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MUTAGENICITY ASSESSMENT OF DAMSISSA (*AMBROSIA MARITIMA, L.*) AS MEDICINAL HERB USING THREE DIFFERENT BIOLOGICAL SYSTEMS.

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ABSTRACT: The mutagenic activity of damsissa (*Ambrosia maritime, L.*), a medicinal herb that has been used as a medication in many developing countries, was assessed. The assessment carried out through three model systems, a bacterial model (prophage induction and transduction assays), a plant model (*Allium cepa*, L. root tips assay) and finally a mammalian model (rat bone marrow assay). The results of the three models proved that this plant has a weak mutagenic activity, but not completely null.

Moreover, the plant seemed to have antioxidant response because the results showed that it has an anti-mutagenic activity against a powerful mutagen agent.

Key words: **Damsissa, mutagenicity, onion, rat, bacteria, assays.**

INTRODUCTION

The use of medicinal plants by the general population is probably as old as humankind and still widespread practice. These plants contain therapeutic substances which can be extracted and can be used in the preparation of drugs. Alternatively, the plant itself can be employed directly as a medication, a practice that is particularly popular in developing countries (Pabon *et al.*, 2003; Riad, 2002 and Souza *et al.*, 2004). A wide variety of medicinal plants are used in Egypt for treating many diseases. One of these plants is the herb of *Ambrosia martima*, L. (*Damsissa*), family compositae, that is widely grown in Egypt (Tackholm, 1974). It is a gray hairy herb with finely dissected, fragrant leaves found on muddy canal banks. Picman *et al.*, 1986 isolated and identified the

sesquiterpene lactone hymenin from the ethanolic extract of *A.maritima, L.* Two, known sesquiterpene lactones, damsin and ambrosin, are also isolated and characterized. Damsissa has a remdy effect for haematuria in *Schistosoma* infection (Kloss and McCullough, 1981); anti-tumor activity (Abdalla *et al.*, 1991); anti-diabetic effect (Al-Okbi *et al.*, 1993; El-Shabrawy and Nada, 1996); using in treatment of gastrointestinal disturbances (Bakhiet and Adam, 1996) and having a hepatoprotective and anti-oxidant effect (Mohamed *et al.*, 2001). *A.maritima, L.* is reported to be the most effective plant having a molluscicidal activity against snails of *Schistosoma spp* (Sherif and El-Sawy, 1962 and Abdel Hamied, 1997). One of the important characteristics of *A.maritima, L.* is its virtual lack of toxicity to organisms other than snail such as cattle, sheep, fish and apparently to man (Sherif and El-Sawy, 1962 and Alard *et al.*, 1991) and it is virtually non toxic to non-target organisms such as rats, rabbits and algae (Geerts *et al.*, 1992).

So, the aim of this study is to evaluate the mutagenic activity of *A.maritima, L.* extract using a battery of three model systems including, bacterial assay, plant assay *(Allium cepa, L.* root tip cells assay) and mammalian assay (rat bone marrow assay).

MATERIALS AND METHODS

The experiment was carried out at Microbial Genetics Lab, Cytology Lab and Animal Lab, Fac. Agric. Zagazig Univ. during 2002- 2004.

1. Damsissa Extract Preparation

A 50 gm of dried whole herbs was added to 60% ethyl alcohol, set up for 2-3 days at room temperature, then filtered. The dried whole herb retreated again using 60 % ethyl alcohol, then filtered and evaporated using a rotary evaporate. The solvent Dimethyl sulfoxide (DMS) added to the dried material in 0.25 % to obtained the aqueous extract of *Ambrosia maritime, L.*

2. Bacteriophage and Bacterial Strains

In this study, two generalized Bacteriophages were used, F_{116} (Holloway *et al.*, 1961) and AMS phage which has been isolated through our Lab. The two phages propagated on *Pseudomonas aeruginosa* strains. *Pseudomonas aeruginosa* bacterial strains PU 21, PU 17 and MAM_2 that have been

used in this study were kindly obtained from M. Day, UWIST University, Wales, UK. PU 21 is auxotrophic and carrying streptomycin resistance gene, the PU 17 is also auxotrophic and lysogen, PU 21 F_{116} which has been isolated in our lab and MAM2 is also auxotrophic and sensitive to streptomycin.

3. Growth Media

Nutrient agar (NA) and nutrient broth (NB) media were prepared according to manufacture's instructions. Soft agar (0.8 % w/v agar) was prepared in distilled water and kept at 45 C^{o} on water bath. The stryptomycin was added as sterilized solution by filtration through 0.2 µm filter membrane to the media after autoclaving at a concentration of 12 mg/ml.

