GENOTOXICITY OF SOME RANGE PLANTS FROM THE NORTH WESTERN COASTAL ZONE OF EGYPT

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Range plants exist mainly in North Western Coastal Zone (N.W.C.Z.) of Egypt. It is well known that N.W.C.Z. of Egypt has the most favorable moisture regime and the best biological potential of the Egyptian deserts (Kassas, 1977). Four range plants that are distributed in this area were chosen in accordance with their different degrees of palatability to local livestock. These range plant species are *Atriplex halimus* L. (Chenopodiaceae), *Salsola tetrandra* Forssk. (Chenopodiaceae), *Retama raetam* Forssk. (Leguminaceae) and *Limoniastrum monopetalum* L. (Plumbaginaceae). Both *A.halimus* and *L. monopetalum* are crynohalophyte plants but *A.halimus* could tolerate drought. *S. tetrandra* is succulent halophyte plant, while *R. raetam* is xerophyte plant although it could manage with salt. Some range plants as *Atriplex* species are excellent livestock fodder during off-season periods when grasses are low in feed value (Mckell, 1989). This is attributed to having high content of protein, vitamins (A, C and D) and minerals such as chromium (chromium supplementation can improve blood sugar control) (Shani *et al.,* 1972). It is tasty and flocks enjoy eating saltbush compared with berseem hay at the same level of barley grains (Shawket *et al.*, 1998). In addition, it has good quality of wood (Aly and El-Darier, 1992).

S. tetrandra contains moderate amounts of crude protein and high levels of ash, silica and fiber. Although it is energy deficient (El-Shaer *et al*., 1997), *S. tetrandra* is used as a good fodder plant. It stores the salts in bladders as *A. halimus* and called "salt excreting" (Salama *et al.,* 1999).

R. raetam also called *Lygos raetam,* has good quality of wood (Aly and El-Dareir, 1992) and it is low palatable plant. Although *R. raetam* contains alkaloids (El-Shazly *et al.,* 1996) and was reviewed as a poisonous common plant in arid countries using livestock (El-Bahri *et al.,* 1999), local livestock use it in desert in hard times when there are no other palatable plants.

R. raetam showed an antifungal activity (Ali and Ghdeib, 1999) which was attributed to the existence of some flavonoids (Abdallah and Saleh, 1983).

L. monopetalum could excrete salt in glands as most species of plumbaginaceae (Salama *et al.,* 1999). It could also accumulate as much as 800 ppm without showing any damage (Ben-Abdallah and Boukhris, 1990). It is considered as unpalatable plant because of its covered leaves with wax layer. Therefore, it used in the present work as a comparative material and to test if it can be used as a nutritive material. This plant has the ability of fixing nitrogen through some genera of bacteria around its roots (Hassouna *et al.,* 1995). The main objective of the present study was to study the probable genetic toxicity of these range plant species on rats through their effect on rats growth (body & organs weights), the structural chromosomal aberrations in bone marrow, electrophoretic patterns of SDS-protein and some isozymes and finally the probable induction of mutation in P^{53} tumor suppressor gene.

MATERIALS AND METHODS

Plant material

Four range plant species, *Atriplex halimus* L. (Chenopodiaceae), *Salsola tetrandra* Forssk. (Chenopodiaceae), *Retama raetam* Forssk. (Leguminaceae) and *Limoniastrum monopetalum* L. (Plumbaginaceae) collected from N.W.C.Z of Egypt .

Animals

Thirty males of albino rats were divided into five groups of six rats in each one. Group one was untreated rats (control), while the other four groups were treated with plant suspension of each of *A. halimus, S. tetrandra, R. raetam* and *L. monopetalum,* respectively, according to Netzer *et al*. (1993). Three rats of each group were treated for 14 days and the other three were treated for 28 days. All animal groups were kept in glass cages with diameters (50 x 35 x 30 cm) and covered with wire cover.

Plant suspension preparation and treatment

The whole plants were dried in oven at 65°C until reaching constant weight, and then the dry plants were powdered and suspended in tap water. The rats were treated daily with oral administration of freshly prepared suspension of plants with doses of 20 mg/kg according to Maghrani *et al.* (2004).

Body and organs weights

During the experiment, untreated and treated rats were weekly weighted. In addition, when the rats were sacrified, the internal organs were collected and weighted at the end of treatments to study the effect of the range plant species on body and organs weights.

Cytogenetic analysis

The investigation of bone marrow cells in the treated rats was the main source of cytogenetic studies, searching for any structural chromosomal aberrations after treatment with plant species. The bone marrow metaphases were prepared according to Yosida and Amando (1965). Metaphases were examined under

the high power lens (X 100) and 50 cells were examined per animal. Structural chromosome aberrations were recorded and photographed.

