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Regular Article

In vivo study of antimutagenic and antioxidant activity of *Glycyrrihza glabra* root extract

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The studying was aimed to inhibition the mutagenecity and oxidation of genomic DNA by use methanol-water extract of *Glycyrrihza glabra* root (GL) against anticancer drug cyclophosphomide in rats by use DNA fragmentation test, the extract characterized by thin layer chromatography TLC, and antioxidant activity by use β- carotene spray, the experiment design to use tow single dose of GL and single dose of the drug in three interaction, extract before, with and after the drug to investigate the mechanism of which extract effect inside cell. Four multiple doses for drug and extract to investigate the accumulation effect in DNA the results show that extract is contain of different polar compound and have antioxidant activity, so the extract is bio-antimutagenesis and poor dismutagenesis activity, the extract can inhibit accumulation effect of mutagenecity and oxidation activity of the drug.

Keywords: DNA fragmentation test, *Glycyrrihza glabra,* cyclophosphomide, oxidation, mutagenecity.

Glycyrrihza glabra (GL) is one of important medical plant use from long time in Babylon and Egypt impair (Fenwike *et al.*,1990), the root is useful part because has phytochemical compound that important in treatment like Glycyrrihetinic acid and Glycyrrhizin (Isbruker and Burdock, 2006). GL have antimutagenesis activity against some alkalating agent like Ethylmethansulfonat (EMS) in aims test (Mistsher *et al.*, 1986), Alekperove (2002) improve that the GL has antimutagenesis activity by use with another plant it was decreased chromosome aberration in mice bone marrow that induced by physical and chemical factor, the GL have antioxidant activity by protect LDL from free radical effect (Fuhrman *et al.*, 1997) and protect the liver tissue from oxidative stress induced by voltarine drug (Hamza, 2007). Because of the GL contain phynolic compound it is have anticancer activity by decrease anti apoptotic suppression protein Bc-12 (Rafi *et al.*, 2003).

Cyclophosphomide (CP) is anticancer drug cause break in single and double strand DNA in rat germ cell treated by multiple dose (Codrington, 2007).

DNA fragmentation test

This test use in wide rang in research it dependant on fragment or lyses of genomic DNA, this can be detection by electrophoresis DNA (Saiful *et al.*, 2009) DNA fragment result

from the effect of chemicals and physicals factors like alkalating agent, chemical compounds , drugs, free radicals, radiation, air and water pollutions. This test use in different studying like effect of Amphetamine drugs, this cusses DNA fragment as smear when treat the Nero cell *in vitro* by this drugs (Stumm *et al*., 1999). It use to show the medical plant affect on cancer cell, it show the treatment of hepatoma cell line HepG2 by Piper *sarmentosum* causes hydrolysis of DNA in 200-180 pb (Hisham *et al*., 2009).

MATERIAL AND METHOD

1- Plant extract : GL root powder homogenize with solvent mixture (methanol: distal water) (20:80 v\v) in blander for 30 min , the mixture are infiltration and dry in oven 50 C for 24 hours , the product store in dark container (sato *et al*., 1990)
2- Plant extract characterized by TLC, by use solvents (methanol: ethyl acetate: DW) (20:60:20 v\v\v) as mobile phase, then bands exam in visible and UV light in 312 wave length.
3- Antioxidant activity : this test performed by use β-caroten spray (Prepare by dissolve 9 gm of β-caroten in 30 ml chloroform and 2 drop of linolic acid with 60 ml ethanol) (Pratt and Miller, 1984). TLC was spry by this mixture and left it in light for 6 hour; the bands have yellow colure for longer time was antioxidant activity.
4- Drug: cyclophosphomide tamplet (Baxter, German).
5- Doses use 20 , 15, 10 , 5 mg\kg of cyclophosphomide, plant extract doses were 1000, 750, 500, 250 mg\kg
6- Animal: use white albino rat 300±50 mg weight and 12± 2 weak.
7- Experimental design : animals was divided in 2 group
   A. First : animal treated in single dose of drug and extract in 3 interaction, 24 male rats divided in to :
      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 48 hours.
      5) Group treated extract with drug. Animal killed after 24 hours.
      6) Group treated extract after drug. Animal killed after 48 hours.
   B. Second : animal treated by Multiple dose of drug and extract, 27 male rats divided in to :
      1) Group treated by 20 mg\kg CP with 1000 mg\kg GL for 7 days.
      2) Group treated by 15 mg\kg CP with 750mg\kg GL for 10 days.
      3) Group treated by 10 mg\kg mg CP with 500 mg\kg GL for 15 days.
      4) Group treated by 5 mg\kg CP with 250 mg\kg GL for 35 days. Animals were killed after finished treatment period, the blood collected by heart punctured in EDTA tube.
8- DNA extraction according to promega (USA) leaflet. The concentration and purity of DNA was calculated according to:
   - C_{DNA} mg\ml = OD_{260} × dilution factor × 50
   - Purity = OD_{260}/ OD_{280} . (Sambrook et *al*., 2001) .
9- DNA Fragmentation detection by DNA electrophoresis in agarose gel (Prifer, 1984).
10- DNA fragment calculated