4. Treatment of *A.maritima, L.* Extract on Prophage Induction

The liquid culture of lysogenic strain (PU 17) was exposed to eight concentrations (0.00, 0.25, 0.50, 2.00, 6.00, 8.00, 10.00, 15.00 %) of *A. maritima* alcoholic extract. After exposure, the survival of PU 17 cells and phage F_{116} particles were assayed, and then a few drops of chlorophorm were added to cultures and centrifugated at 5000 rpm for 30 min. The supernatant was assayed by overlay method of Adams, 1959.

5.Transduction Frequency Assay

Recipient cells (MAM 2) were grown in NB media overnight, then washed 2-3 times by phosphate buffer (pH 7.0) and resuspended. Viable count of the recipient strain was calculated. Equal volumes (1 ml) of phage lysate and recipient cell suspension were mixed and kept for 15-30 min at room temperature, to allow phage adsorption. Serial dilutions were prepared and placed on selective media (NA+streptomycin). Number of transductants were recorded and transduction frequency per recipient was calculated.

6. Anti- mutagenic Activity of *A. maritime, L.* Against Ethylene imine

Ethylene imine was added as sterilized solution by filteration through 0.2 µm filter membrane to a nutrient broth media having 15% of the plant extarct. A serial concentrations of the powerful mutagenic agent were prepared and sterilized through filteration and 15 % of the plant extract was added to each concentration. This mixture was used to detect the

anti-mutagenic activity of *A. maritime, L.* against ethylene imine in prophage F_{116} induction and transduction assays. Data were statistically analyzed using standard deviation.

7. *Allium cepa, L.* Plant Assay

1.0 gm of onion (*Allium cepa* L), variety Giza 2, seeds was germinated in petri-dishes at room temperature. When roots reached 2.-3 cm long they were treated with 10 ml of damsissa alcohol extract solution for 6, 12 and 24 hrs. Root tips of about 0.5 to 1 cm were taken and fixed in a Farmer's solution and then stained with aceto-carmine. Ten prepared slides were examined for each treatment and control to determine mitotic index (MI) and frequencies of cellular division abnormalities.

8. Rat Bone Marrow Assay (Mammalian assay)

The rat bone marrow of the albino rats (*Rattus norvigeicus, L.*) of agouza strain weighting from 100 – 120 gm were used in this study. The rats were obtained from the Animals Lab, Department of Plant Protection, Faculty of Agriculture, Zagazig University, under the supervision of Prof. R. Sherif. Damsissia extract was assayed in rat bone marrow using the widely applied method described by Yosida and Amano, 1965. The dose of 2 mg / kg body weight of the extract was orally chosen. This dose was calculated as 1/40 of the $L.D_{50}$ of the extract as described by Bliss, 1962. The dose was orally administered by using a stomach tube. The extract didn't result any apparent symptoms in the behaviour or the survival percentage of the treated animals.

The effect of the extract was studied at the intervals of 24,72 hr and 7 days after administration. A total number of 24 rats was used in this study. About 150 mitotically divided cells were examined for each rat for evaluating the mitogenic and mutagenic activities. The data of plant and bone marrow assay systems were statistically analyzed using Chi-square test by means of 2 x 2 contingency table.

RESULTS AND DISCUSSION

1. Mutagenic Activity of *A. maritimia,L.* Extract Using Microbial Models

1.1. Prophage induction assay

In order to estimate the mutagenic activity of the medicinal plant, *A.maritima*, L. the induction of two prophages, F_{116} and AMS

has been assessed. Data are shown in Tables 1 and2. The obtained results showed increase in Plaque Forming Unit (Pfu/ml) (from 1.31 up to 1.87 x 10^5 as a result of application the concentrations from 0.00 up to 15.00 % respectively) in prophage F_{116} Table 1 and from 1.45 up to 1.97 x 10^5 following using the same concentrations for prophage AMS Table 2, but this didn't reach the mutagenicity level.

Table 1: Prophage F116 induction from lysogenic strain Pu17 (Pu21 F116) by extract of *A. maritime, L.*

Concentration %	Pfu / ml. 10^5	Fold increase	*Induced phage	Mutagenic responses
0.00	1.31±0.07	0.00	0.00	-
0.25	1.39±0.06	1.06	0.08	-
0.50	1.41±0.05	1.08	0.10	-
2.00	1.56±0.04	1.19	0.25	-
6.00	1.62±0.03	1.24	0.31	-
8.00	1.69±0.02	1.29	0.38	-
10.00	1.74±0.05	1.33	0.43	-
15.00	1.87±0.07	1.43	0.56	-

- Negative mutagenic responses.

