Evaluation of Carcinogenic Potential of Surface Adsorbed Hazardous Chemicals on Vegetables and Fruits

Mrigendra Kumar Dwivedi, Dhananjay Shukla and Atul Tiwari

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 03

Res Jr of Agril Sci (2021) 12: 801–806

CARAS

Res. Jr. of Agril. Sci. (May-Jun 2021) 12(3): 801–806
www.rjas.org

Full Length Research Article

Evaluation of Carcinogenic Potential of Surface Adsorbed Hazardous Chemicals on Vegetables and Fruits

Mrigendra Kumar Dwivedi*[1], Dhananjay Shukla[2] and Atul Tiwari[3]

Received: 02 Mar 2021 | Revised accepted: 07 May 2021 | Published online: 18 May 2021

A B S T R A C T

Increasing numbers of cancer in urban and rural area of Chhattisgarh India since 2011 emphasizes to search possible causes and way to reduce risk factor for Cancer in this region. Objectives of the study to evaluate the carcinogenic potential of surface adsorbed hazardous chemicals on fruits and vegetables. The study was also aimed to find out the best simple washing method to remove maximum amount of carcinogenic substance from vegetables and fruits. Samples were collected from three sites city markets, villages and the fields of the different selected area of Chhattisgarh state. The samples were washed with three different methods and the washed water is used to analyze for mutagenicity and carcinogenicity through AMES test. This study was found that significant amount of the carcinogenic chemicals presents on the samples of different sites. When same sample was washed extensively through method -2 and 3 pesticide come out in the water and shows significant carcinogenicity at P<0.0001 in the experiments.

***Key words*:** Pesticides, Mutagenicity, chemical carcinogenicity, Ames test

The World Cancer Report said that consistent with the estimated cancer burden in India in 2018, there are about 1.16 million new cancer cases, 784,800 cancer deaths, and 2.26 million 5-year prevalent cases in India's population of 1.35 billion. The report said that "one in 10 Indians will develop cancer during their lifetime, and one in 15 Indians will die of cancer." The exponentially increase within the cases of the cancer within the small cities and therefore the villages establishing challenges for the health and research organizations within the 21st century [1].

A study from medical college Raipur (Chhattisgarh) observed registered cancer patients in the duration of 5 years from 2011 to 2016 and found the increase in the number of incident cases from 3028(2011) to 3315(2015) [2-3]. According to report published in Lancet oncology on 2018 in which the study on cancer burden has been done from 1990 to 2016, incident of cancer in Chhattisgarh is 82.0 per 100000 populations and death is 61 per 100000 populations. The 53.6% increase in breast cancer, 11.4% increase in colon and rectum cancer, 35.6% in ovarian cancer, 46.6% in gallbladder and biliary tract cancer and 27.1% in Thyroid cancer. This study also showed that the crude death rates due to cancer increases from less than 35 per 100000 people in 1990 to 55.0 to 64.9 per 100000 in 2016. This study also found that the dietary risk contributes 43.2% colon and rectum cancer, 21.5% Oesophegeal cancer, 33.6% non-Hodgkin's lymphoma, 37% liver cancer, 41.0% bladder cancer, 28.3% kidney cancer and 31.5% malignant skin myloma. During the year from 1990 to 2016 the bladder cancer in male and breast cancer in female was found most increasing cancer in Chhattisgarh population. The average burden of cancer among women in India is 250 per 100,000 women when data is age adjusted. The age-adjusted prevalence of cancer was found higher amongst the respondents from urban areas (270 per 100,000 women) as compared to their rural counterparts (231 per 100,000 women). Although for Chhattisgarh it is 67 per 100000 women [4].

Increasing number of cancer in Chhattisgarh shouting to find out the possible causes and ways to reduce it. The present study aimed to find out the carcinogenic substances present on the surface of vegetables and fruits, carcinogenic potential of these substances through Ames test and ways to remove maximum amount of these substances through simple house hold methods.

