American Journal of Cancer Biology

American Journals of Cancer Biology
http://ivyunion.org/index.php/ajcb
Vol. 1, Article ID 201300183, 5 page

Research Article

Evaluation of Invitro Antimutagenic Potential of Lagenaria Siceraria Using Ame's Test

Thakkar Jalaram H[1*], Patel Chirag A[1], Santani Devdas D[2], Jani Girish K[1]

[1]Department of Pharmacology, SSR College of Pharmacy, India
[2]Department of Pharmacology, ROFEL, Shri. G. M. Bilakhia College of Pharmacy, India

Abstract

Cancer is one of the most life-threatening diseases and widespread in both developed and developing countries. Accumulation of genetic alterations is main etiology for cancer developments. Many of the Cucurbitaceae plants possess antitumor activity traditionally. Methanolic extract of Lagenaria siceraria Standley Fruit was tested for their antimutagenic potential. The extract of plant exhibited varying level of antimutagenicity. Ames test was used in the current study to evaluate antimutagenic activity in TA98 and TA100 strains of Salmonella typhimurium using direct (Sodium azide) acting mutagens. Results of the study showed significant antimutagenicity against mutagen in TA98 and TA100 strains. The antimutagenicity of the extract observed in the present study implies chemopreventive pharmacological importance of Lagenaria siceraria Standley Fruit and encourages its use as a functional food.

Keywords: Antimutagenicity; Sodium azide; Salmonella typhimurium; Ames test; Lagenaria siceraria

Peer Reviewer: Rama K. Mallampalli, MD, Department of Medicine, University of Pittsburgh, United States

Academic Editor: Xiaoning Peng, PhD, Department of Internal Medicine, Hunan Normal University, China

Received: July 17, 2013; **Accepted**: September 23, 2013; **Published**: October 19, 2013

Competing Interests: The authors have declared that no competing interests exist.

[*]**Correspondence to**: Jalaram H. Thakkar, Assistant professor, Department of Pharmacology, SSR College of pharmacy, SSR Memorial Trust, Sayli Road, Silvassa-396230, UT of D & NH, India; E-mail: jay_143143@yahoo.com

Introduction

Humans are exposed to a variety of exogenous and endogenous genotoxic agents. From the past few years' extensive use of various chemicals and many of the drugs have cause insidious damage to the environment and life. A high dose drug causes mutation and the mutation is the first step involve in cancer and other disease [1]. It has recently come to be realized that the high mutation pressure exerts an critical influence on the physical and mental well-being of humans.

It is assumed that 9 out of every 1000 living newborn babies suffer from a disease resulting from gene mutations. This increase in mutation frequency is responsible for genetic disorders, which may cause a great decline in quality of life [2].

Various antimutagenic activities are mediated by compounds from food and plant origin. Some of these compounds possessing antioxidant property which is responsible for antimutagenic activity and therefore prevents initiation and development of cancer [3-6]. So there is an increasing interest in the evaluation of protective biochemical function of natural antioxidants contained in medicinal herbs, which are candidates for the prevention of oxidative DNA damage caused by oxygen-free radical species [7-9]. So therefore, on the basis of above mentioned property, we have decided to investigate the usefulness of indian medicinal plants against chemically induced mutation.

Bottle gourd [Lagenaria siceraria (Mol.) Standl.] is an important gourd having wide range of uses and is largely cultivated in the tropical and subtropical zone for its edible fruits. Affectionate fruits are used as vegetable and also for preparation of sweets and pickles especially in the hills. It has a cooling effect and prevents constipation and has diuretic and cardio-tonic properties. Fruit pulp is used as antidote against certain poisons. Externally the pulp is applied as poultice and cooling application as a means to alleviate delirium and also applied to the soles of feet and palms of hands to diminish the effect of heat [10]. The present study was been undertaken to evaluate invitro antimutagenic potential of methanolic extract of *Lagenaria siceraria* using ames test.

Materials and Methods

Chemicals and Reagents:

Sodium azide (SA), Dimethyl sulfoxide, Histidine, biotin, Magnesium sulfate, citric acid monohydrate, potassium phosphate dibasic anhydrous, sodium ammonium phosphate, Agar, sodium chloride were purchased from Chemdyes corporation, Rajkot. Dextrose was procured from Research-Lab Fine Chem Industries, Mumbai.

Bacterial strains

Clinical strains of two human pathogenic bacteria of Gram-negative bacteria *Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100 were used for the Ames assay. All the microorganisms were obtained from the Institute of microbial technology (IMTECH), Chandigarh and maintained in the Department of Pharmaceutical Microbiology, SSR College of Pharmacy, Silvassa. A fresh nutrient broth culture was grown to a density of 1-2 X 10^9 cells/ml for 12 hour at 37℃ before each experiment.