* Induced phage = Pfu / ml of treatment – Pfu/ml of control

Table 2: Prophage AMS induction from lysogenic strain L3 (Pu21 AMS2000) by extract of *A. maritime, L.*

Concentration. %	pfu/ml. 10^5	Fold increase	*Induced phage	Mutagenic responses
0.00	1.45±0.1	0.00	0.00	-
0.25	1.51±0.2	1.04	0.06	-
0.50	1.59±0.03	1.10	0.14	-
2.00	1.61±0.07	1.11	0.16	-
6.00	1.69±0.04	1.17	0.24	-
8.00	1.78±0.02	1.23	0.33	-
10.00	1.88±0.05	1.30	0.43	-
15.00	1.97±0.04	1.36	0.52	-

- Negative mutagenic responses.

* Induced phage = Pfu / ml of treatment – Pfu/ml of control

It seems that up to 15 % of the plant extract, no mutagenic activity was observed. However, there are many bacterial assays for detecting the mutagenicity of compounds (Ames *et al.*, 1975; Cornwell *et al.*, 2002; Horn and Vargas, 2003; Pabon *et al.*, 2003) but the prophage induction assay is a rapid sensitive and inexpensive assay (Rossman *et al.*, 1984). The genetic end point measured, prophage induction, is a very broad one and does not depend on a particular type of DNA damage (D'Ari, 1985). Agents that damage DNA in bacteria induce a response known as the SOS system. One of the SOS function, the induction of lysogenic phages has been suggested as bioassay for genetic damage (Heinemann, 1971; Elespuru and Yarmolinsky, 1979).

1.2. Transduction assay

Transducing the streptomycin resistance gene was performed to assess the mutagenicity of *A.maritima, L.* extract (Table 3). Number of transductants and subsequently transduction frequency were not significantly increased upon exposure to concentrations of *A.maritima, L.* up to 15%. Transduction frequency at 15 % reached 2.32 x 10^{-6} with fold increase than those observed in control only up to 1.06.

Table 3:Effect of *A. maritime, L.* extract on transducing streptomycin resistance gene

Concentration %	No. of transductants ♂ x 10^{6}	Fold increase	♂ / ♀ x 10^{-6}
0.0	5.87±0.9	0.00	2.19±0.03
2.0	5.89±0.8	1.00	2.20±0.01
6.0	5.93±0.9	1.01	2.21±0.05
8.0	6.02±0.8	1.03	2.25±0.07
10.0	6.11±0.9	1.04	2.28±0.05
15.0	6.22±0.7	1.06	2.32±0.07

- MAM2 strain ($2.68X10^{12}$ cfu/ml) was used as recipient.
♀ = Recipient cells, MAM2 strain ($2.68X10^{12}$ cfu/ml), ♂ = Transductant cell

These results appear to support the finding of prophage induction assay in the conclusion that, this medicinal plant had no mutagenic activity in the range of concentrations that are used in this study.

The mutagenic agent, ethylene imine was used in this investigation as a positive control either in prophage F_{116} induction Table 4 or in transducing streptomycin resistance gene Table 5. It is obvious from these results that the two mechanisms were seriously influenced by ethylene imine when comparing with *A.maritima,L.* extract. A dramatically increased was observed either in prophage (fold increase reached 15.0) or in transduction frequency (fold increase reached 5.68).

Table 4: Ethylene imine activity on prophage F116 induction (positive control)

Concentration %	pfu/ml. 10^5	*Induced phage	Fold increase	Mutagenic response
0.0	4.1±0.07	0.0	0.0	-
2.0	15.7±0.1	11.6	4.0	+
6.0	25.5±0.3	21.4	6.0	+
8.0	26.9±0.4	22.8	7.0	+
10.0	44.8±0.5	40.7	11.0	+
15.0	60.9±0.6	56.8	15.0	+

- and + negative and positive responses, respectively.
* Induced phage = Pfu / ml of treatment – Pfu/ml of control

Table 5: Ethylene imine activity on transduction mechanism (positive control)

Concentration %	No. of transductants ♂ x 10^6	Fold increase	♂ / ♀ x10^{-6}
0.0	2.99±0.07	0.00	1.12±0.03
2.0	4.49±0.3	1.50	3.54±0.07
6.0	11.95±0.8	3.99	4.46±0.2
8.0	12.75±0.9	4.26	4.76±0.3
10.0	14.89±0.8	4.98	5.56±0.6
15.0	16.98±0.7	5.68	6.34±0.7

♀ = Recipient cells, ♂ = Transductant cell

In order to detect the anti-mutagenic activity of the *A.maritima, L.* plant, the maximum concentration (15%) of the extract was used against the same concentration of ethylene imine. Data in Table 6 show that 15 % of *A.maritima, L.* was able to reduce the mutagenic activity of ethylene imine in prophage F_{116} induction. The fold increase was only 1.43 instead of 15.0. Number of transducants was not greatly increased also with fold of increase of about 1.16 instead of 5.68 Table 7.