Ames test is mutagenic test in this test, rat liver extract called S-9 mix that containing of microsomal enzymes and cofactors are often added to the bacteriological medium. The medium contains genetically modified Salmonella typhimurium strains. The presence of mutations in the histidine genes, causing defects in a metabolic pathway leading to the production of histidine, allows positive selection

* Mrigendra Kumar Dwivedi
✉ mrigendradwivedi@rediffmail.com

[1] Government Nagarjuna P.G. College of Science, Raipur - 492 010, Chhattisgarh, India

[2] Guru Ghasidas Central University, Bilaspur - 495 009, Chhattisgarh, India

[3] Government Bhanwar Singh Porte P.G. College Pendra - 495 119, Chhattisgarh, India

of histidine revertants on minimal agar plates lacking histidine. Only mutants that able to restore of this function occurred are able to form colonies on such plates. Usually, the plates containing the tested compound and tester bacteria are incubated for 48 hours and bacterial colonies are counted [5-7]. Vegetables and fruits are excessively treated with pesticides and ripening chemicals during farming and processing. Pesticides and ripening chemicals deeply adsorbed on the surface of vegetables and fruits and can't drain out by simple washing methods. These chemicals entered in the cell through digestive tract. Some of these chemicals are cyclic compound and mimic the nitrogenous bases of DNA. During the replication these chemicals get introduced in to the synthesizing DNA and causes Cancer by different molecular mechanism [8]. Many studies have focused on the analysis of pesticides on fruits and vegetables but little is known about the carcinogenic potential of these pesticides and weather they are removed before cocking through simple washing practicing in the houses or not.

MATERIALS AND METHODS

Sample collection – Three types of routinely supplied vegetables and fruits brought from different districts of Chhattisgarh India-

1. By the market
2. From the farmers
3. Unwashed sample from the fields

Washing vegetables and fruits – The following washing techniques used for the present investigations:

1. Normal washing practicing at home- Normal washing procedure includes; 1 kg of sample washing in one litter of pure drinking water supplied at home. This washed-out water with chemical designated as Chemical-I.
2. Extreme washing with normal water in three times- 1 kg of sample washing in one litter of pure water with consistent stirring for 10 minutes. This washed-out water with chemical designated as Chemical-II.
3. Single washing with lukewarm water at 55^0C 1 kg of sample washing in one litter of pure lukewarm water with consistent stirring for 10 minutes. This washed-out water with chemical designated as Chemical-III.

Mutagenicity analysis

Carcinogenic potential of above extracts (chemical I, II and III) were analyzed by AMES test as following:

1. The experiments were performed with pre incubation method described by Mortelmans and Zeiger in 2000 [9] and guideline given in bioprotocols Urvashi Vijay in 2018 [10]. Before the experiment start, standard strains of S. typhimurium TA 98 were inoculated in nutrient broth oxoid no.-2 and incubate for 16 h at 37°C in an incubator at 120-140 rpm. Strain of S. typhimurium was grown separately in 10 ml Erlenmeyer flasks.
2. Mutagen was freshly prepared for each experiment. We used autoclaved distilled water as a negative control and Sodium azide (1 µg/ml) as positive controls for TA 98 without S9 metabolic activation (S9 mix) and for TA 98 with S9 metabolic activation (S9 mix).
3. We prepared minimal glucose agar (MGA) plates by mixing the medium of minimal glucose agar plates and poured 25 ml into each Petri dish. We prepared all the plates freshly before it uses.
4. Then taken 2 ml Eppendorfs tubed and all minimal glucose agar plates and labeled them before the experiment.
5. In each of the 2 ml sterile Eppendorf tubes, we added the following:
   a. 0.1 ml fresh culture of TA98 Salmonella strains.
   b. 0.2 ml of Histidine solution.
   c. 0.5 ml sodium phosphate buffer (without S9 mix) or 0.5 ml S9 (with S9 mix).
   d. Known amount of 20 µl, 40 µl, 60 µl, 80 µl and 100 µl/plate of test sample or 0.1 ml of positive or negative control.
   e. Added 1 ml autoclaved distilled water to make up to 1ml.
6. Mixed well the contents of Eppendorf tubes and poured them onto Petri plates and spread through L-shaped spreader on the surface of minimal glucose agar plates. Petri plates then covered with sterile aluminum foil to protect the testing sample from photo reactive substances.
7. After incubation of 48 h at 37°C, spontaneous revertants colonies appear and are clearly visible with unaided eyes.
8. Revertants formed like a uniform lawn of auxotrophic bacteria on the surface the background of the medium.