Biochemical genetic analysis of treated rats

The SDS-PAGE technique of water soluble proteins

After sacrificing the treated and non-treated rats, 0.5 g of soluble proteins was extracted by grinding the tissue with 0.5 ml of distilled water until liquefying the tissue and transformed to eppendorf tube, then centrifuged for 10 min at 12000 rpm at 4°C. Supernatants were stored at -20°C until analysis was performed.

The electrophoretic patterns of soluble proteins were detected by sodium dodecylsulfate polyacrylamide gel electrophoresis technique (SDS-PAGE). Then, 50 µl from each liver extract was added to 25 µl 2x lan's buffer, 10 µl mercaptoethanol (10% v/v) and drop of bromophenol blue in each tube. Then all samples were boiled for 5 min and loaded into wells. The gels were run at 200 V/15 min and then the volt was raised to 250 V/until the run ends (3-4 hrs). All buffer solutions, stock solutions, staining and destaining solutions and gel preparation of protein were prepared according to Laemmli (1970) as modified by Studier (1973).

Isozymes electrophoresis

Isozymes were extracted from 0.5g liver from each treated and non-treated rats as previously mentioned for protein preparation. Native electrophoretic patterns of isozymes were detected by polyacrylamide gel electrophoretic technique (PAGE), using vertical Bio-Rad gel electrophoresis apparatus with slab diameter (180 x 200 mm) and 1.5 mm combs. Gel buffer solutions of isozymes were prepared according to Market and Faulhaber (1965), while Gel preparation of isozymes was done according to Bollog and Edelstein (1994). A volume of 30 µl from each liver extract with drop of bormophenol was applied into wells. The gels were run at 110 V/20 min and then the volt was raised to 250 V/until the run ends. α, β-esterase staining was made according to Wendel and Weeden (1989), while glutamate oxaloacetate transaminase was stained using the method of Yamamoto *et al*. (1982) and staining of peroxidase isozymes performed according to Larsen and Benson (1970).

Detection an induced mutation in P^{53} tummor suppressor gene

DNA isolation

DNA was isolated from tail tissues according to Bothwell *et al.* (1990). About one cm of each rat-tail was used to isolate the individuals genomic DNA, then the three replications of each untreated and treated groups were bulked together. Finally, five bulked samples after 14 days and five bulked samples after 28 days of treatment were to be screened for the existence of P^{53} tumor suppressor gene mutation.

PCR reaction

According to Hollishtein *et al.* (1993), three oligo-primers were prepared corresponding to related gene of the same multigene family P^{53} as follows:

P1: 5´- CGC CAT CTT GTG CTA CAT TGC CCG -3´
P2: 5´- ATC TTC TCC TCT TCT GTC TC -3´
P3: 5´- TTC TGG ATT GTA GCA GAT CA -3´

Primers P1 and P2 anneal to P^{53} genes, but P3 specially anneals to GST mutant gene. The three primers were used together in a polymerase chain reaction (PCR) yielding a constant 160-bp fragment seen only in the GSTμ- positive genome (Hollishtein *et al.,* 1993)

The PCR products were applied in agarose gel with concentration of 2.5%in TBE buffer. 100 Kb DNA marker was used as a standard DNA marker.

RESULTS AND DISSCUSION

Effect of range plant species on rats growth

Body and organs weights gain

The body weights of rats were taken weekly before the oral administration. Table (1) and Figures (1 and 2) present the means and standard errors of body weights gain for the treated and untreated (control) groups after two and four weeks of treatments, respectively. Through two weeks of treatments, the two groups showed no significant differences in the average body weight daily gain as compared to the control except for the treatment with *R. raetam* which showed significant reduction in comparison with the other treatments and the control group.

Meanwhile, through four weeks of treatments no significant differences were observed between the treated groups and untreated group.

The analysis of variance revealed significant differences ($P<0.05$) among the treated and untreated groups of the spleen, lungs and tests and no significant differences for both heart and kidneys were found after 14 days of treatment. After 28 days of treatment, the analysis of variance revealed significant differences ($P<0.05$) for most organs and highly significant difference ($P<0.01$) for kidneys.

The results agreed with Netzer *et al*. (1993) who fed rats for 6 weeks with aqueous extract of *A. halimus,* and found that the rate of body weight gain of mature rats increased following *A. halimus* feeding. While, it disagreed with the results obtained by Ibrahim (2001) who found significant decrease in daily weight gain of adult Barki goats after 50 weeks of feeding with *A. halimus*.

The results observed after two weeks of *R. raetam* treatment were similar to the results obtained by Maghrani *et al*. (2004) who found lowering in the activity of body weight gain with single

and repeated oral administration of the aqueous extract of *R. raetam* (20 mg/kg) for two weeks.

