*. The results revealed that *Saraca asoca* leaf extract were non mutagenic in *Salmonella typhimurium* strains (TA100) without metabolic activation. Nonmutagenic nature of leaf extract is evident from the results of mutagenicity assay. The study also revealed that the leaf extracts of *S. asoca* were found to possess strong antimutagenic property against sodium azide. Sodium azide is an inorganic compound with the formula NaN_3. This colorless salt is the gas-forming component in many car airbag systems. It is used for the preparation of other azide compounds. It

is an ionic substance, is highly soluble in water, and is very acutely toxic, affecting the genetic material directly leading to structural damage due to frame shift mutations

Given the outstanding antimutagenic activities revealed in this study, *S. asoca* leaf extracts are good candidates for development of chemo preventive agents. Earlier works have shown the antimutagenic properties of the bark extracts of *S. asoca* (Nag et al., 2013). Human population is exposed to a multitude of mutagens many of which are inevitable, like some mutagenic compounds which form an essential component of drugs used for treatment of diseases which cannot be substituted. In such cases using an antimutagenic or chemo preventive agent can decrease the risk of such chemicals by decreasing their mutagenic potential. In the present study the *S. asoca* leaf extracts showed outstanding antimutagenic ability. It has an 87-97% potential to inhibit the mutagenicity of sodium azide. Using *S. asoca* with similar mutagenic chemicals can help decrease their mutagenicity. Considering that medicinal herbs contain complex mixtures of thousands of components that can act alone or synergistically (Boldrin et al., 2013) it is important to continue phytochemical studies of extracts, to provide the chemical profile of the active species contributing to the antimutagenicity of *Saraca asoca*. The antimutagenic properties have earlier been proven for many commonly used herbs. Vinod, (2011) studied on mutagenic and antimutagenic activity of neem oil using mitomycin and 7, 12-dimethylbenzanthracene as mutagen and found good antimutagenicity. Florinsiah, et al., (2013) studied on the mutagenicity of *Centella asiatica* by Ames test and found it to be nonmutagenic.

The detection of genotoxicity is highly advisable, so as to avoid the risk of genotoxic exposure to mutagens and carcinogens. However, some genotoxic compounds cannot be completely avoided because they are air pollutants, or some might be ingested as food contaminants. Also, some therapeutic drugs belong to an important group of genotoxic compounds. Antimutagenicity studies have been developed to diminish the risk in the event of genotoxic exposure (Arriaga-Alba et al., 2013). There have been several reports in the literature, that medicinal plants or fruit juices have components such as polyphenols, vitamins, chlorophylls, terpenes and unknown organic compounds, which are described as antimutagens and perhaps anticarcinogens (Arriaga-Alba et al., 2008). It can be concluded that *Saraca asoca* leaf should have more effective place in treatment because of high anti-mutagenic property against sodium azide. These results contribute valuable data on the safe use of *S. asoca* leaf extract as medicinal plants. We emphasize the excellent chemo preventive ability of these extracts, especially with respect to compounds that do not require metabolic activation.

IV. ACKNOWLEDGMENT

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