Data analysis

Statistical analysis - The data obtained from Ames test would be analyzed for significant differences between control and test sample by ANOVA, and student's t- test through Prism 3.0.

RESULTS AND DISCUSSION

Carcinogenic potential of chemical - 1

Data obtained from experiments on all the three chemicals were analyzed for- first, association between increasing concentration of chemical and carcinogenicity and second, association between sampling site and carcinogenicity. As shown in (Table 1), increasing concentration of chemical-1 of the sample from city market and from farmers was found insignificant differences for both S-9 negative and S9 positive samples, whereas sample directly from field, found significant differences in number of rivertent colony with increasing concentration of chemical-1. The differences in S-9 +ve as well as S-9 –ve revertant colonies observed for chemical-I for different concentrations among three different sites were found also significant ($P<0.0001$).

Obtained data were also analyzed to find out differences in rivertent colony between negative control and different concentrations of chemical-1 with student's t-test and the results are presented in (Table 2). This is study found that there are insignificant differences has been observed between negative control and chemical-1 (with and without S-9) obtained from city market as well as sample collected from farmers, whereas significant differences ($P<0.0001$) found in the sample directly from the fields, at concentration 20, 40, 60, 80 and 100 µl/plate. These observations indicated that carcinogenic substances are at high concentration on sample at the field.

Carcinogenic potential of chemical - II

The observations in (Table 3) showing that sample collected from the fields were found significant differences in revertant colonies at different concentration of chemical-II. These observations are as same as the observations come out from chemical-I. These observations indicate that the washing method -2 used in this study (this is also practicing in major of

houses in this region) is unable to extract more chemicals than simple washing.

Table 1 Showing results of carcinogenic potential of increasing concentration of Chemical I obtained samples of different sites

Dose (µl/plate)	Chemical -I	Sample collected from city market		Sample collected from farmers		Unwashed sample from the fields		ANOVA (One way)
		TA98		TA98		TA98		
		S9(-)	S9(+)	S9(-)	S9(+)	S9(-)	S9(+)	
Negative control		32.42 ± 5.86	37.36 ± 5.32	42.11 ± 7.22	40.36 ± 5.43	32.42 ± 5.86	38.26 ± 5.71	Insignificant
20		32.92 ± 4.32	37.82 ± 3.11	43.54 ± 5.51	42.82 ± 4.31	66.45 ± 4.19	62.96 ± 4.49	P = 0.0030 for S9(-) P = 0.0030 for S9(+)
40		34.84 ± 4.33	37.44 ± 4.11	37.69 ± 4.48	33.34 ± 4.74	70.41 ± 4.23	73.74 ± 4.83	P = 0.0004 S9(-) P = 0.0004 S9(+)
60		32.45 ± 4.46	35.70 ± 4.48	33.73± 4.46	38.61± 4.64	89.61± 5.98	95.59± 5.96	P = 0.0028 for S9(-) P<0.0001) for S9 (+)
80		34.11 ± 3.12	33.59± 4.71	34.38± 4.27	34.11±4.67	89.34± 5.39	80.47±5.55	P<0.0001 S9(-) P<0.0001 for S9 (+)
100		38.39 ± 4.91	41.45± 4.12	44.45± 5.37	42.45± 5.19	99.15± 5.91	97.45± 5.71	P<0.0001 S9(-) P<0.0001 for S9 (+)
ANOVA		NS	NS	NS	NS	P<0.0001	P<0.0001	
Positive control		425.67±32.86	442.57±35.16	429.82± 31.49	419.28± 30.66	429.52± 31.48	419.28±30.38	Insignificant

