Preventive Effect of Hydroalcoholic Extract of *Salacia Oblonga* Root Bark on Mitomycin-C Induced DNA Damage Using Micronucleus Test System in Rats

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Summary

The extract of root bark of Salacia oblonga (SO) belonging to the family Celastraceae was tested for the anti-mutagenic activity using micronucleus test in Wistar rats. The hydroalcoholic extract (0.5 and 1.0 gm/kg, p.o daily for seven days) was evaluated against mitomycin-C (MMC – 2 mg/kg, ip) induced nuclear damages. The sampling was done after 48 hr and 72 hr of the clastogen treatment. The results indicated that the prior treatment of SO at 1.0 mg suppressed ($P<0.05$) the formation of micronuclei in both the tested time intervals, while the lower dose (0.5 gm/kg) showed inhibitory effect mostly at 48 hr duration. Further, the in vitro radical scavenging activity indicated that SO prevented the DPPH radical formation and the EC_{50} value was found to be 4.5 mcg/ml. The data from the study suggest that SO possess antimutagenic effect against MMC and the activity could be due its antioxidant potential.

Keywords: *Salacia Oblonga*, Bone Marrow Micronucleus test, antioxidant.

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Introduction

Toxicological studies have undergone a significant evaluation during the past decade with much greater emphasis being placed on chronic toxicity, carcinogenicity, teratogenicity and mutagenicity. The environmental pollutants due to various sources are known to cause the generation of reactive oxygen species (ROS) and results in the oxidative damage to tissues and mutation in somatic cells [1]. Many a time's hazards occur not only due to the presence of genotoxic agents but also due to lack of antimutagenic/anticarcinogenic agents in our diet. As we cannot avoid many of these substances, the best way to minimize the effect is by identifying the anti mutagens and desmutagens in our diet and increasing their use [2].

Mutagenicity testing assumes prime importance since chemicals existing in human environment can cause deleterious heritable changes without showing any immediate toxic effects [3]. In vivo bone marrow micronucleus test in rodents is one of the established method to evaluate the mutagens and antimutagens in mammals [4]. The test if performed appropriately can identify the mutagens and also determines the antimutagenic potential [3]. Now-a-days, natural products are driving the attention of researchers by showing comparably therapeutic activity with that of available drugs and with lesser side effects [5]. Several studies in the past indicated that the natural products can be used as chemopreventive agents against the mutagenic damages. These compounds exhibit the preventive effects against the mutagenesis by several pathways and important among them is by quenching the free radicals [1, 5].

Salacia oblonga (Family: celastraceae) commonly known as Saptrangi, is a subtropical under shrub found in the south Asian countries, know to have several medicinal properties such as hypoglycemic, hypolipidemic, anti-inflammatory, anti-oxidant etc [6-9]. The potential genotoxicity of *salacia oblonga* root extract was evaluated using standard battery of tests recommended by US FDA and the results concluded that the extract is safe for human use and do not possess genotoxic potential [10, 11]. However, the role of *Salacia oblonga* root extracts against chemical induced cytogenetic damage in rodents with special reference to its anti-oxidant capabilities has not been found in the literature. Hence the present investigation attempts to evaluate the anti-mutagenic potential of hydroalcoholic extract of roots of *Salacia oblonga* against mitomycin-C by using bone marrow micronucleus test in Wistar rats.

Material and methods

Chemicals

Ascorbic acid was purchased from Loba Chemie, Mumbai and Mitomycin-C is from Khandelwal Labs Pvt. Ltd, Mumbai. Other chemicals and stains used in this study were purchased from local supplier and are of analytical grade.

Animals

Eight week-old healthy, laboratory bred, male Wistar rats weighing 180 ± 10 gm were maintained under standard laboratory conditions such as temperature 20 ± 2^{o} C, 12 hour light / dark cycle and provided water and pellet food *ad libitum*. The experiments were conducted in CPCSEA (Committee for the purpose of control and supervision of experiments on animals, Chennai, India) approved animal house after obtaining the prior approval from the Institutional Animal Ethics Committee.