Cytogenetic effects of the range plant species

Evaluating the mutagenetic effects of the four range plant species, *A. halimus, S. tetrandra, R. raetam and L. monopetalum* was one of the objectives of the present study, especially where the clastogenic and possible carcinogenic potential of such plants was not reviewed before. Male albino rats were treated with plant suspension which was prepared from each of the four range plants under study. In short term of sub-chronic treatment, the daily dose (20 mg/kg) was applied for two different periods (14 and 28 days).The normal rat chromosome set (2n=42) contains metacentric and acrocentric chromosomes. Many cells included different types of aberrations as a result of treatment of previous studies indicated that chromosomal aberrations are considered good biomarkers for the prediction of cancer development (Broegger *et al*., 1990; Kamada *et al.,* 1992; Hagmar *et al.,* 1998; Liou *et al.,* 1999).

Table (2) represents number of cells with different structural chromosomal aberrations such as gaps, breaks, and deletions which were induced in bone marrow metaphases and percentages of aberrant cells for untreated and treated male albino rats with the four range plant species for 14 and 28 days of treatment.

The percentages of aberrant cells (Table 2 and Fig. 3) showed significant differences for each treatment in comparison with the control in both the 14 and 28 days treatments. Figure (4) represents photos of different types of the structural chromosomal aberrations in bone marrow metaphases of the treated groups.

After 14 days of treatment, the control group showed 20.66 % of aberrant cells, while the percentages were 42.66, 35.33, 37.33 and 33.33% for *A. halimus, S. tetrandra, R. raetam and L. monopetalum*, respectively. On the other hand, all these treatments showed an increase in aberration percentages after 28 days, 53.33, 48.66, 52.66 and 50.0% for *A. halimus S. tetrandra, R. raetam and L. monopetalum*, respectively compared with 23.33% for the control. In general, the percentages of aberrant cells of the 28 days treatment were more than the 14 days treatment.

These transformed data were analyzed using one way analysis of variance which revealed significant differences ($P<0.05$) of the mean percentage of aberrant cells between all treatments and the control. Multiple comparisons were done using Duncan's test which showed that all treatments differed significantly from the control. However, the *A. halimus* and *R. raetam* scored the maximum percentages of aberrations.

The high levels of structural chromosomal aberrations which were recorded for *A. halimus* and *R. raetam* after 28 days of repeated doses could be

attributed to the high content of alkaloids in both plants. It is concluded that *A. halimus* and *R. raetam* may be considered as a powerful carcinogen which favors GC→TA transverse type of mutation. The occurrence of such incidences results in impresize base pairing which eventually leads to a replication block. Formation of such blocks, if not repaired, could cause among other things, chromatide gaps. Even "SOS bypass repair system" which is specific for this kind of bulky lesions could itself cause drastic changes leading to deleterious mutations and / or cancer. Similarly, some kinds of alkaloids were recorded to have cytogenetic toxicity and induced aberrant metaphases, chromosomal aberrations excluding gaps and the mitotic index, and micronuclei 30 h after treatment (Choudhury *et al.,* 2004).

Effect of range plant species on rats-gene expression

SDS-water soluble proteins and isozymes were investigated as biochemical indices for the detection of the probable effect of the studied plant species on gene expression in the treated rats.

SDS-proteins analysis

Water-Soluble proteins were analyzed using SDS-PAGE based on the variation in their molecular weights using SDS protein standard marker. The electrophoretic patterns of the SDS- water protein of treated (3 individivals) and untreated groups (2 individivals) exhibited a maximum number of 17 bands in the 14 days treatment and 18 bands in the 28 days treatment. Tables (3 and 4) showed that there were no differences between the treated groups and the control group in the protein electrophoretic patterns of liver after 14 days of treatment.

On the other hand, there were a little bit differences among the treated and untreated groups after 28 days of treatment. The Two bands at 210.4 and 138.61 kDa disappeared in the treated groups with *A. halimus* and *S. tetrandra*, while *S. tetrandra* treated group lost another two protein bands (120.24 and 83.89 kDa). Moreover, in the treated groups with *A. halimus,* band with molecular weight of 91.35 kDa disappeared from this treatment compared with the control only *A. halimus* and *S. tetrandra* treatments exhibited disappearance of some SDS bands in liver tissue which would indicate alterations of gene expressions. However, *R. raetam* showed lower effect where only one band was (210.4 kDa) lost.

