Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env.Pharmacol. Life Sci., Vol 11[10] September 2022 : 32-40

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

In-Vitro Mutagenicity and Antimutagenicity Studies of *Cassia occidentalis* in TA98, TA100, TA102, TA1535, TA1537 and WP2uvrA

Narinder Kumar[1], Mohd Ashaq[2*] and Surjeet Singh[3]
1Department of Zoology, GGM Science College, Cluster University of Jammu, J&K, India -180001
2.Department of Botany, Govt. PG College Rajouri, J&K, India – 185132
3. Pharmacology Division, CSIR- Indian Institute of Integrated Medicine, Jammu, J&K, India - 180001
*Corresponding author: narinderbhagatt@gmail.com

ABSTRACT
Hydroalcohlic extract of leave of Cassia occidentalis, a nontoxic herbal preparation is reported to be hepatoprotective. This study was undertaken to investigate the mutagenic/antimutagenic potential of CO-A002 using Salmonella mutagenicity test. The study will provide an indication about the effect of chemical constituents present in C.occidentalis on genetic damage that leads to gene mutation. Ames Salmonella/Mammalian - microsome mutagenicity test was used to investigate the mutagenic and antimutagenic potential of CO-A002. Salmonella typhimurium tester strains TA98, TA100, TA102,TA1537, TA1538 and E.coli were used for mutagenicity testing. The antimutagenicity study was also carried out in tester strains TA98, TA100, TA102,TA1537, TA1538 and E. coli against various standard mutagens with and without metabolic activation. It reveals that hydro alcoholic leave extract did not induced any mutagenicity but exhibit anti- mutagenicity in tested strains of Salmonella typhimurium and E. coli at the tested doses 0.064, 0.32, 1.6, 8 & 40µg/plate, in the presence and absence of metabolic S9 fraction. CO-A002 was found to be non mutagenic in Salmonella assay, whereas manifested the antimutagenic potential both with and without metabolic S9 activation. The enhanced antimutagenic activity of CO-A002 on preincubation indicates that the antimutagenic factor(s) may be desmutagenic in nature. The precise mechanism by which exerts antimutagenic potential is not known so far. The possibility of having diverse antimutagenic factors in COA002 act by different mechanisms are majorly expressed.
Keywords: *AMES, Cassia occidentalis, Salmonella typhimurium, E. coli, mutagenicity and antimutagenicity.*

Received 15 .08.2022 Revised 22.08.2022 Accepted 21.09.2022

INTRODUCTION

Natural chemicals are known as secondary metabolic products generated by the plants in response to various stresses of an environment. A lot of phytochemicals have been identified isolated and when taking in human diet, may affect the chances of chronic disease. India has been exposed for over a century to the application of allopathic medicines with their definite merits as well as demerits. The ayurvedic herbs have antimutagenic and antiviral properties which occupy an important status in the Ayurvedic system of medicine and are used for the treatment of various diseases either alone or from a part of various formulations [1].

The mutagenicity deems to be an induction of everlasting transmissible alteration in the DNA. The mutations are caused by mutagens like ionizing radiation, ultraviolet radiation and reactive oxygen species (ROS). ROS produced oxidative stress and is known to cause tissue injury include damage to proteins, lipids and DNA. Oxidative injury to DNA takes place when oxygen radicals react with DNA. If not repaired, the changes in nucleic acid bases and the breaks in the DNA chain lead to DNA mutation and mutagenic forms of DNA [2].

The genotoxicity is a broader term than mutagenicity and refers to the ability of the drug or any molecule to affect the DNA and topoisomerases, which are accountable for the genome fidelity. Genotoxic effects on DNA are not always related to mutations. The mutations are majorly produced by external factors such as chemical and physical agents, named mutagens. Mutations can occur spontaneously because of errors in DNA replication, repair, and recombination. The mutagenic alterations take place in germ cells can be transmitted to next generations, whereas somatic mutations may contribute to the pathogenesis of various pathological conditions such as cancer [3].

Antimutagenic components are enough capable to counteract the effects of mutagens. Therefore, a pieces of information's on the mode of action of certain mutagenic agents impart a basis for an explanation of how antimutagenic agents work. Identifying the antimutagenic compounds are among the most promising fields of research in recent years. Mutagenic and antimutagenic potential of CO-A002 was evaluated by employing Ames test with and without metabolic activation in the present study.

C. occidentalis is an annual herb commonly known as *Senna occidentalis* belonging to genus *Cassia* family Leguminosae. It is a common weed and found throughout India (up to an altitude of 1500 m) and used for a variety of purposes in indigenous and folk medicines. Plants belonging to this family have been extensively investigated because of their rich medicinal (anti-inflammatory, anticarcinogenic, antimutagenic, antiplasmodial, antirheumatic and hepatoprotective) and economic use. The leaves reported to have hepatoprotective [7], antimutagenic [4], antimalarial [5], antidiabetes [6] and anti-inflammatory effect [7], antilarvicidal [8], analgesic [9], and antimicrobial [10] activities of different plant parts also reported. The aim of the present study is to determine mutagenicity, antimutagenicity of the hydoalcoholic leaf extract of *C. occidentalis*.

