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Regular Article

Inhibition of cyclophosphomide mutagenicity using *Glycyrrihza glabra* root extract

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The aims of the studying is using methanolic - water extract of the *Glycyrrihza glabra* root for inhibition the mutagenicity of anticancer drug by cytogenetic assays in vivo in three interaction between the drug and extract. The result shows that the extract is bio-antimutagenesis. The treatment by extract after drug causes increase mitotic index and decrease chromosome aberration and sperm head abnormalities. The thin layer chromatography of the extract show that is contains different polar compounds.

Key words : antimutagenesis, *Glycyrrihza glabra*, cytogenetic assay.

DNA is one of the important cell cotenants, it is affecting by physiological, chemical and biological agents that causes increase in mutation rates and rearrangement of chromosomes and induced different type of chromosome aberration (1) the effects of this agents interact with the types, quantity and the exposure to it, so the metabolic amount and its effect on genetic material and repair system enzyme ability to remove this effect (2). *Glycyrrihza glabra (GLY)* is one of the plant that have long historical in medical and therapeutic use since Babylon and Egyptian revolution (3). The studying show *Glycyrrihza glabra* have wide range of phytochemical compounds like terpenoid saponine, phenols, volatile oils, vitamins, minerals, sterols, protein, sugars and starch (4). The research improved the ability of *Glycyrrihza glabra* as anti mutagens (5) show that GLY extract was anti mutagenic to spontaneous and induced mutation in specific locus in genome by chemical factors such as EMS and nitrofurzene using aims and rec assay, so the antimutagenesity of this plant in induced mutation in *Salmonella* spp. by EMS that it have 18-α glycyrrhizin and 18-β glycyrrhizin.

Cyclophosphamide is anticancer drug, alkalating agent that interfere with transcription and translation of nucleic acid as a result this effect on cell proliferation, it has been used as mutagenicity factor in wide range of experiments.

Materials and method

Plant extract : It was prepared according to Sato et al. (9) with modification, powder root homogenized with solvent extract contain from (methanol : DW) (20:80 V\V) in blander for 30 min then it filtered and deride in oven 50C^{o} for 24 hour then it keep in dim container. TLC profile according to (10) using solvents system (chlorophorm : hexan: ethanol) (1:1:1v\v\v).
Mutagenic factor: using cyclophosphamide tablet, dose was 20 mg\kg.

Cytogenetic assay: using chromosome aberration assay, mitotic index assay and sperm head abnormalities assay.

Experimental design : Using white albino rats (male) in Wight 300 ± 50 and age 10 ± 2 weeks was divided in to 5 groups:

1- Group treated by cyclophosphomide 20 mg\kg. Animal killed after 24 hours
2- Group treated by plant extract 500, 250 mg\kg. Animal killed after 24 hours.
3- Group treated by DW as negative control
4- Group treated extract before drug. Animal killed after 48hours
5- Group treated extract with drug. Animal killed after 24hours
6- Group treated extract after drug. Animal killed after 48 hours

After finished treatment animals was killed, cytogenetic assays was performed

Chromosome aberration and mitotic index : according to (11) with some modification, after animal killed femur bone was cleaned the bone end was cut then bone marrow discard by hypotonic solution (KCl 0.05 M) in test tube then it keep it in 37°C for 15 min with shaking after this it centrifugation 2000 rpm to 2 min, discard supernatant and the sedimentation was fixation by fixative solution (methanol : glacial acetic acid) (3:1) then centrifugation 2000 rpm for 2 min, repeat fixation 2 time, at last fixation step sample was distil on cold slide above 75 cm, after dried slide can stained it using gemza stain for determination chromosome aberration and mitotic index (13).

Sperm head abnormality: This performed from epididymis, it cutting in phosphate buffer saline then one drop transfer to slide after dried it fixative by drops of methanol then stand by methylene blue for exam sperm head abnormality (12).

Statistics: using one way ANOVA in probability $p<0.05$ by SPSS software.

Results and dissection

TLC profile

The TLC results in table 1 and figure 1show that extract contain from different polar compound by using visible light and UV light (WL 312n) in other researchers found that GLY contain from comarins, isoflavons such as glabrone and glabrene (14) so they improved that the stolen and root of this plant have large number of phenolic compounds like formonotin, glabridin, isoliquirtigenin, hemileicarpin, paratocarpin and hispaglabridin B so the root have high percentage of (GA) glabridin if it extracted by solvents mixture (ethanol : DW) (70:30 v\v).