Extraction of active ingredient from the root barks of *Salacia oblonga*

The dried root bark of *Salacia Oblonga* (SO) was procured from local market and authenticated at Regional Research Institute (RRI), Bangalore (Ref No.- RRI/BNG/SMP/Drug Authentication/2007-08/214 d). The dried rook bark powder was extracted with the solvent system consisting of methanol and water in the ratio of 1:1. The extract was dried, weighed and suspended in 1% w/v CMC according to the dose and screened for the anti-mutagenic activity.

Dose and Treatment

The animals are divided mainly into three groups' viz., control, challenge and treatment. The control group received saline (0.5 ml/kg) while the challenge group was treated with mitomycin-c (MMC-2 mg/kg, ip) [12]. In the treatment group, SO was tested in two doses (0.5 and 1.0 gm/kg, p.o) [13]. SO was daily administered for 7 consecutive days and on the 7th day MMC was administered. The bone marrow sampling was done after 48 hr and 72 hr of MMC treatment. Ascorbic acid (300 mg/kg, p.o) was used an internal standard antioxidant agent [14].

Bone marrow micronucleus test [15]

The modified method of Schimid was followed to perform the bone marrow MN test (Vijaylaxmi and Venu 1999). The animals after respective treatment were sacrificed by cervical dislocation under light ether anesthesia. Animals were cut open to excise femur and tibia. Bone marrow MN slides were prepared by using the modified method of Schmid. Marrow suspension from femur and tibia bones prepared in 5% w/v bovine serum albumin (BSA), was centrifuged at 1000 rpm for 8 min and the pellet was resuspended in a required quantity of BSA.A drop of this suspension was taken on a clean glass slide and smear was prepared on glass slide and air dried. The slides were fixed in absolute methanol, stained with May-Grunwald-Giemsa and MN were identified in two forms of RBCs (ie, polychromatic erythrocytes as PCEs and normochromatic erythrocytes as NCEs) (Photo-1). About 2000 PCEs and corresponding NCEs were scanned for the presence of MN.

In vitro antioxidant activity by *DPPH method* [16]

A stock solution of 1, 1 diphenyl-2-picryl hydrazyl (DPPH) was prepared such that 75 µl of it in 3 ml of methanol gave an initial absorbance of 0.9 at 515 nm. This stock solution was used to measure the anti-radical activity. Decrease in the absorbance in the presence of test compound at different concentration was noted after 15 min. and the percentage inhibition was calculated by comparing the results of the test compound with the control.

Statistics

The statistical significance of the results was carried out using one-way Anova followed by multiple comparision by Bonferroni test [17]. $p<0.05$ was considered to indicate significance.

Results

A. Effect of the hydro-alcoholic extract of SO on bone marrow micronucleus after the administration of MMC.

The anticlastogenic study indicated that the hyrdoalcoholic extract of SO prevented dose dependently the nuclear damages induced by MMC. Administration of MMC (2 mg/kg) had increased significantly ($p<0.001$) the frequency of MN in both 48 hr and 72 hr time intervals. At lower dose, SO (0.5 mg/kg) had reduced in the number of micronucleated erythrocytes only in 48 hr interval, while in 72 hr, SO did not produced any change compared to the mitomycin-C. SO at higher dose (1 gm/kg) had exhibited significant ($p<0.01$) reduction in the number of micronucleated erythrocytes. The action of SO was found to be more prominent at higher dose compared to the lower dose. Further, administration of SO (1gm/kg) to the control group did not exert any effect on the micronucleated erythrocyte population in both the tested time intervals. Ascorbic acid (300 mg/kg) as a standard antioxidant had showed a significant ($p<0.001$) suppression in the nuclear damage in the MMC treated animals (Table-1).