The numbers indicate the means and standards deviation values
Without (−) and with (+) S9 microsomal fraction of homogenized rat liver
Negative control: phosphate buffer
Positive control: TA98, Sodium azide
Significantly different from the corresponding negative control values (ANOVA test, p < 0.05

Table 2 Showing results of t-test analysis of Chemical I obtained samples of different sites

Dose (µl/plate)	Chemical -I	Sample collected from city market				Sample collected from farmers				Unwashed sample from the fields			
		TA98				TA98				TA98			
		S9(-)	t-test	S9(+)	t-test	S9(-)	t-test	S9(+)	t-test	S9(-)	t-test	S9(+)	t-test
Negative control		32.42 ± 5.86	NS	37.36 ± 5.32	NS	42.11 ± 7.22	NS	40.36 ± 5.43	NS	32.42 ± 5.86		38.26 ± 5.71	
20		32.92 ± 4.32	NS	37.82 ± 3.11	NS	43.54 ± 5.51	NS	42.82 ± 4.31	NS	66.45 ± 4.19	P=0.0002	62.96 ± 4.49	P<0.0001
40		34.84 ± 4.33	NS	37.44 ± 4.11	NS	37.69 ± 4.48	NS	33.34 ± 4.74	NS	70.41 ± 4.23	P<0.0001	73.74 ± 4.83	P<0.0001
60		32.45 ± 4.46	NS	35.70 ± 4.48	NS	33.73± 4.46	NS	38.61± 4.64	NS	89.61± 5.98	P<0.0001	95.59± 5.96	P<0.0001
80		34.11 ± 3.12	NS	33.59± 4.71	NS	34.38± 4.27	NS	34.11±4.67	NS	89.34± 5.39	P<0.0001	80.47±5.55	P<0.0001
100		38.39 ± 4.91	NS	41.45± 4.12	NS	44.45± 5.37	NS	42.45± 5.19	NS	99.15± 5.91	P<0.0001	97.45± 5.71	P<0.0001
Positive control		425.67 ± 32.86		442.57± 35.16		429.82 ± 31.49		419.28± 30.66		429.52± 31.48		419.28± 30.38	

The numbers indicate the means and standards deviation values
Without (−) and with (+) S9 microsomal fraction of homogenized rat liver
Negative control: phosphate buffer
Positive control: TA98, Sodium azide
Significantly different from the corresponding negative control values (ANOVA test, p < 0.05

Carcinogenic potential of chemical - III

To analyze carcinogenic potential of increasing concentration of chemical-III we applied Anova test to find significant differences in number of revertant colonies and the results are presented in (Table 5). This study found significant differences in number of revertent colonies at different concentration of chemical III in samples from city markets and from formers. This study also observed that increasing concentration of chemical-III increases number of revertant colonies which are significantly different at different concentration at level of P<0.0001 in all three sampling sites with and without presence of S9. These results are showing that similar samples which are not showing any carcinogenic potential drained as chemical -I and II are showing carcinogenic potential when drained as chemical –III. Results of table-5 also showing significant difference among revertant colonies of three different sample sides at the concentration level of 20, 40, 60, 80 and 100 µl/plate is showing significant differences at P<0.0001. When vegetables and fruits are washed with method III, chemical III is obtained. Different concentration (20, 40, 60, 80 and 100 µl/plate) of this chemical III is analyzed for carcinogenic potential against

negative control with student's t-test (Table 6). This study found that chemical III is showing carcinogenic potential at concentration of 20, 40, 60, 80 and 100 µl/plate for samples collected directly from fields whereas samples collected from city markets and formers showing carcinogenic potential at concentration of 60, 80 and 100 µl/plate.