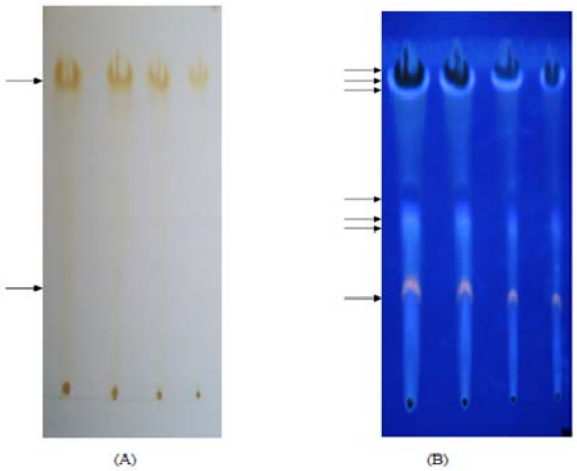

Figure 1. TLC profile of GLY extract 1n visible light A, and UV light B.

Table 1. Band characters of the TLC profile of GLY extract

Bands number	Bands characters		Examining
	R_f	Color	
2	0.34 *	pink	Visible light
	0.90	Brown	
7	0.34 *	orang	UV light
	0.37	Brown	
	0.51	blue	
	0.53	Violet	
	0.69	brown	
	0.81	Violet	
	0.87	brown	

**bands found in visible and UV*

Chromosome aberration assay

The results of this assay that in figure (2) and table (2) show increase in chromosome aberration in drug treatment in all type of it that explanation in figure 5 and decreased in interfere drug with plant extract in all treatment but this decreased is different according to interfere. The reason of this increase was the drug causes harmful effects on DNA it causes break in single and double strand, the research improved the drug was carcinogenetic and induced sister chromatid exchange, chromatid and chromosome break. So it induced micronuclei formation.

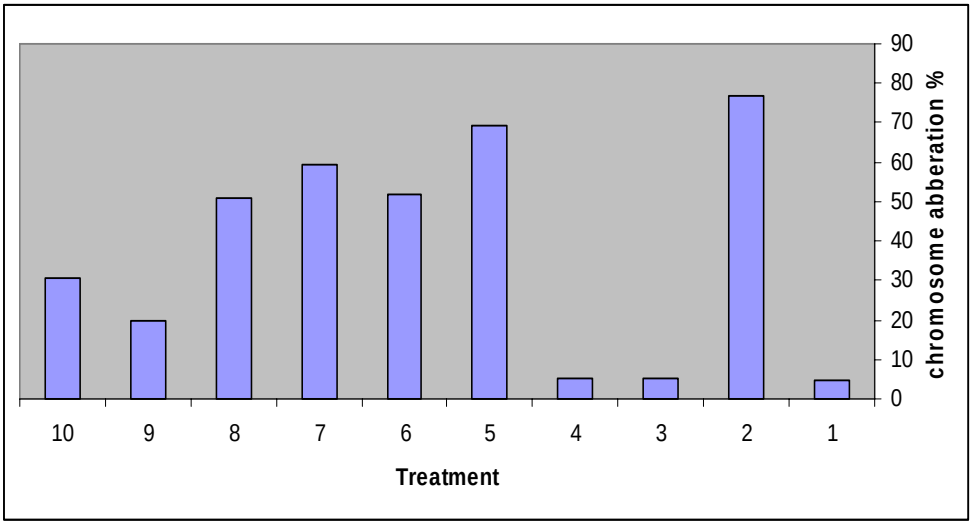

Figure 2. Percentage of chromosome aberration in bone marrow cell of rats treated by CP and GLY.

1-animals treated by DW; 2- CP treatment; 3,4- GLY treatment in 250 and 500 mg\kg respectively; 5,6- treatment GLY with CP in 250,500 mg\kg respectively; 7,8- treatment GLY before CP in 250, 500 mg\kg respectively; 9,10- treatment GLY after CP in 250, 500 mg\kg respectively

Table 2. Chromosome aberration type of rats bone marrow cell treated by CP and GLY.