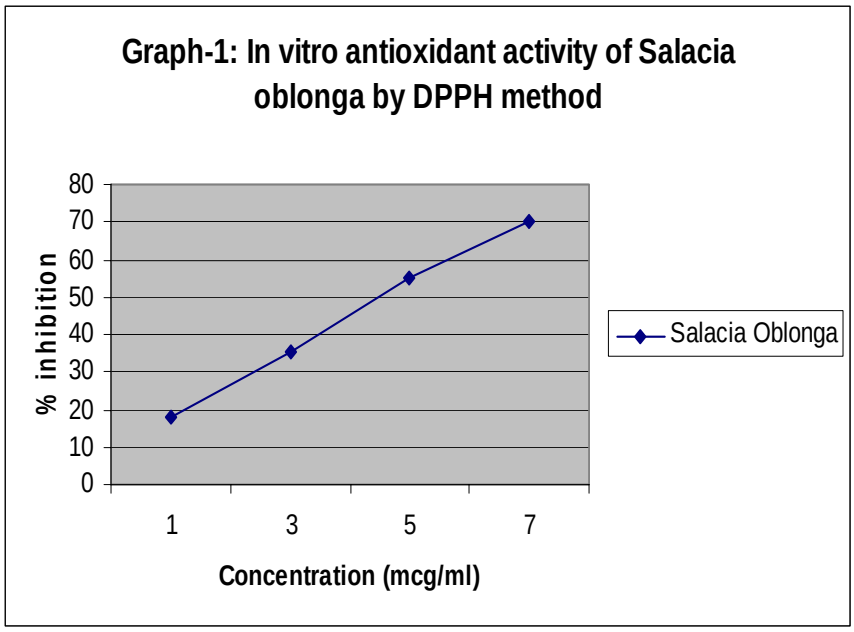
Table-1: Effect of the hydro-alcoholic extract of SO in the frequency of bone marrow micro-nucleated erythrocytes in MMC induced nuclear damage.

Treatment and dosage	After 48 hrs		After 72 hrs	
	% MN in PCE	% MN in NCE	% MN in PCE	% MN in NCE
Control (Saline 0.5 ml/kg)	0.40 ± 0.01	0.31± 0.01	0.40 ± 0.01	0.31± 0.01
S0 (1gm/kg)	0.45 ± 0.01	0.35 ± 0.02	0.44 ± 0.01	0.35 ± 0.01
Ascorbic Acid (300mg/kg)	0.44 ± 0.01	0.37 ± 0.02	0.45 ± 0.018	0.36 ± 0.02
MMC (2mg/kg)	0.77 ± 0.02[a]	0.86 ± 0.01[a]	0.75 ± 0.03[a]	0.85 ± 0.01[a]
MMC + S0 (0.5gm/kg)	0.70 ± 0.01*	0.82 ± 0.01*	0.74 ± 0.02	0.82 ± 0.01
MMC + S0 (1gm/kg)	0.65 ± 0.02**	0.81 ± 0.01**	0.67 ± 0.01*	0.80 ± 0.02*
MMC +Ascorbic Acid (300mg/kg)	0.62 ± 0.02***	0.64 ± 0.03***	0.47 ± 0.02***	0.64 ± 0.03***

Values are expressed as Mean ± SEM, n=6 SO – *Salacia oblonga, MMC - Mitomycin-C*
Statistics: One-way ANOVA followed by Bonferroni test
a $p<0.001$ compared with control
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with MMC

B. In vitro antioxidant activity by DPPH method

The radical scavenging activity of SO was tested in four concentration (1, 3, 5 and 7 mcg/ml). A dose-dependent inhibition in the radical formation was observed and the EC_{50} value was found to by 4.5 mcg/ml (Graph-1).



Discussion

The administration of hydroalcoholic extract of *Salacia oblonga* (SO) has reduced the nuclear damages caused by the MMC in a concentration dependent manner. SO had decreased the cytogenetic damages produced by MMC at the highest tested dose (1gm/kg) in both the durations. Also, SO was found to suppress the formation of DPPH radicals in an in vitro antioxidant assay. Further, ascorbic acid produced significant antimutagenic effect against the MMC induced nuclear damages (Table-1 and Graph-1).