Table 3 Showing results of ANOVA analysis of Chemical II obtained samples of different sites

Dose (µl/plate)		Sample collected from city market		Sample collected from farmers		Unwashed sample from the fields		ANOVA (One way)
		S9(-)	S9(+)	S9(-)	S9(+)	S9(-)	S9(+)	
Negative control	Chemical II	32.42 ± 5.86	37.36 ± 5.32	42.11 ± 7.22	40.36 ± 5.43	32.42 ± 5.86	38.26 ± 5.71	
20		33.62 ± 4.12	37.32 ± 3.61	43.66 ± 5.53	40.74 ± 3.99	59.12 ± 4.11	60.94 ± 4.61	P = 0.0002 for S9 (-) P<0.0001 for S9 (+)
40		34.13 ± 4.55	35.29 ± 4.28	36.96 ± 5.03	37.40 ± 4.70	78.20 ± 4.31	79.29 ± 5.12	P<0.0001 for S9(-) P<0.0001 for S9 (+)
60		32.45 ± 4.46	35.70 ± 4.48	33.73± 4.46	37.01± 4.29	85.39 ± 5.44	80.19 ± 4.49	P<0.0001 for S9(-) P<0.0001 for S9 (+)
80		34.11 ± 3.12	33.59± 4.71	34.18± 4.36	38.39±4.39	91.34 ± 5.25	80.47 ± 5.55	P<0.0001 for S9(-) P<0.0001 for S9 (+)
100		48.39 ± 4.91	41.45± 4.12	43.34± 5.18	41.37± 5.38	97.15 ± 5.51	96.38 ± 5.28	P<0.0001 for S9(-) P<0.0001 for S9 (+)
ANOVA		NS	NS	NS	NS	P<0.0001	P<0.0001	
Positive control		425.67 ± 32.86	442.57± 35.16	429.82± 31.49	419.28± 30.66	429.52± 31.48	419.28 ± 30.38	

The numbers indicate the means and standards deviation values.
Without (−) and with (+) S9 microsomal fraction of homogenized rat liver
Negative control: phosphate buffer.
Positive control: TA98, Sodium azide
Significantly different from the corresponding negative control values (ANOVA test, $p < 0.05$

Table 4 Showing results t- test of carcinogenic potential of increasing concentration of Chemical II obtained samples of different sites

Dose (µl/plate)	Chemical - II	Sample collected from city market				Sample collected from farmers				Unwashed sample from the fields			
		TA98				TA98				TA98			
		S9(-)	t-test	S9(+)	t-test	S9(-)	t-test	S9(+)	t-test	S9(-)	t-test	S9(+)	t-test
Negative control		32.42 ± 5.86	NS	37.36 ± 5.32	NS	42.11 ± 7.22	NS	40.36 ± 5.43	NS	32.42 ± 5.86		38.26 ± 5.71	
20		33.62 ± 4.12	NS	37.32 ± 3.61	NS	43.66 ± 5.53	NS	40.74 ± 3.99	NS	59.12 ± 4.11	P=0.0006	60.94 ± 4.61	P=0.0001
40		34.13 ± 4.55	NS	35.29 ± 4.28	NS	36.96 ± 5.03	NS	37.40 ± 4.70	NS	78.20 ± 4.31	P<0.0001	79.29 ± 5.12	P<0.0001
60		32.45 ± 4.46	NS	35.70 ± 4.48	NS	33.73± 4.46	NS	37.01± 4.29	NS	85.39 ± 5.44	P<0.0001	80.19 ± 4.49	P<0.0001
80		34.11 ± 3.12	NS	33.59± 4.71	NS	34.18± 4.36	NS	38.39±4.39	NS	91.34 ± 5.25	P<0.0001	80.47 ± 5.55	P<0.0001
100		48.39 ± 4.91	P=0.0077	41.45± 4.12	NS	43.34± 5.18	NS	41.37± 5.38	NS	97.15 ± 5.51	P<0.0001	96.38 ± 5.28	P<0.0001
Positive control		425.67 ± 32.86		442.57± 35.16		429.82 ± 31.49		419.28± 30.66		429.52± 31.48		419.28 ± 30.38	

The numbers indicate the means and standards deviation values
Without (−) and with (+) S9 microsomal fraction of homogenized rat liver
Negative control: phosphate buffer
Positive control: TA98, Sodium azide
Significantly different from the corresponding negative control values (ANOVA test, $p < 0.05$