Collection of fruits

The fruits of L. siceraria were procured from the local market of silvassa and its botanical characteristics was confirmed by Mrs Rajeshwary Nair, Vice principal, Head department of Botanical science, SSR College of arts commerce and science, silvassa. The fruits were sundried, finely powdered and stored in airtight polythene bag at room temperature.

Preparation of extracts

The fresh fruits were chopped into small pieces and dried at ambient temperature. The dried

pieces of fruits were milled using an electric grinder to get fine powder. The 180 gm powder was soaked in 100% methanol for 18 hours before being filtered and concentrated under vacuum at 50°C to afford 14.5g of a dirty brown extract. The extracts were kept in the refrigerator for further use.

Determination of antimutagenicity against direct acting mutagens

Salmonella mutagenicity assay was carried out as previously described by Mortelmans K and Zeiger E, 2000[11]. Plate incorporation method was done for antimutagenicity assay without microsomal activation. Fresh bacterial cultures of S. typhimurium strains TA 100 and TA 98 (1-2x10^9cells/ml) were mixed with 2ml of molten agar containing 0.5 ml histidine/biotin solution, different concentration of *lagenaria siceraria* methanolic extract (25, 50, 100 and 200 µg/0.1 ml/plate) and direct acting mutagens such as sodium azide (2.5µg/plate). Further it was spread over minimal glucose agar plates. Plates were incubated for 48 hours at 37℃ and the revertant colonies were counted.

Statistical analysis

Results were expressed as Mean ± S.E.M. Statistical significance was tested using one way ANOVA as appropriate using computer based statistical program (GraphpadPrism 4.0.). Differences were considered to be statistically significant when $p < 0.05$

Results and Discussion

Different doses of *Lagenaria siceraria (LS)* in triplicate was selected for evaluation purpose. LS 25, LS 50, LS 100 and LS 200 groups were given 25 µg, 50 µg, 100 µg and 200 µg per plate respectively. Sodium azide (SA) serves as positive control. Results of antimutagenic studies revealed that methanolic extract of *Lagenaria siceraria* Standley fruit was highly effective in reducing the mutagenicity caused by the mutagen sodium azide. (Figure 1, Figure 2)

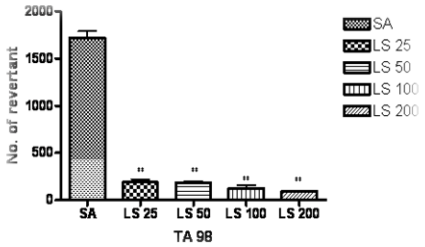

Figure 1 Comparison of No. of Revertant of TA 98. n=3 Trial, ***p<0.001 extremely significant, **p<0.01 very significant, *p<0.05 significant, nsp>0.05 nonsignificant, significant compared to positive control, values are expressed as mean±SD.

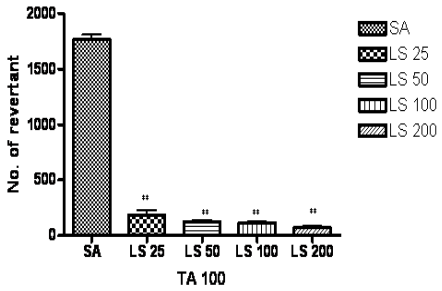

Figure 2 Comparison of No. of Revertant in TA 100. n=3 Trial, ***p<0.001 extremely significant, **p<0.01 very significant, *p<0.05 significant, nsp>0.05 nonsignificant, significant compared to positive control, values are expressed as mean±SD.

The percent inhibition of sodium azide induced mutagenicity was recorded as 89.07±1.58, 89.51±1.05, 92.77±1.40, and 94.39±0.06 in TA 100 and 89.39±2.57, 92.67±0.93, 93.37±0.64, 96.08±0.80 in TA98 for dose 25, 50, 100,and 200 µg/plate respectively. (Table 1)

According to one way ANOVA the protective effect of *Lagenaria siceraria* against SA induced mutagenicity in TA 98 and TA 100 was verified and found significant ($p < 0.0001$)

Anticarcinogenic and antimutagenic activity of medicinal plants may be due to a variety of mechanisms such as inhibition of genotoxic effects, inhibition of cell proliferation, signal transduction

modulation, scavenging of free radicals, induction of detoxification enzymes, induction of cell-cycle arrest and apoptosis, modulation of cytoskeletal proteins that play a key role in mitosis, and the inhibition of topoisomerase I or II activity [12].

Hence, the possible mechanism of the demonstrated antimutagenic behaviour could be due to the bioactive constituents like flavonoids, alkaloids, saponins, glycosides and alkaloids present in the methanol fraction which might inactivate the reactive intermediates formed from mutagens [13].