Isozymes analysis

Three isozymes systems were investigated in the liver of treated and untreated albino male rats, i.e., α-β esterase (EST), glutamate oxaloacetate transaminase (GOT) and peroxidase (PRX), using Native-PAGE. Three individuals (A, B and C) were used in each group (treated &untreated) for the two treatment periods (14 and 28 days).

a) α, β Esterase isozymes (α, β EST)

The electrophoretic patterns of α, β esterase isozymes in of 12 albino male rats (3 individuals for each treatment and

control group) either after 14 or 28 days are displayed in Figs (5 and 6), respectively. All the individuals of treated and untreated groups showed α, β esterase activity, which gave a maximum number of six bands in the 14 days treatment and reached to seven bands in the 28 days treatment (Tables 5 and 6).

In the 14 days treatment, there were three monomorphic from the exhibited six bands of α, β esterase isozyme bands with RF values of 0.30, 0.33 and 0.38. On the other hand, band 1 with RF value of 0.27 disappeared in *A. halimus* and *S. tetramdra* treated groups. Band 2 with RF value of 0.29 was absent in *R. raetam* treated group and band 6 with RF value 0.43 was absent in *S. tetrandra* and *L. monopetalum* treatments. At the 28 days treatment, there were seven detected bands, four of which were monomorphic ones with RF values of 0.27, 0.36, 0.39 and 0.46. However, band 2 with RF value of 0.29 did not appear in *R. raetam* and *L. monopetalum* treated groups. *A. halimus* causes missing of band 3 with RF value of 0.33. Moreover, all treated groups except *A. halimus* lost band number 6 with RF value of 0.42.

A. halimus and *S.tetrandra* treated groups were more similar than any other treated group to the control in both periods of treatments (14 and 28 days). The results revealed that *S. tetrandra*, *R. raetam* and *L. monopetalum* treatments affected the activity of α, β easterase isozymes to catalyze the cholesterol in the liver than *A. halimus* did.

According to Allian (1974), the esterase plasma total cholestrol catalyzes the following reaction inside the organism: Sterol easter ↔ Sterol + fatty acid.

The previous results may be supported by the results of Maghrani *et al.* (2004) who stated that the oral administration of the *R. raetam* aqueous extract for one week decreased the plasma cholesterol levels in diabetic rats.

b) Glutamate oxalate transaminase (GOT)

The enzymatic reactions of GOT isozymes involving intermolecular transfer of amino groups are important in metabolic processes. In mammalian tissues, the enzyme is especially concentrated in heart and liver tissues, when either organ is damaged there is a marked elevation of serum GOT. This phenomenon is of diagnostic significance, the enzymatic reaction of GOT isozyme inside the organism was described by Braunstein (1973) as follows: L-aspartate + 2- oxoglutarate ↔ oxaloacetate + L-glutamate

All the transaminases are involved in the formation of amino acids inside the cell, which could synthesis the 2-keto-acid. GOT isozyme is one of transaminases; and its existence means that the cellular function was activated. This function is involved with carbon compound transport, i.e., synthesis of sugar (glucose) and keeps blood sugar at a normal level when there has been no recent intake of carbohydrates in food.

The electreophoretic patterns of liver GOT isozyme of all treated and untreated individuals were in active forms after both 14 and 28 days of treatments, which are shown in Figs (7 and 8), respectively. In Tables (7 and 8), two electrophoretic bands were detected in untreated group, while the entire treated groups exhibited only one band after the 14 days of treatments. However, the second band of the untreated group disappeared after 28 days of treatments. Nevertheless, increasing the period of treatment for 28 days showed more intensity of band in all treated groups, this might be due to their ability of increasing GOT expressions in liver and the synthesis of glucose.

The results of *R. raetam* treated group disagreed with the indicated results of Maghrani *et al*. (2003) who found that oral administration of aqueous extract of *R. raetam* with the same dose as in the present study (20 mg/kg) for 6 h and 2 weeks showed significant reduction in blood glucose. This contradiction might be due to the different periods of treatments.

c) Peroxidase isozymes (PRX)

Peroxidase is a hemoprotein catalyzes the oxidation by hydrogen peroxide. It labeled immunoglobulins and used successfully as immunhistological probes for the demonstration of tissue antigens (Greenwalt *et al.,* 1975). All treated and untreated individuals after 14 and 28 days of treatments showed peroxidase isozymes activity as seen in Figs (9 and 10), respectively. All individuals in the untreated group and the four treated groups exhibited two electrophoretic band after both 14 and 28 days of treatment as shown in Tables (9 and 10), respectively. However, this band appears as a zone in all treated and untreated groups.

In 14 days treatments, peroxidase band with RF value of 0.31 was a monomorphic band, but it was faint in *A. halimus* and *S. terandra* treated groups and dark in *R. raetam* and *L. monopetalum* treated groups.