Table 6: Anti-mutagenic activity of 15% of *A. maritime, L.* extract against ethylene imine in prophage F116 induction

Concentration %	pfu/ml. 10^5	Fold increase	*Induced phage	Mutagenic response
0.0	2.91±0.03	0.00	0.00	-
2.0	3.11±0.04	1.07	0.20	-
6.0	3.41±0.05	1.17	0.50	-
8.0	3.99±0.04	1.37	1.08	-
10.0	4.07±0.05	1.40	1.16	-
15.0	4.15±0.06	1.43	1.24	-

- Negative mutagenic responses.

* Induced phage = Pfu / ml of treatment – Pfu/ml of control

Table 7:Anti-mutagenic activity of 15% of *A.maritima, L.* against ethylene imine in transducing streptomycin resistance gene

Concentration %	No. of transductants ♂ x 10^6	Fold increase	♂ / ♀ x 10^{-6}
0.0	18.37±0.2	0.00	6.90±0.3
2.0	19.79±0.3	1.08	7.38±0.4
6.0	19.99±0.4	1.09	7.46±0.4
8.0	20.11±0.4	1.09	7.50±0.6
10.0	20.57±0.3	1.12	7.68±0.5
15.0	21.34±0.5	1.16	7.96±0.6

♀ = Recipient cells, ♂ = Transductant cell

These results suggested that the medicinal plant *A.maritima, L.* (Damsissa) had no mutagenic activity in the bacterial models and had a remarkable anti-mutagenic response. It seems that the plant might have some anti -oxidant properties. Especially, it has been reported that many medicinal plants exhibited inhibition of the mutagenicity of many potent mutagens or carcinogen agents in many microbial tests (Pannala and Rice, 2001; Tsai *et al.*, 2002).

2. Mutagenic Activity Using *A.cepa, L.* Plant System

The second model that has been chosen in this study to evaluate the mutagenicity of *A.maritima, L.* was the *A.cepa, L.* root tips assay. Generally, the mitotic index did not show any increasing upon using the plant extract than that in control experiment Table 8. The declined of the mitotic index value was dose dependent for 12 hrs. exposure time only since it was 12.77 , 12.71 and 12.59 as a result of application 2, 8 and 15 % of the plant extract, respectively. Mean-while following 6 hrs. exposure period, M.I decreased following 2 and 8 % of plant extract treatments, afterwards it increased as a result of the highest dose application but still less than in the control experiment. The same trend was detected following the highest exposure period application, i.e., 24 hrs. The frequency of mitotic phases was variable without constant trend among the different concentrations treatments.

The number of cells having micronuclei was higher in all concentrations during each treatment period than those observed in the control experiments (Table 9). The highest frequency (0.53) was observed at 15 % of plant extract with time of treatment reached 24 hrs. Regarding the total number and percentages of cells containing chromosomal aberrations, it was remarkable that no constant trend could be noticed as a dose or exposure period application. Following all exposure times, the percentages of cells containing chromosomal aberrations decreased following 2 % then it increased gradually higher than the control following 8 and 15 % plant extract applications. The percent of compact micronuclei increased by dose increasing at all of exposure time with exception of 8 % for 24 hrs. exposure time. On the other hand, the non-compact

Table 8: Mitotic index (M.I) and frequency of mitotic phases in *Allium cepa,L.* root tips growing in extract of *A.maritima, L.* medicinal herb

Soaking period (hr)	Concentration	No. of studied cells	No. of divided cells	Mitotic index	Frequency of mitotic phases		
					Prophase	Metaphase	Ana- telophase
6	Control	13876	2000	14.41	56.4	15.5	28.1
	2	11248	1198	10.65	62.77	13.52	23.7
	8	13726	1262	9.19	70.28	10.69	19.01
	15	12860	1518	11.80	59.61	13.83	26.54
12	Control	15375	2255	14.67	51.49	20.31	28.20
	2	13333	1703	12.77	71.93	11.63	16.44
	8	12442	1582	12.71	63.72	13.53	22.76
	15	12865	1620	12.59	72.84	10.80	16.36
24	Control	11766	1551	13.18	72.08	8.89	19.01
	2	12056	1306	10.83	60.10	11.17	28.71
	8	12423	1288	10.37	67.15	8.92	23.91
	15	13718	1515	11.04	64.42	10.04	19.54

Table 9: Mitotic abnormalities in *Allium cepa, L.* root tips growing in extract of *A.maritima, L.* medicinal herb