Pesticides are excessively used for crop production and preservation. Industries are also tried to increase durability of pesticides on the surface of vegetables and fruits, which increases the chances of entry of pesticides to our food table. The pesticides which were used to drain out from simple wash now they are remain on the surface of vegetables and don't drain out without extensive wash and may be one of the major causes of increasing cancer cases in Chhattisgarh India. Although the region Chhattisgarh has lower the average of cancer patients than the national average of the India [3] but increasing incidences in cases of the breast cancer and cancers associated with digestive tract is at alarming condition and emphasizes to pay attention to find out and reduce the causes of cancer.

In the present study the observations are showing that there are some carcinogenic substances in different amount are present on vegetables and fruits at three different sites. This study observed that the same sample from same sampling site showing carcinogenic potential through chemical III but not through chemical I and II. It means that surface adsorbed

hazardous chemicals remain on the surface and don't extract without extensive and proper washing. Hazardous chemical which adsorbed on surface may be carcinogenic, especially when eaten without cooking like coriander, chilies, reddish, etc. A Report from Hidustan Times (Delhi) 20014 stated that the High Court had acted on a report presented by NGO Consumer, which had found that 35 different varieties of fruits and vegetables, selected from Delhi markets and analyzed for pesticide content, had toxins exceeded the permissible limits [11].

Table 5 Showing results ANOVA test of carcinogenic potential of increasing concentration of Chemical III obtained samples of different sites

Dose (µl/plate)		Sample collected from city market		Sample collected from farmers		Unwashed sample from the fields		ANOVA (One way)
		S9(-)	S9(+)	S9(-)	S9(+)	S9(-)	S9(+)	
Negative control	Chemical III	32.42 ± 5.86	37.36 ± 5.32	42.11 ± 7.22	40.36 ± 5.43	32.42 ± 5.86	38.26 ± 5.71	
20		32.92 ± 4.32	37.82 ± 3.11	43.54 ± 6.51	42.82 ± 5.31	96.45 ± 6.19	92.96 ± 6.49	P<0.0001 S9(-) P<0.0001 S9 (+)
40		34.84 ± 6.33	37.44 ± 6.11	57.69 ± 7.48	53.34 ± 6.74	190.41 ± 7.23	183.74 ± 7.83	P<0.0001 S9(-) P<0.0001 S9 (+)
60		102.45 ± 8.46	115.70 ± 8.48	103.73± 7.46	98.61± 8.64	189.61± 7.98	195.59± 8.96	P<0.0001 S9(-) P<0.0001 S9 (+)
80		144.11±11.12	163.59± 12.71	134.38±11.27	154.11±12.67	289.34± 11.39	280.47±12.55	P<0.0001 S9(-) P<0.0001 S9 (+)
100		198.39±13.91	201.45± 12.12	194.45±13.37	182.45±12.19	350.15± 13.91	347.45± 12.71	P<0.0001 S9(-) P<0.0001 S9 (+)
ANOVA		P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	
Positive control		425.67±32.86	442.57±35.16	429.82±31.49	419.28±30.66	429.52±31.48	419.28±30.38	

The numbers indicate the means and standards deviation values.
Without (−) and with (+) S9 microsomal fraction of homogenized rat liver
Negative control: phosphate buffer.
Positive control: TA98, Sodium azide
Significantly different from the corresponding negative control values (ANOVA test, p < 0.05