Gene mutations cause a number of genetic disorders in human. The Ames test is used worldwide as an initial screen to determine the mutagenic potential of extract, new chemicals or drugs and has been deemed to be over 90% precision in genotoxicity prediction. The strains *of Salmonella typhimurium* and *Escherichia coli* recommended for use in the bacterial reverse mutation test [11], have been considered as non-GMOs (genetically modified organisms) because they can be constructed by self-cloning or naturally occurring bacterial strains, or do not disturb the biological diversity [12-16]. The identification of substances capable to induce mutations has become an important procedure in safety assessment. This study was undertaken to investigate the mutagenic/antimutagenic potential of CO-A002 using Salmonella mutagenicity test. The study will provide an indication about the effect of chemical constituents present in *Cassia occidentalis* on genetic damage that leads to gene mutation.

MATERIAL AND METHODS

Materials

Extract Preparation:

Leaves of *Cassia occidentalis* were collected from Akhnoor Chenab bank of Jammu in the month of August and September identified from botany Department of CSIR-IIIM Jammu India, shade dried and crushed into fine powdered. The powdered leaves (quantity in gms) were extracted with 500 ml 50% Ethanol. The extract was filtered and filtrate was freeze dried using freeze dryer into amorphous powder.

Chemicals:

Nutrient broth and agar were purchased from Hi-Media Laboratories Pvt. Ltd. 2-nitro-fluorene, 2-anthracene (2-AN), sodium azide, Mitomycin, 2-amino-acridine, Mitomycin-C, 2-aminoanthracene, phenobarbitone sodium, D-biotin, and L-histidine were purchased from Sigma Chemical Company; 0.1MNADP and glucose -6- phosphate were from Sisco Research Laboratories, India, and Dextrose, KCl, MgCl2 , 0.2M Sodium phosphate buffer and other buffer components were from SD Fine Chemicals, India. S9 was obtained from Molecular Toxicology. INC, Boone. NC 28607, USA and stored at -80°C. S9 fraction was added to obtain working concentration 5% (v/v) of complete S9-mixture.

Media: Bacto agar, Nutrient broth (Oxoid) and Agar powder extra pure (BD) were used in the study.

Salmonella, E.coli tester strains and mutagenicity test

Salmonella typhimurium and *E.coli* tester strains (TA98, TA100, TA102, TA1537, TA1538, and WP2uvrA) were kindly supplied from Bruce N Ames Laboratory, University of California, USA. An optimum association of standard tester strains for maximum detection of mutagens was used in this study. The plate incorporation and pre-incubation modification method using tester strains with and without metabolic activation were carried out essentially.

Metabolic activation

The tester strains S. typhimurium and E. coli do not possess enzyme systems which are present in mammals to metabolize promutagens to active electrophilic metabolites capable of reacting with DNA. Sometimes, these pro-mutagens interact with a mammalian enzyme system and produced mutagenic metabolites. Hence, it is necessary to add external metabolic activation system which is post-mitochondrial fraction (S9) that is characterized by protein and cytochrome P450 content. S9 fraction was mixed with cofactor solution (D-Glucose –6-phosphate 0.80g, β NADP: 1.75g, magnesium chloride: 0.90g, potassium chloride: 1.35g, sodium phosphate, dibasic: 6.40g, sodium phosphate, monobasic: 1.40g were dissolved in 450ml distilled water and filter sterilized) 10% v/v in the S9 mixture. This 10% v/v S9 mixture is used for the study.

The mutagenicity test was used to determine the number of spontaneous revertants and positive control revertants. Spontaneous revertants are determined without the addition of test chemical and are used as

a baseline to compare with the test chemicals (05 concentrations)/positive control on TA98 and TA100, TA102, TA1535 TA1537 and *E. coli*. Sodium azide and 2-aminoanthracene are used as a positive control in order to confirm the reversion characteristic and specificity of the bacteria.

Method:

Ames /Salmonella/mammalian-microsome mutagenicity test (an in-vitro short-term mutagenicity test) was used in this study. S. typhimurium and E.coli tester strains TA98, TA100, TA102, TA1537, TA1538, and WP2uvrA were used for mutagenicity testing. The antimutagenicity study was conducted in the same above said strains against various standard mutagens with and without metabolic activation.

Mutagenicity assay:

Mutagenicity test was performed by following plate incorporation method with 6 testers strains (TA98, TA100, TA102, TA1537, TA1538, and WP2uvrA) at doses 0.064, 0.32, 1.6, 8 and 40 µg/plate, in triplicates both in the absence and presence of metabolic activation system (S9). The negative control plates are treated with 100µl of DMSO/plate. The positive mutagenic controls including 2-nitroflourene for TA98, sodium azide for TA100, mitomycin for TA102, sodium azide for TA1535, 2-aminoacridine for TA1537 and mitomycin-C WP2uvrA were treated in triplicate in order to confirm the reversion characteristic and specificity of the bacteria.

After treatment, plates were kept in inverted position for a period of 48 hrs. at 37±2°C. Colony counts were compared with negative control. Criteria for determining a positive result is dose-dependent increase or a reproducible increase at one or more concentration, in the number of revertant colonies per plate in at least one strain in the presence or absence of metabolic activation system. Two-fold (TA98, TA100, TA102)or more than two-fold (TA1535,TA1537) increase in revertant colonies at one or more concentrations corresponding to negative control will be considered as positive result. The result which does not meet this criterion is deemed as non mutagenic in this assay.