Soaking period (hr)	Concentration	No. of studied cells	No. of divided cells	Total no. of cells with micronuclei	Total no. of cells with chromosomal aberrations	Distribution of micronuclei types		Frequency of chromosomal aberration types			No. of binucleat cells
						Compact	Non - compact	Fragment	Lag	Bridge	
6	Control	13876	2000	38(0.27)	35(1.75)	22(0.15)	16(0.11)	10(0.5)	11(0.55)	14(0.7)	67(0.56)
	2	11248	1198	45(0.40)	17(1.41)	25(0.22)	20(0.18)	3(0.25)	4(0.33)	10(0.83)	73(0.73)
	8	13726	1262	50(0.36)	26(2.06)	38(0.28)	12(0.08)	6(0.48)	4(0.32)	16(1.26)	78(0.63)
	15	12860	1518	58(0.45)	33(2.17)	43(0.33)	15(0.12)	7(0.46)	8(0.53)	18(1.18)	85(0.75)
12	Control	15375	2255	41(0.27)	42(1.86)	29(0.19)	12(0.08)	14(0.62)	10(0.44)	18(0.79)	75(0.57)
	2	13333	1703	49(0.37)	23(1.35)	36(0.26)	13(0.11)	4(0.23)	4(0.23)	15(0.88)	80(0.69)
	8	12442	1582	63(0.51)	37(2.34)	47(0.38)	16(0.13)	3(0.19)	7(0.44)	27(1.71)	86(0.79)
	15	12865	1620	70(0.54)	46(2.84)	53(0.41)	17(0.13)	5(0.31)	6(0.37)	35(2.16)	97(0.86)
24	Control	11766	1551	36(0.31)	39(2.51)	22(0.19)	14(0.12)	14(0.90)	10(0.64)	15(0.97)	68(0.67)
	2	12056	1306	52(0.43)	32(2.4)	43(0.36)	9(0.07)	3(0.22)	9(0.68)	20(1.53)	84(0.78)
	8	12423	1288	68(0.55)	44(3.41)	44(0.35)	24(0.19)	6(0.46)	6(0.46)	32(2.48)	93(0.84)
	15	13718	1515	73(0.53)	48(3.17)	53(0.39)	20(0.15)	4(0.26)	3(0.19)	41(2.71)	108(0.89)

( ) = Percentage
Lag = Laggard chromosome
Binucleate cells calculated relative to total interphase cells.

Table 10: Chi-square values in comparison between the effects of *A.maritima, L.* medicinal herb within each soaking period on mitotic index (M.I), frequency of cells containing micronuclei and chromosomal abnormalities as well as binucleate cells

Type of comparison	X^2 values			
	MI	Micronuclei	Chromosomal aberration	Binucleate cells
		6 hrs. Soaking period		
Control % - 2 %	61.18**	2.97	0.49	2.22
Control % - 8 %	135.76**	1.75	0.38	0.37
Control % - 15 %	30.53**	5.8*	0.79	3.01
2 % - 8 %	12.09**	0.20	1.40	0.82
2 % - 15 %	6.34*	0.3	2.03	0.04
8 % - 15 %	39.11**	1.22	0.04	1.3
		12 hrs. Soaking period		
Control % - 2 %	16.34**	2.3	1.52	1.31
Control % - 8 %	16.71**	10.52**	1.01	4.26*
Control % - 15 %	19.34**	13.65**	3.87*	7.21**
2 % - 8 %	0.02	2.85	4.33*	1.75
2 % - 15 %	0.15	4.48*	8.68**	2.24
8 % - 15 %	0.06	0.18	0.75	0.02
		24 hrs. Soaking period		
Control % - 2 %	24.46**	2.51	0.02	0.94
Control % - 8 %	36.47**	8.15**	1.89	2.01
Control % - 15 %	21.42**	7.55**	1.19	3.38
2 % - 8 %	1.10	1.66	1.98	0.18
2 % - 15 %	0.12	1.33	1.25	0.72
8 % - 15 %	2.08	2.02	0.12	0.15

*, **: Significant at 0.05 and 0.01 probability levels, respectively.

Table 11: Chi-square values in comparison between the effects of *A.maritima, L.* medicinal herb between the same concentration of the different soaking period on mitotic index (M.I), frequency of cells containing micronuclei and chromosomal abnormalities as well as binucleate cells

Type of Comparison	X^2 values			
	MI	Micronuclei	Chromosomal aberration	Binucleate cells
		6/12 hrs. Soaking period		
Control % - Control %	0.28	0.02	0.07	0.00
2 % - 2 %	20.85**	0.17	0.02	0.11
8 % - 8 %	67.04**	3.04	0.09	2.26
15 % - 15 %	2.03	3.11	1.21	2.35
		6/24 hrs. Soaking period		
Control % - Control %	20.85**	0.17	0.02	0.11
2 % - 2 %	0.17	0.14	3.33	0.19
8 % - 8 %	8.39**	4.82**	4.16*	3.53
15 % - 15 %	3.01	0.87	2.75	1.31
		12/24 hrs. Soaking period		
Control % - Control %	9.19**	0.00	1.93	0.82
2 % - 2 %	17.99**	0.65	4.8*	0.66
8 % - 8 %	26.60**	0.18	2.84	0.12
15 % - 15 %	12.06**	0.02	0.27	0.04

*, **: Significant at 0.05 and 0.01 probability levels, respectively.

ones exhibited variable trend. The chromosomal aberrations that observed in this study were fragments, laggard, and bridges. Percent of fragments was lower than the control. A remarkable increasing in the number of binucaleate cells was recorded Table 9. Some of these chromosomal aberrations are shown in Fig.1. The statistical analysis of these results are shown in Tables 10 and 11.