Table 6 Results of t-test analysis of Chemical III

Dose (µl/plate)	Chemical - III	Sample collected from city market				Sample collected from farmers				Unwashed sample from the fields			
		TA98				TA98				TA98			
		S9(-)	t-test	S9(+)	t-test	S9(-)	t-test	S9(+)	t-test	S9(-)	t-test	S9(+)	t-test
Negative control		32.42 ± 5.86		37.36 ± 5.32		42.11 ± 7.22		40.36 ± 5.43		32.42 ± 5.86		38.26 ± 5.71	
20		32.92 ± 4.32	NS	37.82 ± 3.11	NS	43.54 ± 6.51	NS	42.82 ± 5.31	NS	96.45 ± 6.19	P<0.0001	92.96 ± 6.49	P<0.0001
40		34.84 ± 6.33	NS	37.44 ± 6.11	NS	57.69 ± 7.48	NS	53.34 ± 6.74	NS	190.41 ± 7.23	P<0.0001	183.74 ± 7.83	P<0.0001
60		102.45 ± 8.46	P<0.0001	115.70 ± 8.48	P<0.0001	103.73 ± 7.46	P<0.0001	98.61± 8.64	P<0.0001	189.61± 7.98	P<0.0001	195.59± 8.96	P<0.0001
80		144.11 ± 11.12	P<0.0001	163.59± 12.71	P<0.0001	134.38 ± 11.27	P<0.0001	154.11±12.67	P<0.0001	289.34± 11.39	P<0.0001	280.47±12.55	P<0.0001
100		198.39 ± 13.91	P<0.0001	201.45± 12.12	P<0.0001	194.45 ± 13.37	P<0.0001	182.45± 12.19	P<0.0001	350.15± 13.91	P<0.0001	347.45± 12.71	P<0.0001
Positive control		425.67 ± 32.86		442.57± 35.16		429.82 ± 31.49		419.28± 30.66		429.52± 31.48		419.28± 30.38	

The numbers indicate the means and standards deviation values
Without (−) and with (+) S9 microsomal fraction of homogenized rat liver
Negative control: phosphate buffer
Positive control: TA98, Sodium azide
Significantly different from the corresponding negative control values (ANOVA test, p < 0.05

Another study from Malwa region of Panjab India has fund that the high use of pesticides, along with social factors and environmental, is responsible for the high concentration of pesticide residues in the food chain of this region. There are many banned and restricted pesticides are also in practice in this region [12]. A similar study from metro city Hyderabad also found exposure of urban populations to different classes of fenitrothion, acephate, organophosphate, and phosalone pesticides due to the consumption of different types of fruits and vegetables. The study found that there are eighteen fenitrothion phosalone, organophosphate and acephate, pesticides found in vegetable samples (tomato, ladyfinger, cabbage, eggplant, cauliflower and chili) at concentration of more than the permissible limit [13].

A similar study from Uttar Pradesh revealed that a total of 244 samples of different cereals (rice, wheat flour, and maize), pulses (moong, arhar, gram, black gram and lentil), spices (black pepper, chili, coriander, and turmeric), vegetables (cabbage, brinjal, potato, onion, tomato and spinach,) fruits (guava, apple, mango, and grape), Deshi ghee, milk, butter, and edible oils (groundnut, vegetable, mustard, and sesame) was tested for the presence of organochlorine pesticide residues. The levels of 2,2-bls(p-chlorophenyl)-1,1,1-trlchloroethane residues and hexachlorocyclohexane (HCH) detected high in oil, wheat flour, and fat samples analyzed [14]. Another investigation from National Capital Region (NCR), was found that most of the organochlorine pesticides residues on vegetable samples recorded in these studies exceeded the maximum residue levels set by international and national regulatory agencies [15]. Another study from Patna (Bihar) found increased level of DDT and Endosulfan than the permissible limit [16].

CONCLUSION

The present Study shows a simple wash can drain only loosely bound pesticides and not able to able to drain complete amount of pesticides. When same sample washed extensively more pesticide come out in the water and shows carcinogenicity in the experiment. Because when we again wash the same sample with simple washing method it shows no carcinogenicity in majority of samples (not all). Samples collected from city market and direct from the farmers are also shows carcinogenicity in the extensive wash not in simple wash.