The fragment was smear therefore lyses level was calculated according to distance between beginning and end the smear compare with negative control.

Results

Plant extract characterization by TLC

TLC profile results shows the GL extract contain of different polar compounds have different color and different retardation factors (R_f) in figure (1, A, B) and table (1).

Table (1) Characteristic of TLC profile of methanolic –water extract of *Glycyrrihza glabra.*

Bands characters		Exploration
R_f Value	**Color**	
*0.55	Brown	Visible light
*0.68	Yellow	
0.79	Yellow	
0.82	Brown	
*0.88	Brown	
0.50	Wight	Ultra violet
0. 52	Blake	
*0.55	Brown	
0.58	Violet	
0.64	Brown	
*0.68	Yellow	
0.73	Wight	
0.76	Yellow	
0.85	Violet	
*0.88	Brown	

** Bands found in visible light and UV light*

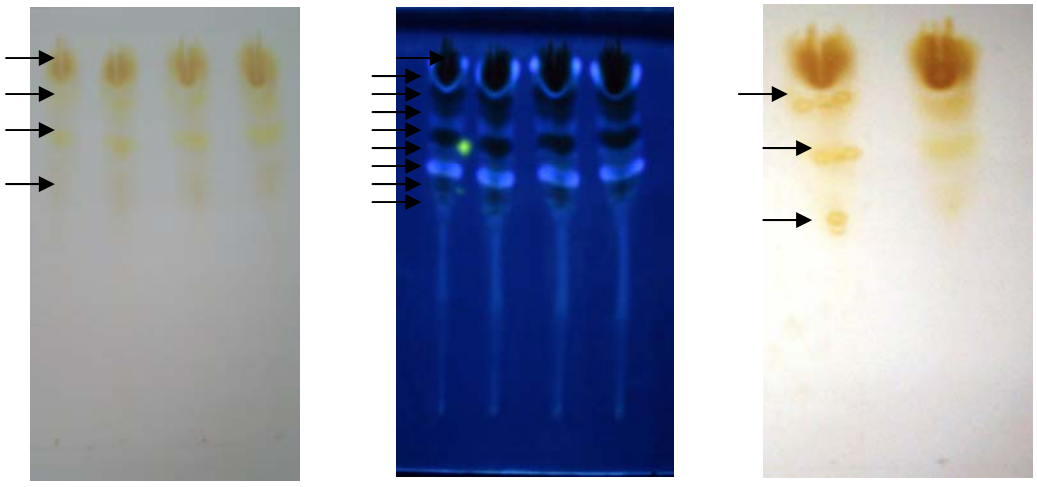

Figure 1. TLC profile of *Glycyrrihza glabra* extract by use (methanol: Ethyl acetate: distal water) (20:60:20 v\v\v). A- Exam in visible light. B - Exam in UV light. C - Antioxidant activity.

Antioxidant activity

The β-carotene spray assay show three bands have antioxidant activity figure (1C) different R_f , Table (2) .

Table 2. R_f value of antioxidant activity of *Glycyrrihza glabra* extract.

Antioxidant test	R_f
-	0.50
-	0.52
+	0.55
-	0.64
+	0.68
-	0.73
-	0.76
+	0.79
-	0.82
-	0.85
-	0.88

+ Bands have antioxidant activity.