This was correlated with the model of *Allium cepa* assay which has been used extensively as a tool for detecting the genotoxic activity of a wide range of medicinal plants (Grant, 1982; Williams and Omoh, 1996; Yajia *et al.*, 1999; Dovgaliuk *et al.*, 2001; Biscardi *et al.*, 2003 and Marcano *et al.*, 2004).

3. Mutagenic Activity Using the Rat Bone Marrow Model

The third model, bone marrow in rats was used in this study in order to assess the mutagenic activity of *A.maritima, L.* medicinal plant. The mitotic index was lower than spontaneous event at dose 2 % after 24 hr of treatment, although a small increase was obtained (8.33 up to 8.53 and 9.00) at higher dose, i.e. 8 and 15% respectively Table 12-a. However, no chromosomal aberrations have detected during 24 hrs. of treatment. The same observation of M.I. was occurred after exposure time of 72 hrs. Table 12-b on the other hand, some chromosomal aberrations were detected after 72 hrs. treatments. In addition, after 7 days treatment the mitotic index and percentages of chromatid aberrations value were increased gradually by dose increasing. Moreover, some chromosomal aberration were detected following 8 and 15 % treatments value has increased Table 12-c.

Some chromosomal aberrations including ring, dicenteric and sticky abnormalities were observed at high concentration, 8.00 and 15.00 % only. Some of these chromosomal abnormalities are shown in Figs. 2 - 7. The rat bone marrow assay has been applied to evaluate the toxicity and genotoxicity of various medicinal plants (Ramos *et al.*, 1998; Chen *et al.*, 2001, Ramos *et al.*, 2001; Chacon *et al.*, 2002; Kirkland and Marzin, 2003). In spite of the recommendation of Zeiger *et al.*, 1985 that a positive or negative result at any simple short- term test is regarded as sufficient, Nagabhusham and

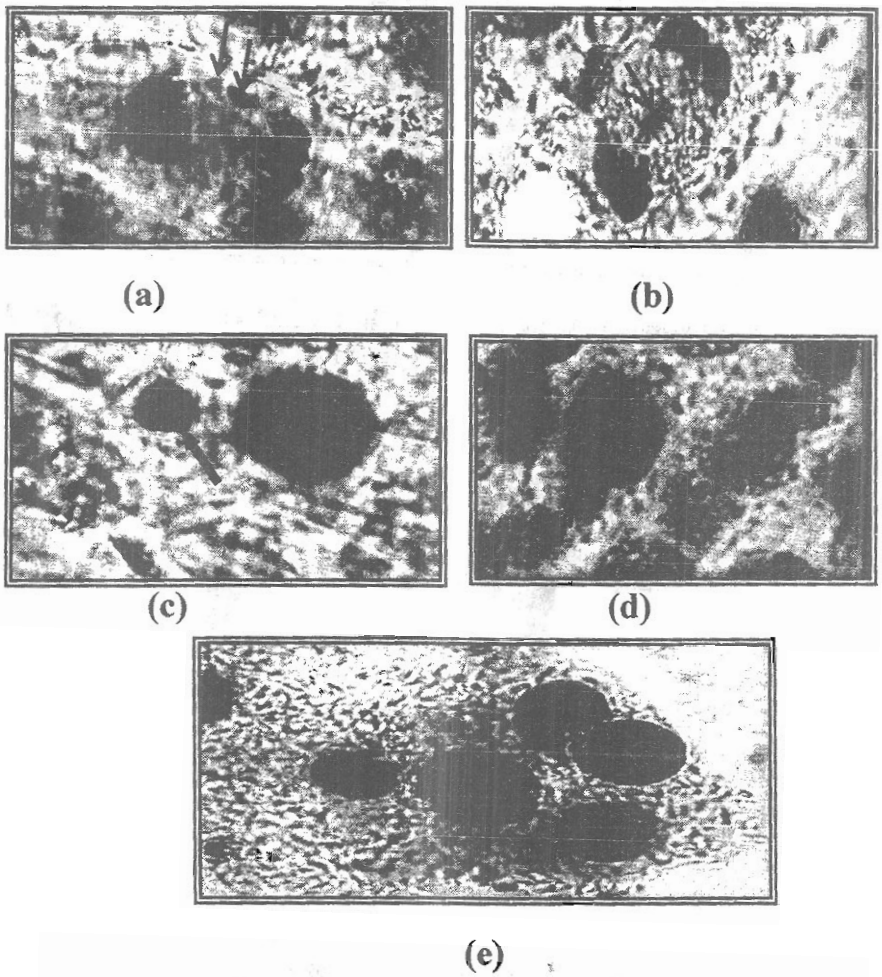

Fig. 1: Mitotic abnormalities in *Allium cepa* L. root tip cells.
(a) Anaphase with fragments (b) Anaphase with laggard
(c) Interphase cell with compact micronucleus
(d) Interphase cell with non-compact micronucleus
(e) Binucleate cells