Treatment mg\kg	Breaking	Ring	Analytical	Polyploidy	Aneuploidy	Chromosome break	Chromatid break
N C	** 1.33±1.33	*6.66±3.52	0.00	4.00±2.30	* 8.00±2.30	9.33±1.33	* 17.00 ± 1.00
Cp 20	70.66±30.66	50.66±15.02	48.33±27.95	2.66±2.6	425.33±144.11	25.33±6.66	146.66 ±24.6
Gly 500	** 14.00±1.15	* 10.00±1.15	8.00±3.30	4.00±0.57	* 12.33±0.33	4.00±0.00	* 10.00±3.46
Gly 250	** 10.00±3.43	* 8.00±2.30	4.00±2.30	4.00±0.57	* 12.00±1.15	** 2.00±1.15	* 12.00±0.57
Before 500	94.66±10.41	** 17.33±3.52	** 168.00±58.56	2.66±1.33	276.00±29.48	26.66±1.33	** 109.33±7.42
Before 250	97.33±12.71	** 21.33±5.81	** 136.00±46.13	0.00	** 236.00±70.31	29.33±7.05	125.33±13.13
With 500	62.66±11.85	** 22.66±8.11	74.00±17.08	5.33±1.33	286.66±31.18	22.66±10.66	121.33±10.91
With 250	69.33±40.68	** 21.33±1.33	53.33±22.43	5.33±1.33	** 239.33±76.87	18.66±8.74	** 100.00±4.00
After 500	34.66±1.33	* 14.66±4.80	17.33±2.66	5.33±3.52	* 70.66±17.93	16.00±12.22	* 37.33±13.13
After 250	112.00±25.45	** 16.00±6.11	62.66±31.35	2.66±2.66	* 72.00±8.32	40.00±11.54	* 81.33±15.37

Me±Se * significant at p<0.005; ** significant at p<0.001

The aim of these interfering between plant extract an drug is for determination the how that extract can protect the cellular compound against the effect of mutagenicity the result in table 2 show that treatment after drug was the best specially in 500 mg\kg thus the GLY was bio-antimutagenesis this mean that extract may be increased the DNA replication fidelity or induced repair system enzyme as result decreased in mutation frequency (17). Gly extract contain of phytochemicals can be protect cell component, other research improved that extract causes decrease in chromosome aberration in mice bone marrow cell that treatment by chemical and physical factors so the extract induced repair system or phytochemical may interfere with mutagenicity but the mechanism of this has been unknown(19).

Mitotic index assay

Table (3) and figure (3) show the results of bone marrow proliferation that effected by CP treatment and GLY extract, CP causes decreased in MI because it inhibit cell division by its effect on DNA in S phase or by DNA -DNA cross link or DNA – protein cross link , this crosslink interfere with DNA replication and transcription thus it causes stopped in cell divided (20) when treatment by extract with CP it causes increased in MI especial in treatment after drug , this can be clarified that extract have ability to effect on cell proliferation rate when use different extraction method of *Glycyrrihza uralensis* on cell *in vitro* , the water and alcohol extract causes increased in MI by increase nucleic proteins and stimulating DNA polymerase for DNA replication thus its reflect on cell proliferation so this extract especially ethanolic extract stimulated estrogen independent cell proliferation, ethyl acetate that contain of mid polar compound large effect in cell division by induced proliferating cell nuclear antigen in S phase an G2 phase that consider as cell proliferation (21).

Table 3. Mitotic index of rats bone marrow cell treated by CP and GLY

M ± SE	Treatment Mg\kg
*5.06 ±0.57	N C
1.40±0.49	Cp 20
5.53±0.49	Gly 500
5.20±0.34	Gly 250
1.56±0.44	Before 500
1.36±0.34	Before 250
1.73±0.83	With 500
1.80±0.46	With 250
*6.06±0.43	After 500
*4.76±0.17	After 250