In the 28 days treatment, the peroxidase band with RF value of 0.30 which seems as a zone still monomorphic band. However, it became clearer in only *A. halimus* treated group but *S. tetrandra* treated group still has the faint band.

Suneja *et al*. (1989) studied the effect of toxic materials on peroxidase isozymes in liver and found that these toxic materials have a significant increase in liver lipid peroxidation. So increasing the peroxidase isozymes activity in *A. halimus*, *R. raetam* and *L. monopetalum* treated groups, which was observed through the clearly and darkly stained bands after 28 days of treatment may be due to the toxic effects of these plant species.

Effect of range plant species on P^{53} mutations induction

The tumors are kind of uncontrolled cell division that could result from mutations in some genes or loss in their

activity. P^{53} is a tumor suppressor gene; it has ability to suppress the activity of the mutant alleles. It is also related to cancer. Burdon (1999) found mutations in P^{53} genes in 50% of all human cancers. Koga *et al*. (2000) observed that about 37-62% of human bladders cancers contain P^{53} gene mutations.

No mutant effects on the existence of P^{53} gene were observed from all the four treated plants comparable to the normal existence of P^{53} in the untreated group. The PCR product of the amplified gene (P^{53}) displayed in Figs (11 and 12), which shows the characteristic fragment with constant size (160 bp) presented in all treatments compared to control group. This fragment refers to the presence of P^{53} gene.

Therefore, the results of DNA analysis of the present study indicated the absence of the mutagenic and carcinogenic effects of each of *A. halimus, S. tetrandra*, *R. raetam* and *L. monopetalum* plant species on P^{53} gene in the treated plants compared with control.

SUMMARY

Four range plants that are distributed in the North Western Coastal Zone (N.W.C.Z.) of Egypt were chosen in accordance with their different degrees of palatability to local livestock. They were *Atriplex halimus* L. (chenopodiaceae), *Salsola tetrandra* Forssk. (chenopodiaceae), *Retama raetam* Forssk. (Leguminaceae) and *Limoniastrum monopetalum* L. (Plumbaginaceae).

This study aimed to evaluate these four range plant species and display their genetic toxicity using sample of male albino rats. Rats were treated with oral daily administration of plant suspensions for 14 and 28 days to study their effect on rats growth (body weight and organs weight), gene expression of treated rats, and finally the presence of mutation in P53 tumor suppressor gene.

In general, the results showed reduction effects on the daily body weight gain and organs weights of the treated rats with these plants. However, *L. monopetalum* had the least effect followed by *R. raetam*, while *A. halimus* and *S. tetrandra* had similar effects.

On the level of cytogenetic effects of these 4 plants on the bone marrow of rats, they had the ability to induce structural chromosomal aberrations, especially *A. halimus* and *R. raetam*. This would indicate the probabilities of more induced tumors with continuous feeding on such plants.

When studying the 4 plants effects on gene expression, the electrophoretic analysis of the liver total proteins showed no effect of any treatment after 14 days, while after 28 days; *A. halimus* and *S. tetrandra* affected the presence of high protein bands (disappearance). After studying three isozymes α, β EST, GOT and PRX, the results indicated that α, β

esterase isozymes activity was not affected by any treatment while *R. ratam, S. tetrandra* and *L. monopetalum* lost few of the electrophoretic bands after both 14 and 28 days. GOT isozymes were not affected in all the treatments after 14 days, while all of them lost an electrophoretic band comparable to the control after 28 days of treatment. Peroxidase isozyme was expressed in all the treated groups, although the density of the electrophoretic bands was different. However, after 28 days, *R. raetam*, *A. halimu*s and *L. monopetalum* had more dark bands than *S. tetrandra* which had faint bands. The dark bands of liver peroxidase isozymes may be related to the toxic contents.

On the gene level, no plant treatments were able to induce mutations in P^{53} tumors suppressor gene

The present study preveal precaution for animal producers when using *A. halimus* and *R. raetam* plants as fodders for feeding because of their serious effects on mammalian biological system on different levels.

These effects were seen although low dose of these plants was used (20 mg/kg) per day from concentrate suspension with 2% (w/v) of plant for short term (14 & 28 days). *S. tetrandra* can be recommended to be used as fodders but not with high quantity or for long period. *L. monopetalum* is the recommended plant as a result of this study to present new fodder plant, although it is unpalatable to animals that may be due to its morphological characteristics or taste. However, this plant could be used as a part of diet with other favorable components to animals.

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Table (1): Means and standard errors of body weight gain in rats for the treated and control groups after two and four weeks of treatments.