Fig. 2: Centromeric fusion, (↗) Chromatid exchange (↗↗) and Gap (➔➔) in metaphase spread of *Rattus norvegicus, L.*

Fig. 3: Chromatid break (↗) in metaphase spread of *Rattus norvegicus, L.*

Fig. 4: End to end association (↗) in metaphase spread of *Rattus norvegicus, L.*

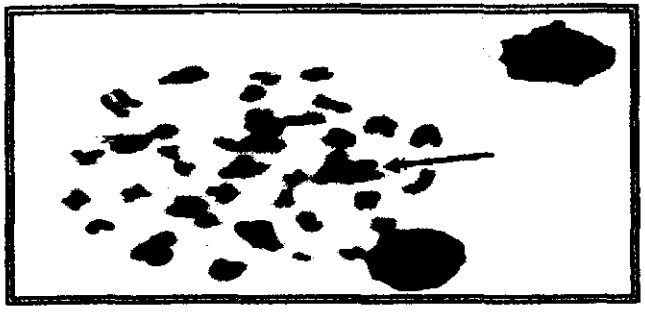

Fig. 5: Dicentric chromosome (↗) in metaphase spread of *Rattus norvegicus, L.*

Fig. 6: Ring chromosome (↗) in metaphase spread of *Rattus norvegicus, L.*

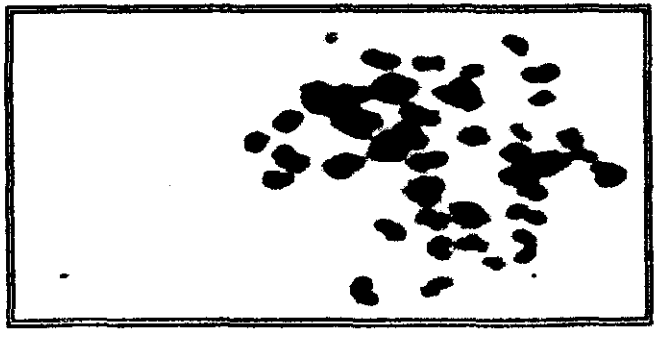

Fig. 7: Sticky chromosomes in metaphase spread of *Rattus norvegicus, L.*

Table 12: Mitogenic and clastogenic effects of oral administration of *A.maritima, L.* medicinal herb extract on rat bone marrow cells after 24, 72 hr. and 7 days treatment

Concentration		No. of studied cells	No. of divided cells	Mitotic index	% of abnormal cells	Percentage of chromatide type aberrations					Percentage of chromosome type aberrations				
						Gap	Break	CH.E	E.E.A	Total	Ring	Centromeric fusion	Dicentric	Sticky	Total
						(a)									
Control	No	1500	125		4	1	2	0.0	1	4	0.0	0.0	0.0	0.0	0.0
	%	100	0.08	8.33	3.2	0.8	1.6	0.0	0.8	3.2	0.0	0.0	0.0	0.0	0.0
2.00	No	1500	120		8	5	2	0.0	1	8.0	0.0	0.0	0.0	0.0	0.0
	%	100	0.08	8.0	6.67	4.17	1.67	0.0	0.83	6.67	0.0	0.0	0.0	0.0	0.0
8.00	No	1500	128		12	5	4	2	1	12	0.0	0.0	0.0	0.0	0.0
	%	100	0.09	8.53	9.38	3.91	3.13	1.56	0.78	9.38	0.0	0.0	0.0	0.0	0.0
15.00	No	1500	135		12	5	4	0.0	3	12	0.0	0.0	0.0	0.0	0.0
	%	100	0.09	9.00	8.89	3.70	2.96	0.0	2.40	9.06	0.0	0.0	0.0	0.0	0.0
						(b)									
Control	No	1500	130		5	2	2	1	0.0	5	0.0	0.0	0.0	0.0	0.0
	%	100		8.7	3.8	1.5	1.5	0.7	0.0	3.7	0.0	0.0	0.0	0.0	0.0
2.00	No	1500	125		12	5	3	1	1	10.0	0.0	0.0	0.0	2.0	2.0
	%	100		8.3	9.6	4.0	2.4	0.8	0.8	8.0	0.0	0.0	0.0	1.6	1.6
8.00	No	1500	135		15	6	2	2	2	12	1.0	1.0	1.0	0.0	3.0
	%	100		9.0	11.11 *	4.4	1.5	1.5	1.5	8.9	0.7	0.7	0.7	0.0	2.1
15.00	No	1500	135		18	6	3	2	3	14	1.0	1.0	1.0	1.0	4.0
	%	100		9.0	13.3 *	4.4	2.2	1.5	2.2	10.3	0.7	0.7	0.7	0.7	2.8
						(c)									
Control	No	1500	130		4	2.0	1.0	1.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0
	%	100		8.5	3.1	1.6	0.8	0.7	0.0	3.1	0.0	0.0	0.0	0.0	0.0
2.00	No	1500	125		14	6	3	2.0	2.0	13.0	1.0	0.0	0.0	0.0	1.0
	%	100		9.0	10.3 *	4.4	2.2	1.5	1.5	9.6	0.7	0.0	0.0	0.0	0.7
8.00	No	1500	135		18	6	4	2.0	2.0	14.0	1.0	0.0	1.0	2.0	4.0
	%	100		9.3	12.8 **	4.3	2.9	1.4	1.4	10.0	0.7	0.0	0.7	1.4	2.8
15.00	No	1500	135		25	6	6	4.0	4.0	20.0	2.0	1.0	1.0	1.0	5.0
	%	100		9.7	17.3 **	4.1	4.1	2.8	2.8	13.8	1.4	0.7	0.7	0.7	3.5