DNA fragmentation test

Single dose; figure (2) explain DNA extracted from animal blood that treated by CP and GL in three interaction, the lyses level was calculated in table (3). CP causes DNA lyses, interaction between CP and GL decreased lyses in different level according to the type of interaction. Plant extract don't affect on DNA.

Multiple dose; figure (3) explain DNA extracted from animal blood that treated by different dose for different time of CP and GL, DNA lyses level in table (4), CP causes different lyses level, GL decreased DNA lyses caused by CP in different level according to dose concentration and time.

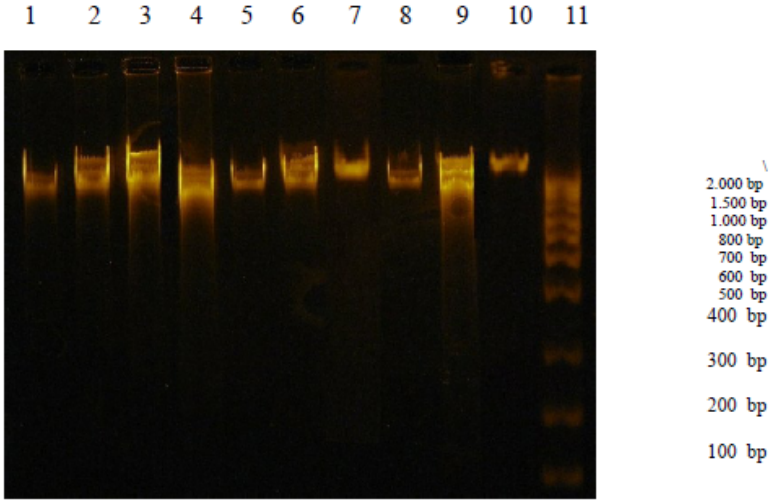

Figure 2. Electrophoresis of DNA that extract from animal blood. Lane 1, 2 animal treated the GL before drug in 500, 250 mg\kg. Lane 3, 4 animal treated the GL with drug in 500, 250 mg\kg. Lane 5, 6 animal treated the GL after drug in 500, 250 mg\kg. Lane 7, 8 animal treated GL in 500, 250 mg\kg only. Lane 9 animal treated CP 20mg\kg only. Lane 10 negative control. Lane 11 DNA marker.

Table 3. DNA lyses level in animal treated by GL and CP in three interactions.

DNA Lyses level	Smear size (bp)	Treatment mg \ kg	Lane number In figure (1)
1200	800 – 2000	Before 500	1
1250	750 – 2000	Before 250	2
1400	600 – 2000	With 500	3
1600	400 – 2000	With 250	4
500	1500 – 2000	After 500	5
1200	800 – 2000	After 250	6
500	1500 – 2000	GL 500 only	7
400	1600 – 2000	GL 250 only	8
1900	100 – 2000	CP 20 only	9
0	2000 – 2000	Negative control	10
-	100- 2000	DNA Marker	11