The molten soft agar of 2 ml, 0.1 ml of overnight bacterial culture, and 0.5 ml of S9 mixture (wherever needed) were added, gently mixed and poured over minimal glucose agar medium and added 2ml of L-Histidine or D-Biotin (0.5 mM), 0.1ml of the fresh overnight bacteria culture(TA98,TA100, TA102,TA1535,TA1537 and WP2uvrA), 0.5 ml of sodium phosphate buffer and various concentrations of the test compound (0.064,0.32, 1.6, 8 and 40µg) of CO-A002 mixed and added to minimal glucose agar plate incubated at 37°C for 48 hrs. After incubation, the plates were counted for his+ revertants. The experiment was performed in triplicate using the similar procedure for all the concentrations. The results were determined as mean of six plates per point with standard deviation and viable cell count as approximately 1–2 $\times 10^9$ cfu/ml. All the solutions were prepared freshly by dissolving in dimethyl sulfoxide(DMSO). In the positive control assay, 0.1ml of 2-nitroflourene for TA98, sodium azide for TA100, mitomycin for TA102, sodium azide for TA1535, 2-aminoacridine for TA1537 and mitomycin-C for WP2uvrA (10µg/plate)was used in place of test chemical and 0.1 ml of DMSO was used in case of negative control. All plates were incubated at 37ºC for 48hr, and the numbers of revertant colonies were determined and the entire experiment was replicated in triplicate. All the tester strains of *S.typhimurium* and E.coli were analyzed for mutagenicity assay with and without S9 mix.

Mutagenic Index=

No. of revertants at different conc. of test substance / No. of revertants in negative control

Antimutagenicity

The antimutagnic assay used was the Salmonella/microsome assay developed by Maron and Ames [17] and as modified by Ruan *et al*, [23]. BruceM.Ames (University of California, Berkeley,CA) provided bacterial strains TA98, TA100,TA102, TA1535,TA1537 and WP2uvrA. An antimutagenicity assay was performed using plate incorporation method and preincubation method by incorporating the tester strains TA98, TA100, TA102, TA1535, TA1537 and WP2uvrA. For plate incorporation assay, 2 ml of the molten soft agar various concentrations of test compound (10 to 500 µl), 0.1 ml of overnight bacterial culture, 0.1 ml of known appropriate positive mutagen control, and 0.5 ml of S9 mix (wherever used) were added aseptically and poured over minimal glucose agar medium and incubated at 37°C for 48 hrs..

For preincubation method, 0.1 ml of overnight culture, various concentrations of test compounds, optimal concentration of appropriate known mutagen control, and 0.5 ml of S9 mix were added in sterile tubes,mixed thoroughly using vortex mixture and incubated at 37°C for 20-30 minutes. This mixture was added to molten top agar and poured on the surface of minimal glucose agar and continued further as in plate incorporation method. The results were expressed as the mean of six plates from two independent experiments with standard deviation.

Antimutagenicity or percentage of inhibition of mutagenicity by CO-A002 was calculated using equation from [18, 19] as given below:

Inhibition rate (%) = 1-(T/M) x100

Where T- is the number of revertants per plate in the presence of mutagen and plant extract

M- is the number of revertants per plate in positive control.
No antimutagenic effect was considered if inhibition rate is less than 25%, a moderate effect lies between 25-40% and a strong antimutagenicity is considered when the inhibition rate is greater than 40%.
Statistical evaluation
Statistical analysis was performed using Karl Pearson Correlation Coefficient method. Statistical Package for the Social Sciences (SPSS) ver.15.0 was used.

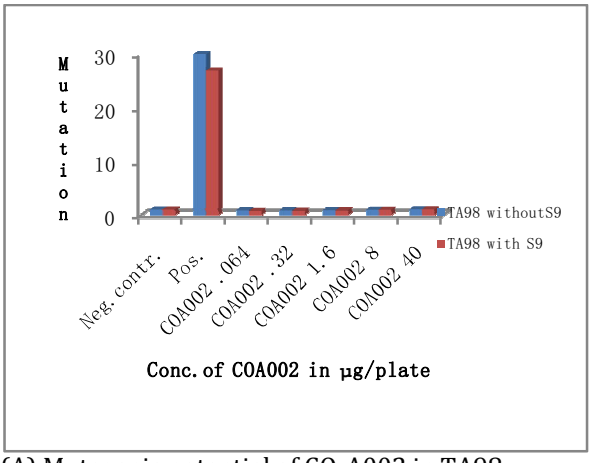
RESULTS

The test preparation was found to be nontoxic to the tester strains at different doses with or without metabolic activation fraction. The characteristics his+ revertants patterns of the standard tester strains to various standard mutagens were shown in Table 1. The absence of mutagenicity of CO-A002 against the recommended tested strains shows that DNA does not appear to be an appropriate target for active phytocompounds of extract. However, results obtained against TA98, TA100, TA102, TA1535, TA1537, and WP2uvrA exhibited that CO-A002 is non- mutagenic and did not produce any change against these strains of bacteria which suggest mutagenicity of CO-A002. CO-A002 failed to induce his+ revertants in all the six tester strains either in the presence or absence of metabolic activation by plate incorporation method thereby found to be non-mutagenic in Ames test (Table 1).
The present investigation depicts the antimutagenic potential of various concentrations of CO-A002 in S. typhimurium and E.coli reverse mutation assay with or without S9 (Table 2). The maximum inhibitory effect shown by CO-A002 was 45.12% against TA1537 and 43.2% towards TA100 at a dose of 40 µg per plate.