Table 1 Antimutagenic activity of *Lagenaria siceraria Standley* Fruit against sodium azide on Salmonella typhimurium strain TA 98 and TA 100.

Treatment	Dose	TA 98		TA 100	
	(µg/plate)	No of his+ revertant/plate	% Inhibition	No of his$^+$ revertant/plate	% Inhibition
Positive Control	SA	1717±76.33	---	1765±47.4	---
	25	189±34.27**	89.07±1.58**	185.3±42.74**	89.39±2.57**
Co-incubation	50	180±20.07**	89.51±1.05**	128±13.3**	92.67±0.93**
(SA + LS)	100	126±30.12**	92.77±1.40**	116.3±8.373**	93.37±0.64**
	200	96.33±4.41**	94.39±0.06**	69.67±15.59**	96.08±0.80**

n=3 Trial, ***p<0.001 extremely significant, **p<0.01 very significant, *p<0.05 significant, ^{ns}p>0.05 nonsignificant, significant compared to positive control, values are expressed as mean±SD.

Conclusion

Antioxidant, cytoprotective activity and anti cancer activity of *Lagenaria siceraria* Standley fruit has been proven [14]. It is well known that antioxidants are almost universal antimutagenic agents. A reason for this effect is the genotoxicity of reactive oxygen species (ROS) and antioxidants in such cases can act as stabilizers of homeostasis. So there is an increasing interest in the protective biochemical function of natural antioxidants contained in medicinal herbs, which are candidates for the prevention of oxidative damage caused by oxygen-free radical species. Keeping in mind number of previous investigation about antioxidant and anticancer nature of *Lagenaria siceraria* Standley fruit, it can be anticipated that antimutagenic activity observed in the present study may be via antioxidant mechanism. Further study is needed to find out bioactive compound its exact mechanism responsible for antimutagenic activity.

Reference

1. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. ***J Natl Cancer Inst***. 1981, 66:1191-1308
2. Raymond JM, Niesink JV, Mannfred AH. Genetic toxicology. In: ph. Van parys., Th M.C.M dekuk, editor. Toxicology principles and applications. 1st ed. CRC press; 1996.p.314-319
3. Weisburger JH. Antimutagenesis and anticarcinogenesis from the past to future. ***Mutat Res***. 2001, 480-481:23-35
4. Flora SD, Izzotti A, Agostini FD, Balansky RM, Noonan D, Albini A. Multiple points of intervention in the prevention of cancer and other mutation-related diseases. ***Mutat Res***. 2001, 480:9-22
5. Flora SD. Mechanisms of inhibitors of mutagenesis and carcinogenesis. ***Mutat Res***. 1998, 402:151-158
6. Mateuca R, Lombaert N, Aka PV, Decordier I, Kirsch-Volders M. Chromosomal changes: Induction, detection methods and applicability in

human biomonitoring. ***Biochimie***. 2006, 88:1515-1531

7. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. ***Free Radic Res***. 1995, 22:375-383
8. Kim NY, Song EJ, Kwon DY, Kim HP, Heo MY. Antioxidant and antigenotoxic activities of korean fermented soybean. ***Food Chem Toxicol***. 2008, 46:1184-1189
9. Santosh KV, Viswanatha GL, Ramesh C, Nandakumar K, Srinath R. Antimutagenic (anticlastogenic) and antioxidant activities of bark extract of Terminalia arjuna. ***Journal of Genetic Toxicology***. 2008, 1:1-7
10. Harika M, Gasti VD, Shantappa T, Mulge R, Shirol AM, Mastiholi AB et al. Evaluation of bottle gourd genotypes [Lagenaria siceraria (Mol.) Standl.] for various horticultural characters. ***Karnataka J Agric Sci***. 2012, 25:241-244
11. Mortelmans K, Zeiger E. The ames salmonella/microsome mutagenicity assay. ***Mutat Res***. 2000, 455:29-60
12. Zahin M, Ahmad I, Aqil F. Antioxidant and antimutagenic activity of carum copticum fruit extracts. ***Toxicol In Vitro***. 2010, 24:1243-1249.
13. Singh MK, Faisal M, Ahmad A, Sonkar A, Yadav J. Protective effect of lagenaria siceraria against doxorubicin induced cardiotoxicity in wistar rats. ***International Journal of Drug Development & Research***. 2012, 4:298-305
14. Amit K, Sangh P, Sharma N, Jha KK. Phytochemical, Ethnobotanical and Pharmacological Profile of Lagenaria siceraria : A Review. ***Journal of Pharmacognosy and Phytochemistry***. 2012, 1:27-35