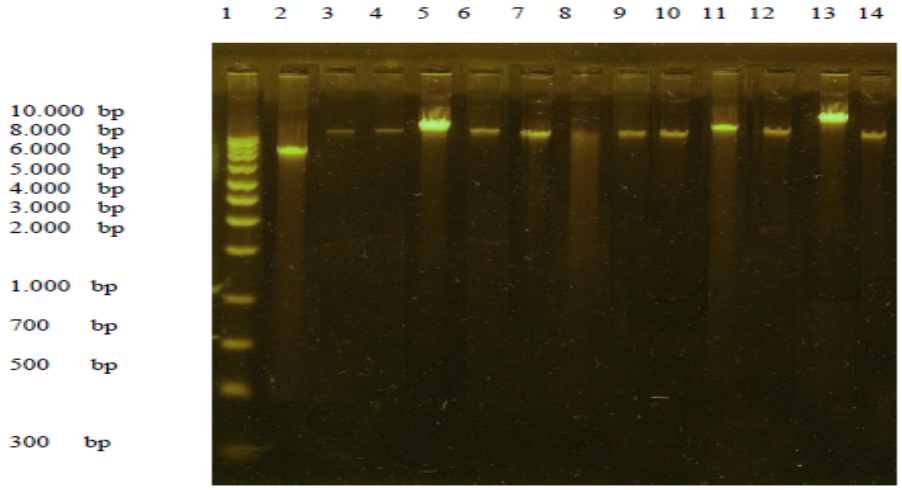

Figure 3. Electrophoresis of DNA that extract from animal blood in different dose and time. Lane 1 DNA marker. Lane 2, 5, 8, 11 animal treated by 20, 15, 10 , 5 mg\kg of drug. Lane 3, 6, 9, 12 animal treated by 1000, 750, 500 , 250 mg\kg of extract . Lane 4, 7, 10, 13 animal treated by drug and extract (20,1000), (15,750), (10,500), (5,250) for 7, 19, 15, 35 days respectively.

Table 4. DNA lyses level in animal treated by different dose of drug and *Glycyrrihza glabra* extract for different time.

DNA Lyses level	Smear size (bp)	Treatment mg \ kg	Lane number in figure (2)
_	300 - 10000	DNA Marker	1
9.500	500 – 10000	CP 20	2
0	10000 – 10000	GL 1000	3
0	10000 -10000	CP + GL	4
9.300	700 – 10000	CP 15	5
1000	9000 -10000	GL 750	6
8000	2000 – 10000	CP + GL	7
7.500	2.500 – 10000	CP 10	8
0	10000 – 10000	GL 500	9
5.700	4300- 10000	CP + GL	10
9.200	800 - 10000	CP 5	11
500	9.500 – 10000	GL 250	12
8.000	2000 - 10000	CP + GL	13
1000	9000 - 10000	Negative control	14

Discussion

The results show that plant extract have different polar compound in TLC profile, Meena *et al*. (2010) improved the GL contain of different phytochemicals like tannin, polysaccharide, pectin, amino acid, and minerals like pb, cd, the TLC in previous study show 12 bands in UV and 5 bands in visible light.

Methanolic –water extract have antioxidant activity in Three bands in β-carotein sepray assay, some studies improve the phenolic compound extract from root and stolen of GL like glabridin, isoliquirtignin and 4- o-methylglabridin can scavenger peroxnuitit radical (Young-won, 2007) and can repair necrosis in liver tissue that induced by oxidative stress (Hamza, 2007).

The treatment by CP causes hydrolysis in DNA because this drug is alkalating agent cusses DNA-DNA cross link and DNA –protein cross link (springer et al., 1998). Suman and Jamil (2006) use comet assay to study effect CP and other anticancer drug on human lymphocyte, they found the DNA tail length increased by treatment of this drug. CP causes generation free radical and reveled 8-oxo-dG in DNA that causes oxidative stress (Ibrahim *et al*., 2007). Hydrolysis of DNA may be because of apoptosis in all cells because of treatment by CP (Schwartz and Waxman, 2001).

Plant extract don't affect on DNA this improve that extract don't have any cytogenetic toxic in the concentration uses in studying, it don't cusses malformations in bone marrow chromosome in rats. Sasaki *et al*. (2002) found that treated mice by 2000 mg\kg of glycyrrhizin don't affect on DNA extracted from different body organs by comet assay.

When interaction between CP and GL, in single dose treatment GL after CP is the best treatment , thus GL is consider as bio- antimutagenesis, the reasons of this are GL can induced repair enzyme system of DNA (Isbruker and Burdook, 2006). The interaction extract with and

before drug inhibited lyses in DNA but less than interaction GL after CP this is may be because the phytochemicals compounds such as phenolic compounds and terpin that protect cellular compound from alkalating agent (Liang *et al*., 2007).

In treatment by multiple dose for different time, plant extract decrease DNA hydrolysis in different level according to concentration and time of dose, this result can be clarify the antioxidant activity of GL, or enhanced antioxidant enzyme gene expression Super oxide dismutase (SOD) and catalase (CAT) (Russo, 2005), Letha and Raesh (2004) improve the powder of GL root cusses increased the SOD and CAT and glutathione (GSH) in rat liver and kidney that suffer from oxidative stress. Flavonoid that extracted from GL can decrease 8-oxo dG level in blood (Davis, 2007)

Rafi *et al*. (2003) explained the mechanism of phenolic compound against oral cancer cell that GL induced apoptosis in cancer cell only by inhibition anti apoptotic Bc12 protein.

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