Parameters			Treatments				
			Control	*A. halimus*	*S. tetrandra*	*R. raetam*	*L. monopetalum*
Organs	Lungs	14 days	1.77±0.096^{a}	1.50±0.096^{ab}	1.45±0.096^{ab}	1.63±0.096^{ab}	1.37±0.096^{b}
		28 days	1.50±0.100^{ab}	1.72±0.100^{a}	1.40±0.100^{ab}	1.62±0.100^{ab}	1.34±0.100^{b}
	Kidneys	14 days	1.98±0.175^{a}	1.68±0.175^{a}	1.83±0.175^{a}	1.64±0.175^{a}	1.95±0.175^{a}
		28 days	1.97±0.150^{a}	1.59±0.150^{ab}	1.37±0.150^{b}	1.90±0.150^{a}	1.87±0.150^{a}
	Heart	14 days	0.75±0.066^{a}	0.66±0.066^{a}	0.66±0.066^{a}	0.76±0.066^{a}	0.76±0.066^{a}
		28 days	0.82±0.070^{a}	0.63±0.070^{ab}	0.56±0.070^{b}	0.64±0.070^{ab}	0.67±0.070^{ab}
	Spleen	14 days	0.84±0.066^{a}	0.73±0.066^{ab}	0.77±0.066^{ab}	0.85±0.066^{a}	0.61±0.066^{b}
		28 days	0.81±0.110^{a}	0.56±0.110^{a}	0.49±0.110^{a}	0.59±0.110^{a}	0.59±0.110^{a}
	Testes	14 days	4.52±0.300^{a}	3.88±0.300^{a}	3.80±0.300^{a}	3.99±0.300^{a}	4.22±0.300^{a}
		28 days	4.65±0.190^{a}	4.27±0.190^{ab}	3.91±0.190^{b}	4.20±0.190^{ab}	4.18±0.190^{ab}
Average body weight daily gain in		Two weeks	0.61±0.250^{a}	0.83±0.250^{a}	0.93±0.250^{a}	0.02±0.250^{b}	1.05±0.250^{a}
		Four weeks	1.06±0.260^{a}	0.59±0.260^{b}	0.24±0.260^{b}	0.55±0.260^{b}	1.04±0.260^{a}

Table (2): Number of cells with different structural chromosomal aberrations in bone marrow cells of control and treated male albino rats with the four range plant species for 14 and 28 days.

Treated Group	Sampling time	No. of Treated	No. of Examined Cells	No. of cells showing satirical chromosome					Percentage of aberrant cells (%)	Mean transformed
				F	R	G	B	D		
Control	14 days	3	150	24	3	14	0	17	20.66	6.89 ± 1.64^{b1}
	28 days	3	150	25	19	19	6	12	23.33	7.77 ± 1.29^{b2}
A. halimus	14 days	3	150	43	32	27	16	37	42.66	14.22 ± 1.64^{a1}
	28 days	3	150	30	3	23	37	40	53.33	17.77 ± 1.29^{a2}
S. tetrader	14 days	3	150	30	10	13	17	20	35.33	$11.77 \pm 1.64^{a1.b1}$
	28 days	3	150	37	20	13	27	43	48.66	16.22 ± 1.29^{a2}
R. raetam	14 days	3	150	28	0	9	19	37	37.33	12.44 ± 1.64^{a1}
	28 days	3	150	26	0	35	35	35	52.66	17.55 ± 1.29^{a2}
L. monopetalum	14 days	3	150	0	12	12	12	50	33.33	$11.11 \pm 1.64^{a1.b1}$
	28 days	3	150	0	0	25	0	50	50.00	16.66 ± 1.29^{a2}

F: Fragments R: Ring chromosomes G. Gaps B: Breaks D: Deletions

Table (3): The presence of liver SDS protein bands of treated groups and the control group after 14 days of treatment.

Band No.	MW (kDa)	Control		*A. halimus*			*S. tetrandra*			*R. raetam*			*L. monopetalum*		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	217.99	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	121.97	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	87.68	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	77.35	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	69.84	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	51.94	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	43.30	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	36.92	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	33.70	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	32.57	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	31.12	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	29.40	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	23.95	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	13.10	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	11.43	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	8.03	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	7.33	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(MW) Molecular weight (+) presence of band (-) absence of band

Table (4): The presence of liver SDS protein bands of treated groups and the control group after 28 days of treatment.