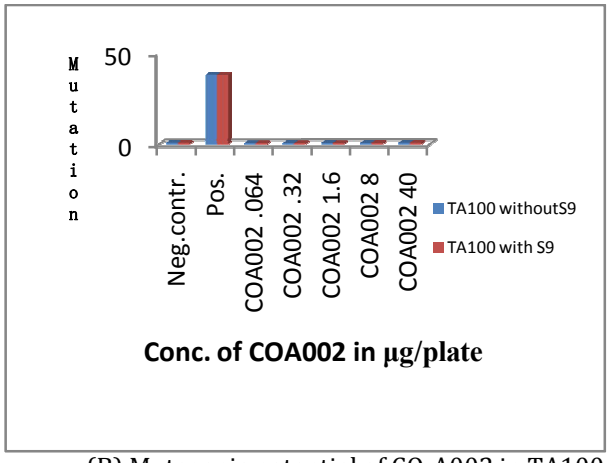
Table 1: Evaluation of CO-A002 in *Salmonella typhimurium* and *E. coli* (wp2uvrA) for mutagenesis without and with S9 metabolic activation (Mutagenic index).

Treatment	Concentration	Strains											
		TA98		TA100		TA102		TA1535		TA1537		*E. Coli*	
		Without Out	With	Without Out	With	Without Out	With	Without out	With	Without Out	With	Without Out	With
Negative	Vehicle	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Positive		29.93	26.86	8.28	8.24	40.62	38.29	35.34	36.43	42.11	40.62	35.91	32.58
CO-A002	0.064	0.88	0.73	0.71	0.66	0.89	0.76	0.8	0.81	0.78	0.7	0.73	0.78
CO-A002	0.32	0.88	0.75	0.77	0.68	0.93	0.83	0.82	0.83	0.87	0.84	0.73	0.8
CO-A002	1.6	0.89	0.83	0.83	0.66	0.94	0.92	0.86	0.9	0.93	0.88	0.72	0.84
CO-A002	8	0.97	0.95	0.8	0.9	0.99	1.00	0.99	1.04	0.98	0.97	0.91	0.88
CO-A002	40	1.01	1.02	1.03	0.99	1.19	1.13	1.08	1.17	1.07	1.04	1.07	1.08

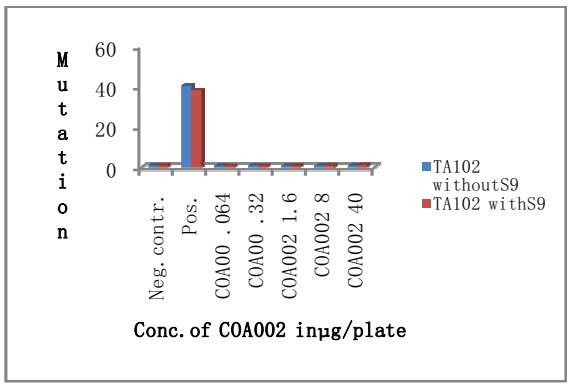
N=6; *P<0.05 Student-Newman-Keuls Test.



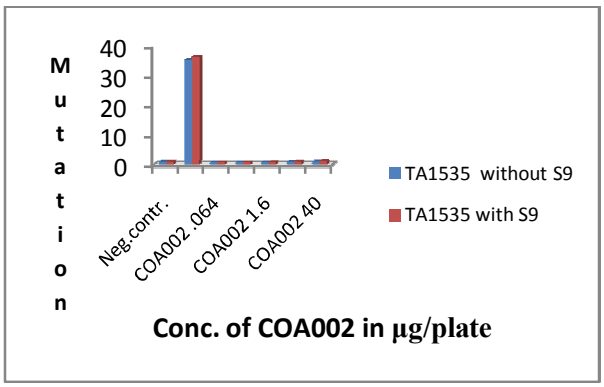
(A).Mutagenic potential of CO-A002 in TA98



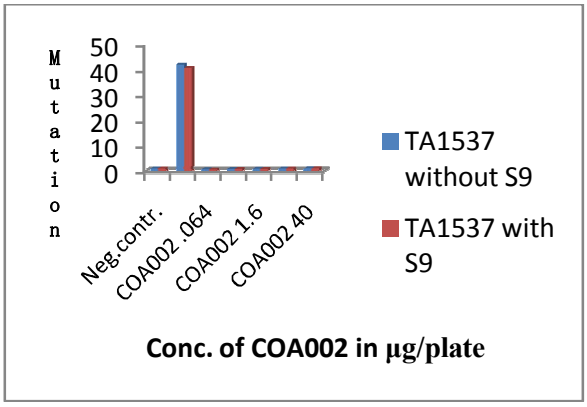
(B) Mutagenic potential of CO-A002 in TA100