*,** = Significant at 0.05 and 0.01 probability levels, respectively.
CH.E : Chromatid exchange
E.E.A : End to end association
(a) : 24 hr., (b) : 72 hr. and (c) : 7 days

Bhide, 1985 recommended to use a mammalian assay to confer the possibility of the exploration of the results obtained by either the bacterial or plant model. The results of this investigation clearly demonstrated that the medicinal plant *A.maritima, L.* (Damsissa) has low mutagenic compounds. This conclusion was confirmed through a battery of short systems including a prokaryotic one representing prophage induction and transduction assays followed by a lower eukaryotic model, the root tips of *A.cepa, L.* assays, and finally with a high eukaryotic assay, the bone marrow assay of rat. These results are in agreement with previous studies that confirmed the lack of toxicity of *A.maritima* to non-target organisms (Sherif and El-Sawy, 1962, Alard *et al.*, 1991 and Geerts *et al.*, 1992).

The statistical analysis, using X^2 test, showed a positive effect in either the *Allium cepa, L.* or rat bone-marrow assays, although the two microbial assays showed no mutagenic activity. This might due to either to the differences in the time of exposure that has been used in the three models, or to the sensitivity of the type of assay that has, plant or mammalian cells, been treated. This could affect the induction of mutagenicity (El-Gorashi *et al.*, 2002 and Kirkland and Marzin, 2003). Although, *A. maritime, L.* had no mutagenic effects in the *Sallmonella* Ames test (Alard *et al.*, 1991) our results might reflect a clastogenic mechanism.

Moreover, positive response in the microbial assays was recommended to be corresponded to a three fold increase in each concentration over the background according to Rossman *et al.*, 1985; Houk and De Marini, 1988 and De Marini *et al.*, 1990. When applying this recommendation in the other two models, no positive response was detected in the plant model (Data are not shown here). A positive response was only detected after 7 days of exposure time in the percent of abnormal cells, chromatide type aberration and chromosome type aberration at 15 % only of the plant tested.

Finally, this conclusion needs further investigations for the proper assessment of mutagenicity for this medicinal plant.

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تحديد القدرة الطفوريةلنبات الدمسيسة (*Ambrosia maritime, L.*) كنبات مبى باستخدام نظم بيولوجية مختلفة

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تم تقدير النشاط الطفرى لنبات الدمسيسة والذى يمثل أحد الأعشاب الطبية التى تستخدم لأغراض طبية فى عديد من الدول النامية وذلك باستخدام ثلاث نظم وهى:

أولا: النظام البكتيرى باستخدام اختبار Prophage induction واختبار Transduction

ثانيا: النظام النباتى باستخدام اختبار خلايا القمم النامية فى البصل وأخيرا أحد الأنظمة الثديية باستخدام اختبار نخاع العظام فى خلايا الفئران وقد أثبتت النتائج فى الثلاث أنظمة أن نبات الدمسيسة له تأثير طفرى منخفض وليس تأثير منعدم نهائيا خاصة عند استخدام جرعات عالية من هذا المستخلص النباتى (مثلا عند استخدام تركيز ١٥ % من المستخلص النباتى)

بالإضافة لذلك فإن هذا النبات يمكن أن يستخدم كمضاد للأكسدة نتيجة لقدرته المضادة عند استخدامه مع أحد المواد المعروف قدرتها الطفرية.