Band No.	MW (kDa)	Control		*A. halimus*			*S. tetrandra*			*R. raetam*			*L. monopetalum*		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	210.4	-	-	-	-	-	-	-	-	-	-	-	+	+	+
2	138.61	+	+	-	-	-	-	-	-	+	+	+	+	+	+
3	120.24	+	+	+	+	+	-	-	-	+	+	+	+	+	+
4	91.35	+	+	-	-	-	+	+	+	+	+	+	+	+	+
5	83.89	+	+	+	+	+	-	-	-	+	+	+	+	+	+
6	75.58	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	57.42	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	48.41	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	37.84	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	36.09	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	34.74	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	33.45	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	27.94	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	25.90	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	16.91	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	14.94	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	11.80	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	10.52	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(MW) Molecular weight (+) presence of band (-) absence of band

Table (5): Densitometric analysis for α, ß esterase isozymes for treated and untreated groups after 14 days of treatments.

Band Number	RF values	Control			*A. halimus*			*S. tetrandra*			*R. raetam*			*L. monopetalum*		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	0.27	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1
2	0.29	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1
3	0.30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	0.33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	0.38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	0.43	1	1	1	1	1	1	0	0	0	1	1	1	0	0	0

Table (6): Densitometric analysis for α, ß esterase isozymes for treated & untreated groups after 28 days of treatments.

Band Number	RF values	Control			*A. halimus*			*S. tetrandra*			*R. raetam*			*L. monopetalum*		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	0.27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	0.29	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
3	0.33	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1
4	0.36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	0.39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	0.42	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
7	0.46	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table (7): Densitometric analysis for GOT isozyme for treated &untreated groups after 14 days of treatments.

Band Number	RF values	Control			*A.halimus*			*S. tetrandra*			*R.raetam*			*L.monopetalum*		
		*A	*B	*C	A	B	C	A	B	C	A	B	C	A	B	C
1	0.22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	0.24	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0

Table (8): Densitometric analysis for GOT isozyme for treated &untreated groups after 28 days of treatments.

Band Number	RF values	Control			*A. halimus*			*S. tetramdra*			*R. raetam*			*L.monopetalum*		
		*A	*B	*C	A	B	C	A	B	C	A	B	C	A	B	C
1	0.31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table (9): Densitometric analysis for PRX isozymes for treated &untreated groups after 14 days of treatments.

Band Number	RF values	Control			*A. halimus*			*S. tetramdra*			*R. raetam*			*L. monopetalum*		
		*A	*B	*C	A	B	C	A	B	C	A	B	C	A	B	C
1	0.04	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	0.31	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table (10): Densitometric analysis for PRX isozymes for treated &untreated groups after 28 days of treatments.

Band Number	RF values	Control			*A. halimus*			*S. tetramdra*			*R. raetam*			*L. monopetalum*		
		*A	*B	*C	A	B	C	A	B	C	A	B	C	A	B	C
1	0.03	1	0	0	1	1	0	1	1	1	1	1	1	1	1	1
2	0.30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

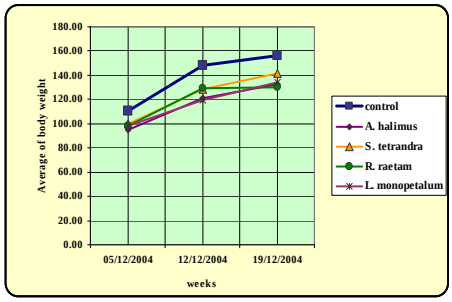


Fig. (1): Differences between means of body weights of treated groups and untreated group after 14 days of treatment.

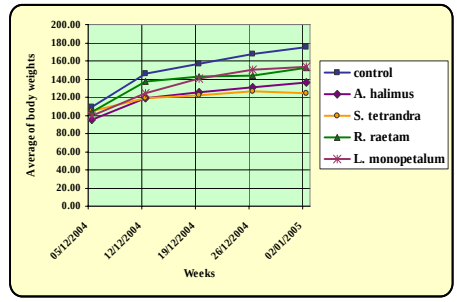


Fig. (2): Differences between means of body weights of treated groups and untreated group after 28 days of treatment.