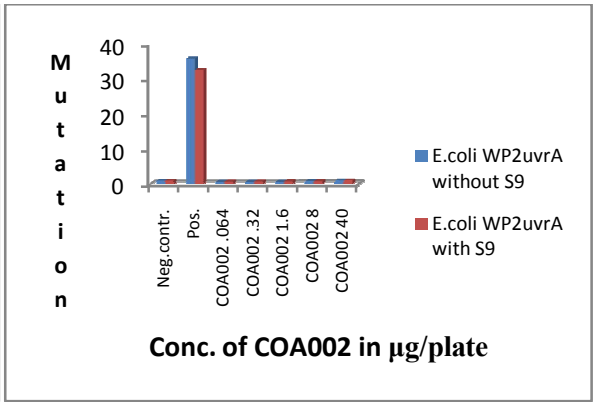
(C) Mutagenic potential of CO-A002 in TA102



(D) Mutagenic potential of CO-A002 in TA1535



(E) Mutagenic potential of CO-A002 in TA1537



(F) Mutagenic potential of CO-A002 in *E.coli* (WP2uvrA)

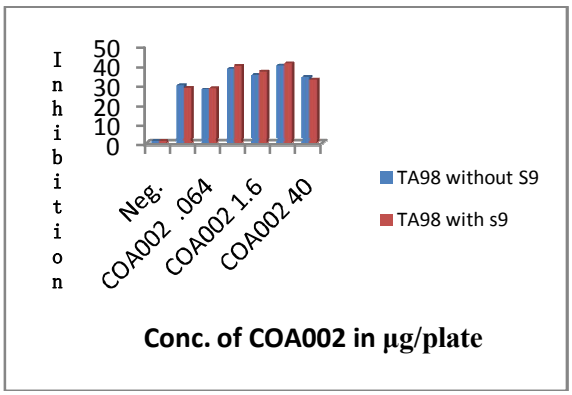
Fig.1 (A-F): Graphs of mutagenicity of CO-A002 from different strains of *Salmonella typhimurium* and E.coli (A-TA98, B -TA100, C-TA102, D-TA1535, E-TA1537&F-Wp2uvr.

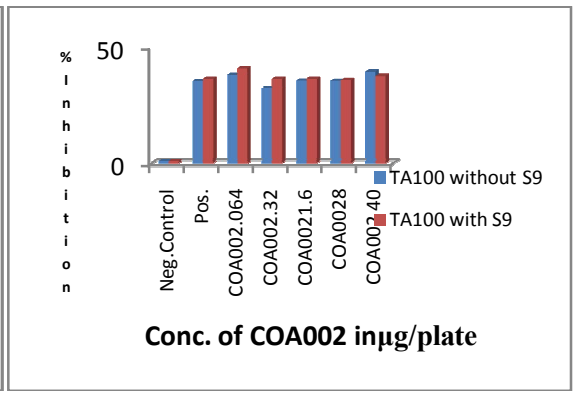
Table 2: Evaluation of CO-A002 in Salmonella typhimurium and E. coli (wp2uvrA) for Antimutagenesis without and with S9 metabolic activation (Mutagenic index).

Treatment	Concentration (µg)	Strains											
		TA98		TA100		TA102		TA1535		TA1537		E. coli	
		With Out S9	With S9	With Out S9	With S9	With outS9	With S9	With Out S9	With S9	With Out S9	With S9	With Out S9	With S9
Negative	Vehicle	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Positive		29.84	28.58	35.47	36.44	37.29	41.52	35.33	36.43	42.11	40.62	36.52	39.64
CO-A002	0.064	27.63	28.2	38.07	40.8	35.11	43.35	34.56	35.79	37.6	35.98	36.18	35.18
CO-A002	0.32	38.19	39.82	32.4	36.40	38.44	42.84	35.62	34.92	39.25	36.88	35.37	38.85
CO-A002	1.6	34.9	36.94	35.59	36.44	35.84	35.52	35.5	36.48	43.76	41.22	36.07	39.53
CO-A002	8	39.92	40.99	35.49	35.8	36.31	38.33	35.88	37.4	42.88	40.60	38.25	39.76
CO-A002	40	33.92	32.62	39.58	37.58	34.48	41.62	39.08	36.99	44.12	42.00	38.92	36.84