*Significant at p<0.001

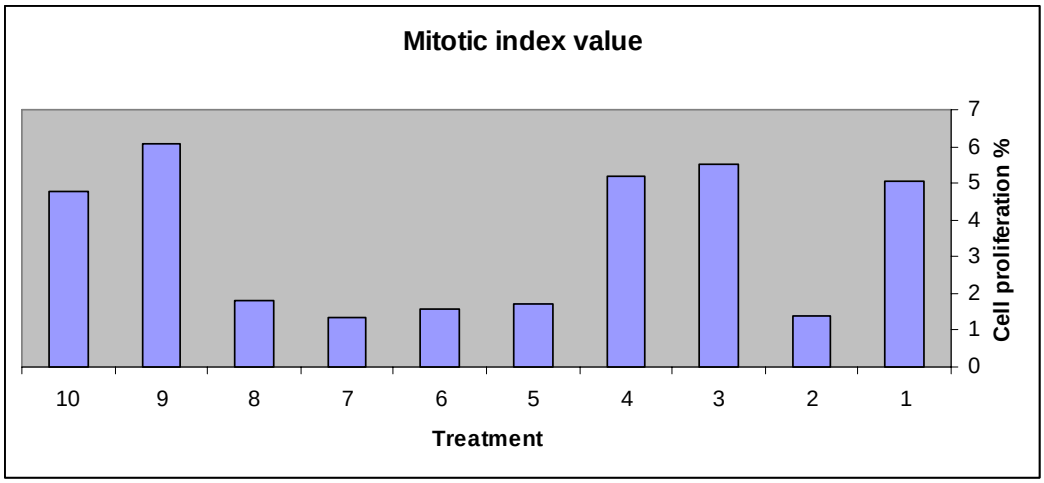

Figure 3. Mitotic index value of rats bone marrow that treated by CP and GLY

1- Animals treated by DW, 2 – CP treatment; 3,4 – GLY treatment in 250 and 500 mg\kg respectively; 5,6- treatment GLY with CP in 250,500 mg\kg respectively; 7,8- treatment GLY before CP in 250 , 500 mg\kg respectively

Sperm head abnormalities

The treatment by CP causes increased in the abnormality of sperms heads that is explanation in table 4 and figure 4 the reasons of this that CP is alkylating agent changes in DNA and induced chromosome aberration in germ cell (22) . when interfered drug and plant extract the results are same in all assays that use in our studying and the mechanism of plant extract protection was identical, the reasons of the decrees in abnormalities was that extract effect on hormones that responsible of testes functions or regulatory of protein synthesis and repair system of germ cells, by this mechanism clarified the *Chelidonium majus* ability in decreased malformation of sperm head, so the decreased may be because the interfere or link between phytochemical compound with drug such as thyme with gemcitabine(21).

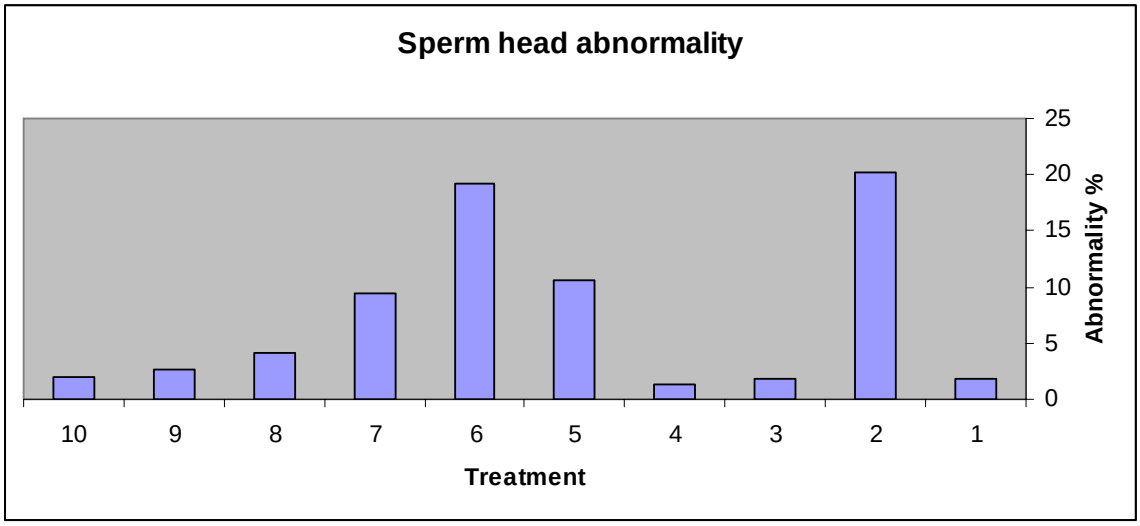

Figure 4. Sperm head abnormality in rats treated by CP and GLY

1 -animals treated by DW, 2 – CP treatment; 3,4 – GLY treatment in 250 and 500 mg\kg respectively; 5,6- treatment GLY with CP in 250,500 mg\kg respectively; 7,8- treatment GLY before CP in 250 , 500 mg\kg respectively.