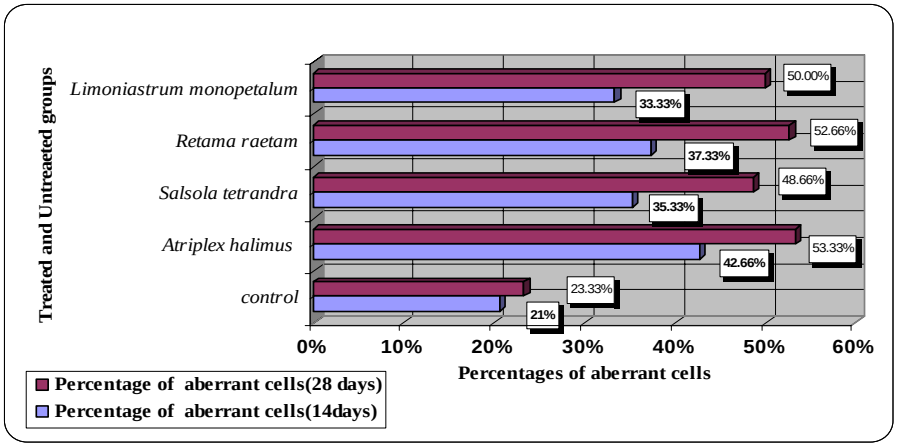
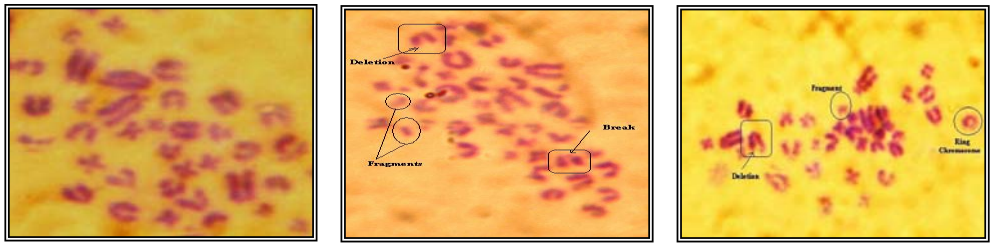


Fig. (3): Percentages of aberrant cells of treated groups compared with the control after 14 and 28 days of treatment.



(a) Untreated rat metaphase (b) Treated with *A. halimus* (c) Treated with *S. tetrandra*

Fig. (4): Represents photos of different types of the structural chromosomal aberrations.

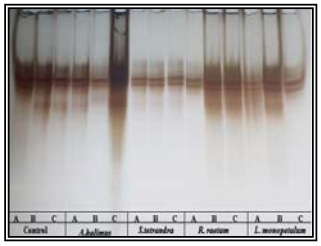


Fig. (5): α, β esterase isozymes for treated & untreated groups after 14 days of treatment.

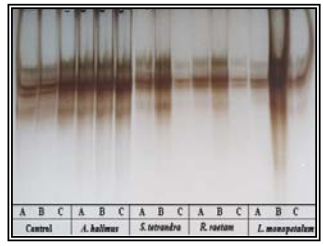


Fig. (6): α, β esterase isozymes for treated & untreated groups after 28 days of treatment.

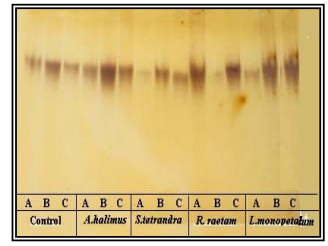


Fig. (7): GOT isozyme for treated & untreated groups after 14 days of treatment.

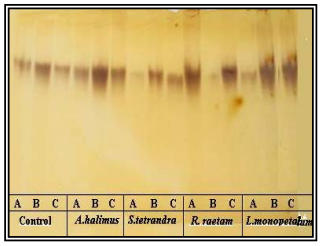


Fig. (8): GOT isozyme for treated & untreated groups after 28 days of treatment.

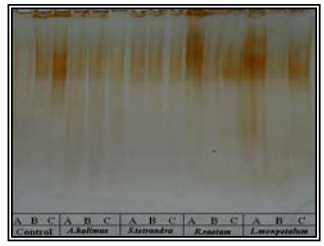


Fig. (9): PRX isozymes for treated & untreated groups after 14 days of treatment.

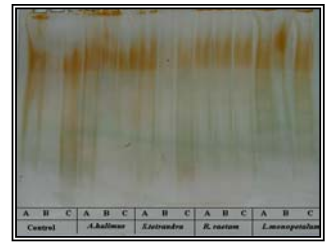


Fig. (10): PRX isozyme for treated & untreated groups after 28 days of treatment.

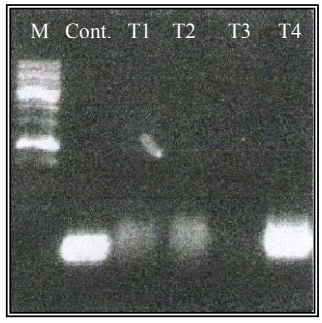


Fig. (11): PCR product of P53 gene amplified with two oligo primers for different treated groups and untreated group after 14 days of treatment.

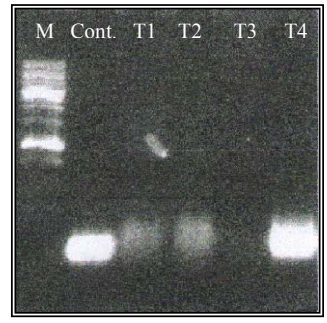


Fig. (12): PCR product of P53 gene amplified with two oligo primers for different treated groups and untreated group after 28 days of treatment.