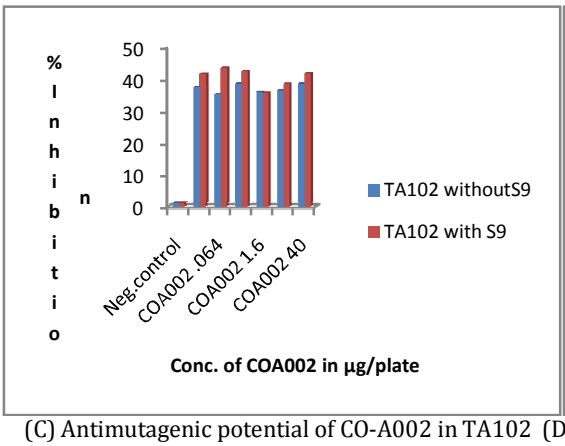
N=6; *P<0.05 Student-Newman-Keuls

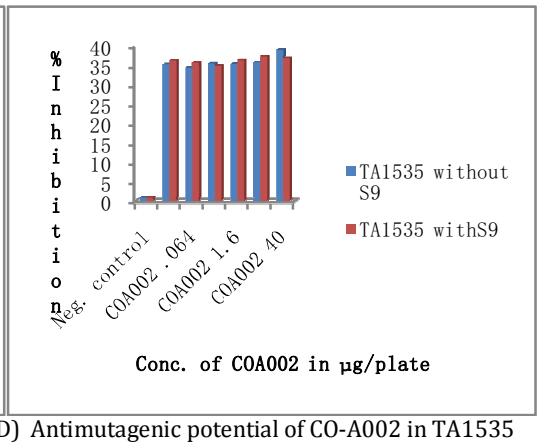
Antimutagenicity of CO-A002



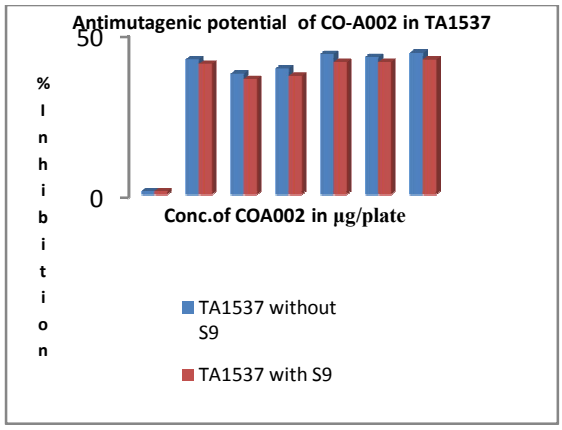


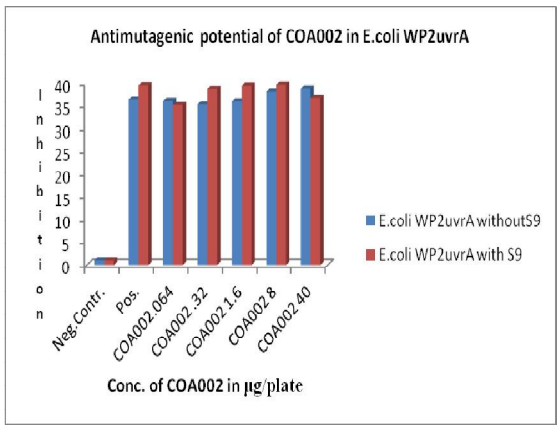
(A) Antimutagenic potential of CO-A002 in TA98 (B) Antimutagenic potential of CO-A002 in TA100





(C) Antimutagenic potential of CO-A002 in TA102 (D) Antimutagenic potential of CO-A002 in TA1535





(E) Antimutagenic potential of CO-A002 in TA1537 (F) Antimutagenic potential of CO-A002 in WP2uvrA

Fig. 2 (A-F): Graphs of antimutagenicity of CO-A002 from different strains of Salmonella typhimurium and E.coli (A-TA98, B-TA100,C- TA102,D-TA1535,E-TA1537&F-Wp2uvrA).

DISCUSSION

CO-A002, an herbal ayurvedic preparation was found to be non-mutagenic in Ames test and did not produce any change against these strains of bacteria which suggest non-mutagenicity of CO-A002. This is due to the fact that even at high concentration, it did not induce an increase in number of revertants when compared to the number of spontaneous revertants. The absence of a mutagenic activity of CO-A002 against *S. typhimurium* and *E.coli* bacterial strains in the Ames assay is a positive step and a mutagenic activity requires to be investigated to determine its genotoxicity to humans as its safe use in traditional medicine is questionable. The lack of mutagenicity is not a property of all natural products in use, because some medicinal plants assayed with Ames test, with or without the S9, have shown positive for mutagenicity [25-27].

CO-A002 exhibited antimutagenic potential against TA98, TA100, TA102, TA1535, TA1537 and WP2uvrA in the presence or absence of metabolic activation fraction S9 in both the tester strains. These results indicate that CO-A002 inhibits his+ revertants arising by base pair substitution more effectively as compared to the frameshift mechanisms. The exact mechanism, by which CO-A002 exerts antimutagenic activity, is not clearly understood. The antimutagenicity may be due to the large amount of flavonoids, polyphenols, tannins, and terpenoids found in CO-A002. Flavonoids, polyphenol, tannins, and terpenoids are known to have strong antioxidants characteristics by inhibiting lipoxygenase, superoxide anion and single oxygen formation in indeed. Flavonoids inhibit lipid peroxidation showed its effect as an antioxidant or free radical scavengers and chelator of divalent cations. All the test strains have shown signs of significant inhibition of the induced mutagenic effect in the presence and absence of S9 metabolic activation system as compared to positive control. CO-A002 may stimulate or facilitate the transmembrane export system of bacteria in order to eliminate the mutagens; or it can also interfere with the uptake of mutagens into bacteria. The results of experiments confirm known antioxidant activities of flavonoids [24]. The aqueous and ethyl acetate leaf extract of *C. occidentalis* were found to possess flavonoids, saponins, tannins, phenolics in a previous study from West Africa region [20-23]). So, the same extract showed high free radical scavengers and antimutagenic activities. It is presumed that extract is an antimutagenic, and then it may also be anticancer in nature. These activities are correlated with the already studied polyphenolic nature of extract/compounds [29]. Botanical remedies and phytotherapy drugs possessing active principles and are currently developed to protect against electrophile (e.g Reactive Oxygen Species) that attack on DNA and is responsible for causing cancer and even aging. The occurrence rate of cancer is increasing at a very high rate throughout the world and the determination of chemoprophylaxis compound is crucial to alleviate the cancer chances [28]. The determination of antimutagenicity of plant extracts or pure compounds is very much crucial for the discovery of new effective anticarcinogenic treatments.