Limitations

Ames assay consists of Salmonella typhimurium strains then it's not an ideal model for human. Mice liver S9 hepatic fraction is employed to attenuate the mammalian metabolic activations formed within the hepatic system in order that the mutagenicity of metabolites are often assessed. There are several differences between human and mice metabolism which may affect the mutagenicity of testing substances. Major disadvantages of fluctuation test is slower and slightly more laborious than Ames protocol. The test is primarily used for testing aqueous samples containing low levels of mutagen and thus, this test is well adapted for evaluating the mutagenicity of wastewater samples. After cocking pesticides may destroy hence their carcinogenicity may be challenged.

Declaration of conflicting interests

The authors declared there are no conflicts of interest with respect to the authorship, research, and/or publication of this article.

Funding

This study was supported with a grant from the Central Regional Office Bhopal (CRO) of University grant commission (UGC) in the year 2017-19 under the Minor research project scheme.

Acknowledgments

Authors would like to thank the Department of Biochemistry, Govt. Nagarjuna P.G. College of Science where the work was carried out.

LITERATURE CITED

1. Anonymous. 2019. Word Health Report 2019.
2. Thakur H, Kawale SK, Dhruw V, Singh L. 2018. Morbidity and mortality trend and pattern of cancer in newly started cancer unit of tertiary care hospital in Bilaspur, Chhattisgarh. *International Journal of Medical Science and Public Health* 7(8): 238-242.
3. Sinha AL, Jain RR, Pradhan SK. 2018. Epidemiological trend of cancer among patients at regional cancer center, Dr. B. R. Ambedkar Memorial Hospital, Raipur: A Tertiary Care Hospital of Central India. *International Journal of Health Sciences and Research* 8(2): 53-59.
4. Anonymous. 2016. The burden of cancers and their variations across the states of India: The Global Burden of Disease Study 1990–2016. *Lancet Oncol* 2018 (Supplementary appendix). pp 1-54.
5. Ames BN. 1972. Chemical Mutagens, Principles and Methods for Their Detection, *Springer publishers*. 1:851-863.
6. Ames BN, McCann J, Yamasaki E. 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutat Research* 31(6): 347-364.
7. Barnes W, Tuley E, Eisenstadt E. 1982. Base sequence analysis of His+ revertants of the hisG46 missense mutations in Salmonella typhimurium. *Enviorn Mutagen* 4: 297-302.
8. Claudia B, Morasso G. 2000. Genotoxicity of pesticides: potential risk for consumers. *Trends in Food Science ans Technology* 11(4/5): 182-187.
9. Mortelmans K, Zeiger E. 2000. The Ames Salmonella/microsome mutagenicity assay. *Mutat Research* 455(1/2): 29-60.
10. Vijay U, Gupta S, Mathur P, Suravajhala P, Bhatnagar P. 2018. Microbial Mutagenicity Assay: Ames Test. *Bio Protocol* 8(6): 1-15.
11. Singh SR. 2014. Hindustan Times; Delhi, Feb 06, 2014.
12. Mittal S, Kaur G, Vishwakarma GS. 2014. Effects of environmental pesticides on the health of rural communities in the Malwa region of Punjab, India: A review. *Human and Ecological Risk Assessment. An International Journal* 20 (2): 366-387.
13. Sinha SN, Raob VV, Vasudeva K. 2012. Distribution of pesticides in different commonly used vegetables from Hyderabad, India. *Food Research International* 45(1): 161-169.
14. Kaphalia BS, Takroo R, Mehrotra S, Nigam U, Seth TD. 2020. Organochlorine pesticide residues in different Indian cereals, pulses, spices, vegetables, fruits, milk, butter, deshi ghee, and edible oils. *Journal of Association of Official Analytical Chemists* 73(4): 509-512.
15. Chourasiya, Khillare PS, Jyethi DS. 2015. Health risk assessment of organochlorine pesticide exposure through dietary intake of vegetables grown in the periurban sites of Delhi, India. *Environmental Science and Pollution Research* 22: 5793-5806.
16. Nath A, Vendan AE, Priyanka, Singh JK, Singh CK, Kumar S. 2013. Carcinogenic pesticides residue detection in cow milk and water samples from Patna, India. *Current Trends in Biotechnology and Chemical Research* 3(1): 2249-407.