In this study, administration of MMC has increased the percentage micronuclei in both polychromatic erythrocytes and normochromatic erythrocytes compared to the control group (Table-1). MMC is one of the clinically used toxic anticancer drugs. It produces the cytotoxicity by an electrophilic attack on the nucleophilic site in the DNA. MMC is commonly used as a clastogen in pre-clinical studies as it produces chromosomal damage in variety of cells [15]. Further, the administration of MMC has been reported to suppress the levels of antioxidant enzymes which contribute in the oxidative stress [18]. As reported, the free radical in the oxidative stress initiates a chain of reaction in the body which ultimately damages the cellular components. The injury to the protein structure results in the formation of wrong amino acids while the damage to the lipids results in lipid peroxidation. However, the damage to the nucleus contributes in more severe effect in form of mutations. It is well established that the consequence of mutations leads to carcinogenesis, heart ailment, neurological defects and congenital abnormalities [19, 20].

In vivo rodent micronucleaus (MN) assay has been widely used to detect genotoxicity. The MN test is devised for evaluating the ability of test agents to induce structural and/or numerical chromosomal damage [15]. Both the kinds of damages are associated with the appearance and /or progression of tumors and with adverse reproductive and development outcomes. MN known as Howell-Jolly bodies are generally smooth, round remnants of nuclear chromatin seen in the erythrocytes [3]. During erythropoiesis, the cell continue to divide at which time a given test agent administered may act and cause chromosome damage, such as breaks and exchanges. These anomalies (a fragment or a whole chromosome) may lag behind in the cell during division and may not become integrated in the daughter nuclei, rather may form MN, which can be seen in the cytoplasm [4]. The frequency of MN is evaluated in two types of erythrocytes viz., polychromatic erythrocytes (PCE, young erythrocytes still containing RNA) and normochromatic erythrocytes (NCE, mature erythrocytes) [20].

The observations from the study indicated that the pretreatment of extract of SO before the administration of MMC has reduced the incidences of MN formation. The non-significant prevention of SO against the MMC mediated changes after 72 hr indicated that the dose and duration of SO treatment might not be sufficient to prevent the MMC induced DNA damages. The inhibitory effect exerted by SO suggested that SO could be a potential agent to reduce the nuclear damages induced by the environmental factors. Previous studies suggest that the compounds' possessing the scavenging activity against the free radicals prevents the nuclear damages [1, 5]. The research conducted on certain plants like *Tilia cordata, Mentha piperita, Valeriana officinalis* etc revealed that there herbs inhibited the nuclear damages due to he antioxidant effect [2, 5, 21].

Earlier study suggested that the administration of ascorbic acid produced the antimutagenic effect against the MMC due to its ability to protect the electrophilic attack on the nucleophilic sites on the nucleus, primarily by reducing the concentration of free radicals [1,2,15]. The DPPH scavenging method is widely used to assess the ability of the compound to scavenge the free radicals [16]. Since, in our study SO oblonga inhibited the formation of DPPH radical and the earlier reports has also indicated the antioxidant potential of SO, hence, it can be suggested that SO could have exerted similar mechanism as like ascorbic acid to prevent the nuclear damages induced by MMC. Future studies on SO might help to find the exact mechanism for antimutagenesis using different clastogens and also to identify SO as a potential agent for chemoprevention.

Conclusion

The present study indicate that the hydroalcoholic extract of *Salacia Oblonga* (SO) root bark possess antimutagenic effect against the cytonuclear damage caused by mitomycin-C (MMC). The extract prevented the incidence of micronuclei formation induced by the clastogen. The in vitro antioxidant study indicated that SO reduced the formation of DPPH radical. The ability of the extract of SO to prevent the nuclear damage caused by the known mutagens could play an important role in overcoming the mutations related defects caused by the environmental factors.

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