Further investigation on phytochemical screening and extensive genotoxic assays must be carried out, in order to determine its safe use on the basis of in- vitro evaluation of the plant. Thus, due to the proven antioxidant activity of CO-A002, it is an important to assess whether the consumption of hydroalcoholic extracts of these constituents can assist in the prevention or repair of cellular changes caused by the exposure to potentially mutagenic agents, in addition, to the proven beneficial effects for health and well-being. The protective effect of hydroalcoholic extracts of any medicinal products was determined by testing their antimutagenicity potential.

Most of carcinogens are inactive when present in the environment, on entering the system, they are converted into active metabolites by the carcinogen-metabolizing enzymes, further the protocol have improvised a method for detecting chemicals which are potential human carcinogens or mutagens by adding homogenates of rat liver directly to the Petri plates thus incorporating an important aspect of mammalian metabolism into the in-vitro test . In this study, antimutagenicity of CO-A002 was studied by adding homogenates of rat liver to the Petri plates along with the extracts to understand the important aspects of mammalian metabolism in the in- vitro testing. This, ayurvedic product may have better antioxidant activity endow with the ability to intercept the free radicals produced by cellular metabolism or exogenous sources,

Further, CO-A002 having antimutagenic potential and it may play a crucial function in neutralization of various mutagens of diet. Thus; the consumption of this ayurvedic/herbal product can bring more benefits and protection to individuals and also ameliorating their quality of health and life.

CONCLUSION

CO-A002 was found to be non-mutagenic in nature when tested against various bacterial strains through AMES, whereas manifested the antimutagenic potential both with and without metabolic activation. The enhanced antimutagenic activity of CO-A002 on preincubation indicates that the antimutagenic factor(s) may be desmutagenic in nature. The exact mechanism by which CO-A002 exerts antimutagenic potential is not known. It is presumed that the presence of flavonoids, polyphenols, tannins, and terpenoids may be responsible for the anti-mutagenic activity through antioxidant potential. The absence of mutagenic and presence of the promising antimutagenic feature of CO-A002 suggests that the extract may possess a positive pharmaceutical potential. As a consequence, the present result may encourage additional and more in-depth investigation to understand the exact mechanism by which extract act and to assess the pharmacological characteristics of extract. Hence, it is suggested that CO-A002 is potentially safe to use as natural supplement to cure different ailments of human.

ACKNOWLEDGEMENTS

We express our sincere thanks to the Director, IIIM, Jammu, for providing facilities to execute this study. Further, I (Narinder Kumar) am grateful to University Grants Commission for the award of fellowships (FIP).