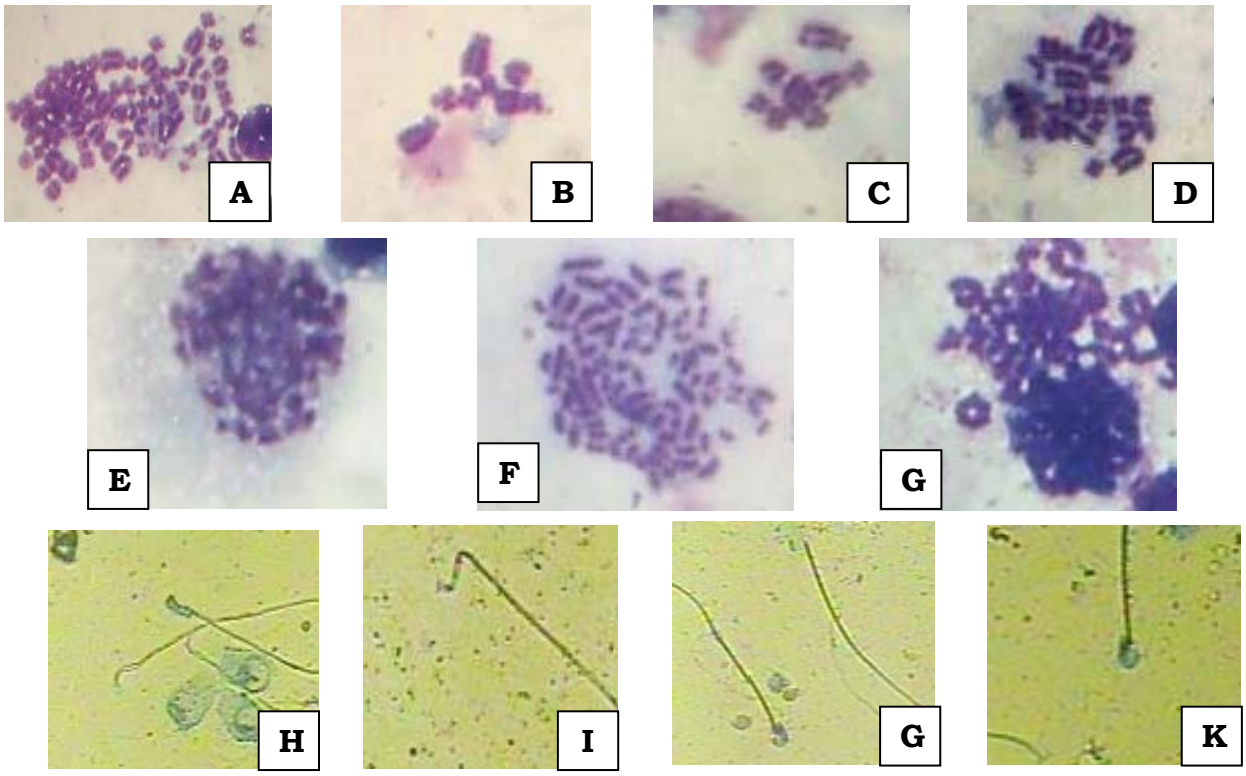

Figure 5. Chromosome aberration in bone marrow cell and abnormality in sperm head in rats that treated by cyclophosphamide and plant extract. *A*, polyploidy ; *B*, aneuploidy ; *C*, chromatids break ; *D* , chromosome break ; *E*, analytical ; *F*, breaking ; *G*, ring ; *H*, lubeled head ; *I*, curved head ; *J*, missed head ; *k*, globular head.

Table 4. Sperm head abnormality in rats treated by CP and GLY

Treatment	Globular head	Curved head	Lobule head	Missed head
N.C DW	**1.00 ± 0.57	**0	**2.66 ± 1.33	*14.66±2.18
CP 20	11.33 ± 6.35	6.00 ± 1.15	47.00 ± 35.59	138.33±44.75
Gly 500	**0.66 ± 0.33	**0.33 ± 0.33	**1.33 ± 0.66	**15.33±1.20
Gly 250	**0.66 ± 0.66	**0	**2.66 ± 1.45	*10.33±1.45
Before 500	6.33 ± 2.18	1.00 ± 0.57	17.33 ± 11.14	97.66±11.79
Before 250	17.66 ± 4.97	6.00 ± 1.15	43.66 ± 17.37	124.66±37.82
With 500	**2.00 ± 1.15	6.66 ±5.69	10.33 ± 8.83	75.33±38.76
With 250	**1.66 ± 0.33	**0	**5.33 ± 1.66	**42.00±5.50
After 500	**1.00 ± 0.57	**0	**6.66 ± 4.25	**19.66±2.33
After 250	5.66 ± 3.17	2.33 ± 0.33	12.66 ± 0.33	**49.33±4.33

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