REFERENCES

1. Vashishtha VM, John TJ, Kumar A:(2009). Clinical & pathological features of acute toxicity due to Cassia occidentalis in vertebrates. Indian J Med Res, 130(1):23-30.
2. Yadav JP, Arya V, Yadav S, Panghal M, Kumar S, Dhankhar S: (2010). Cassia occidentalis L.: A review on its ethnobotany, phytochemical and pharmacological profile. Fitoterapia, 81(4):223-230.
3. Yadava RN, Satnami DK: (2011). Chemical constituents from Cassia occidentalis Linn. Indian J Chem, Sect B: Org Chem Incl Med Chem, 50B(8):1112-1118.
4. Tona L, Mesia K, Ngimbi NP, Chrimwami B, Okond'ahoka, Cimanga K, de BT, Apers S, Hermans N, Totte J et al:(2001). In-vivo antimalarial activity of Cassia occidentalis, Morinda morindoides and Phyllanthus niruri. Ann Trop Med Parasitol, 95(1):47-57.
5. Sharma A, Tripathi P, Kumar S: (2016). Evaluation of the Larvicidal Efficacy of Five Indigenous Weeds against an Indian Strain of Dengue Vector, Aedes aegypti L. (Diptera: Culicidae). J Parasitol Res, 2016:2857089.
6. Tasaka AC, Weg R, Calore EE, Sinhorini IL, Dagli ML, Haraguchi M, Gorniak SL:(2000). Toxicity testing of Senna occidentalis seed in rabbits. Vet Res Commun, 24(8):573-582.
7. Jafri MA, Jalis SM, Javed K, Singh S: (1999). Hepatoprotective activity of leaves of Cassia occidentalis against paracetamol and ethyl alcohol intoxication in rats. J Ethnopharmacol, 66(3):355-361.
8. Bin-Hafeez B, Ahmad I, Haque R, Raisuddin S: (2001). Protective effect of Cassia occidentalis L. on cyclophosphamide-induced suppression of humoral immunity in mice. J Ethnopharmacol, 75(1):13-18.
9. Vadnere GP, Patil AV, Jain SK, Wagh SS: (2011). Investigation on in-vitro antioxidant activity of whole plant of Cassia occidentalis Linn. (Caesalpiniaceae). Int J PharmTech Res, 3(4):1985-1991.
10. Sadique J, Chandra T, Thenmozhi V, Elango V: (1987). Biochemical modes of action of Cassia occidentalis and Cardiospermum halicacabum in inflammation. J Ethnopharmacol, 19(2):201-212.
11. Rani MS, Emmanuel S, Sreekanth MR:(2010). Evaluation of the antipyretic and anti-inflammatory activities of methanolic fraction and chrysophenol of Cassia occidentalis Linn. Res J Pharm Technol, 3(3):888-893.
12. Eguale T, Tadesse D, Giday M: (2011). In vitro anthelmintic activity of crude extracts of five medicinal plants against egg-hatching and larval development of Haemonchus contortus. J Ethnopharmacol, 137(1):108-113.
13. Sini KR, Karpakavalli M, Sangeetha PT: (2010). Analgesic and antipyretic activity of Cassia occidentalis Linn. World Appl Sci J, 11(10):1216-1219.
14. Brown JP: (1980). A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. Mutat Res , 75(3):243-277.
15. Sadiq IS, Shuaibu M, Bello AB, Isah SGTA, Izuagie T, Nasiru S, Kamaru MB: (2012). Phytochemistry and antimicrobial activities of cassia occidentalis used for herbal remedies. J Chem Eng, 1(1):38-41, 34.
16. Weisburger JH, Hara Y, Dolan L, Luo F-Q, Pittman B, Zang E: (1996). Tea polyphenols as inhibitors of mutagenicity of major classes of carcinogens. Mutat Res, Genet Toxicol, 371(1,2):57-63.
17. Maron DM, Ames BN: (1983). Revised methods for the Salmonella mutagenicity test. Mutat Res, 113(3-4):173-215.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: (1951). Protein measurement with the Folin phenol reagent. J Biol Chem, 193:265-275.
19. Mazel P: (1971). Experiments illustrating drug metabolism in vitro. In: Williams and Wilkins: 546-582.
20. Hakura A, Suzuki S, Satoh T: (1999). Advantage of the use of human liver S9 in the Ames test. Mutat Res, Genet Toxicol Environ Mutagen, 438(1):29-36.
21. Kinoshita E-I, Yoshimoto M, Motomura K, Kawaguchi T, Mori R, Baba T, Nishijo K, Hasegawa T, Momoi T, Yorihuji T: (1997). DAX-1 gene mutations and deletions in Japanese patients with adrenal hypoplasia congenita and hypogonadotropic hypogonadism. Horm Res; 48(1):29-34.
22. Leettola CN, Knight MJ, Cascio D, Hoffman S, Bowie JU: (2014). Characterization of the SAM domain of the PKD-related protein ANKS6 and its interaction with ANKS3. BMC Struct Biol, 14:17/11-17/15, 15 pp
23. Ruan X, Liu L, Chen X, He Z, Li H: (2008). Cloning and signature analysis of six novel DNA fragments of ribosome inactivating protein from different plants. Huazhong Nongye Daxue Xuebao , 27(1):7-11.
24. Sankam P, Punvittayagul C, Sringam K, Chaiyasut C, Wongpoomchai R: (2013). Antimutagenicity and anticlastogenicity of glutinous purple rice hull using in vitro and in vivo testing systems. Mol Cell Toxicol, 9(2):169-176.
25. Kul L, Kook KHY (1987). Effect of bacterial growth-inhibiting ingredientson the Ames mutagenicity of medicinal herbs. Mutat. Res. Lett.,192(2): 99-104.
26. Sohni Y, Davis C, Champs A, Kale P (1995). "Frameshift mutations in salmonella induced by the extracts of medicinal herbs Lannea edulis(sond.) engl. and Monotes glaber sprague." Environ. Mol. Mutagen., 25(1): 77-82.
27. Diana AN (1997). Modulating effects of flavonoids on food mutagens inhuman blood and sperm samples in the comet assay. Teratogen.Carcinogen. Mutagen, 17(2): 45-58.

28. Kundu J, Mossanda K (2005). Inhibitory effects of the extracts of *Sutherlandia frutescens* (L.) R. Br. and *Harpagophytum procumbens* DC. On phorbol ester-induced COX-2 expression in mouse skin: AP-1and CREB as potential upstream targets. Cancer let. , 218(1): 21-31.
29. Faller, A.L.K. and Fialho, E. (2009).The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking. Food Research International. 42: 210–215.

CITATION OF THIS ARTICLE

N Kumar, M Ashaq and S Singh. *In-Vitro* Mutagenicity and Antimutagenicity Studies of *Cassia occidentalis* in TA98, TA100, TA102, TA1535, TA1537 and WP2uvrA .Bull. Env. Pharmacol. Life Sci., Vol 11 [10] September 2022: 32-40