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Comparative Biochemical Study of Aloe-emodin and Barbaloin by Antioxidative and Antiglycation Evaluations in Mice Liver Tissue

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Aloe vera is widely used for medicine, dietary supplements, and cosmetic purposes. In recent years, the consumption of this plant's juice and gel has promoted and with entering them to the body different systems are encountering their effects. Liver as an important organ may response to the main active components of this plant, Aloe-emodin and Barbaloin. The aim of the present study was to investigate the antioxidant and antiglycation properties of these compounds on mice liver tissue at concentrations ranging from 0 to 12 mM. In this study various concentrations (0-12 mM) of Aloe-emodin and Barbaloin were added to liver tissue cultures and after 24h incubation the tests were performed. Enzymatic activities, Malondialdehyde level and DPPH radicals scavenging activity were evaluated by spectrophotometric method. Dityrosine level was measured by HPLC method. Antiglycation properties were evaluated by pentosidine and glyoxal tests. *in vitro* studies indicated that Aloe-emodin and Barbaloin scavenged free radicals in a dose dependent manner. Also, the results revealed that Aloe-emodin and Barbaloin could increase liver antioxidant enzymes activity including superoxide dismutase, catalase, and glutathione peroxidase at low (0.75-1.5 mM) and high (3-12 mM) concentrations respectively. In other words, the levels of malondialdehyde and dityrosine, as oxidative damage biomarkers, significantly decreased at these concentrations, compared with the control value. Moreover at these doses, these two compounds had significant antiglycation property on the liver tissue as they evaluated by pentosidine and glyoxal tests. Therefore, as these compounds had significant antioxidant and antiglycation properties on the liver tissue, it can be expected that usage of them can be protected the liver tissue against chronic liver disease that is resulting from diabetes, non-alcoholic steatohepatitis (NASH).

Key words: Aloe-emodin, Barbaloin, Antioxidant enzymes, Oxidative damage biomarkers, Antiglycation.

Diabetes mellitus is a heterogeneous metabolic disorder [Jagtap and Patil, 2010] which is a major source of illness all over the world and its incidence is expected to increase by 5.4% in 2025 [Amouoghli Tabrizi and Mohajeri, 2012].

This disease is characterized by hyperglycemia, where alterations in the carbohydrate, fat, and protein metabolisms accompanied by absolute or relative deficiencies in insulin secretion and/or it′s action [Ramachandraiahgari *et al*., 2012]. In addition, the production of free radicals increases and these enhanced species in diabetes, especially in uncontrolled diabetes, can lead to the autooxidation of glycosylated proteins, induction of membrane damage, and oxidation of cellular lipids and proteins [Can *et al*., 2004].

As diabetes is often accompanied with

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chronic hyperglycemia and enhanced oxidative stress, the rate of advanced glycation end products (AGEPs) is usual enhanced. AGEPs and oxidative stress have been implicated in the pathogenesis of diabetic complications including neuropathy, nephropathy, retinopathy, cardiovascular disease, and non-alcoholic steatohepatitis (NASH). Therefore, natural compounds or extracts particularly from medical plants that posses both antioxidant and antiglycation activities might have great potential for preventing diabetic complications [Chen *et al*., 2011].

Aloe vera as medicinal plants is a perennial succulent plant belonging to the Aloeaceae family [Bozzi *et al*., 2007]. It is native to North Africa [Bolkent *et al*., 2005 and Helal *et al*., 2003] and cultivated in warm climates areas [Abuelgasim *et al*., 2008].

This plant is made of turgid green leaves joined at the stem in a rosette pattern [Miladi and Damak, 2008]. The internal part of the leaf, called gel, is colorless, mucilaginous, and has a slightly bitter flavor. It is composed mainly of large polysaccharide chain and also contains proteins and anthraquinones in low quantities. The yellow-brown, bitter-flavored exudate obtained after cutting the leaves is known as juice, and has a high content of polyphenolic compounds, mainly Aloe-emodin and Barbaloin [Perez *et al*., 2007].

Aloe-emodin ($C_{15}H_{10}O_5$, 1,8-Dihydroxy-3-(hydroxymethyl) anthraquinone) [Dorsey and Kao 2007] (Fig. 1a) presents in the leaves and roots of a number of plants such as aloe and senna. Although this compound is a minor constituent of most botanical raw materials, many studies have Shown that it is the pharmacologically active metabolite of aloin and sennosides, both botanical products used for their cathartic activity [Wamer *et al*., 2002]. Also, Aloe-emodin has more recently garnered significant interest as a naturally-derived anti-cancer agent [Dorsey and Kao, 2007].

Barbaloin (10-beta-D- glucopyranosyl-1,8 dihydroxy-3-hydroxymethyl-9(10H)-anthracenone) (Fig.1b) is C-glucoside of Aloe-emodin anthrone, found in the outher rind of the aloe plant [Patel *et al*., 2012]. It has been reported to constitute up to 30% of the aloe plant′s dried leaf exudates [Wamer *et al*., 2002] and play an important part in the defense mechanisms against herbivores. This compound demonstrates anti-inflammatory, antiviral, antioxidant, and cathartic effects [Patel *et al*., 2012].

There is increasing evidence indicating that these active components have different biochemical effects at various concentrations. Thus, the aim of our investigation was to study antioxidant and antiglycation properties of these compounds over a wide range of concentrations in mice liver tissue.

MATERIALS AND METHODS

Materials

Aloe-emodin and Barbaloin purchased from Sigma company (99% purity). Balb/C mice were purchased from Pasteur Institute of Iran and all of them were healthy, adult, and 4-6 weekly with 25-30g estimated weight. Dulbecco·s Modified Eagle Medium (DMEM) which was suitable for animal cells culture such as mice was bought from Sigma company. All chemicals, solvents, and reagents were 98.5% purity and were purchased from Sigma company.

Liver tissue culture

Rats were sacrificed by decapitation and their livers were removed in sterile conditions. The collected livers immediately were washed two times with cold normal saline and then minced and sliced into small pieces. 0.5g of tissue was placed in Nunclon peteridishes which contained 5ml of DMEM culture media. These culture media were subsequently supplemented with 30 mg/ml of asparagine, antibiotics (200 U/ml penicillin and 200 mg/ml streptomycin), and 10% heat inactivated fetal calf serum pH 7.4. Aloe-emodin and Barbaloin were added at the concentrations of 0.35, 0.75, 1.5 , 3, 6, and 12 mM to these culture media and incubated for 24h at 37°C in a fully humidified atmosphere of 5% CO_2 in air.

Preparation of liver tissue for biochemical analyse

After 24h incubation of tissue cultures, all tissues were separately homogenized in 1:4 volumes of ice-cold Tris-HCL buffer (50 mmol/L, pH 7.4) using a glass Teflon homogenizer. The homogenate was then centrifuged at 2500×g for 15 min to remove debris. Supernatant was taken and used for evaluation of liver antioxidant enzymes activity and oxidative damage biomarker levels.

Free radicals scavenging activity of Aloe-emodin and Barbaloin

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay

DPPH free-radical scavenging activities of Aloe-emodin and Barbaloin samples were evaluated by the method of Blois (1958) at concentrations ranging from 0.35 to 12 mM. DPPH is a well-known free-radical. Antioxidant compounds reduce the methanolic solution of DPPH free radical through the donation of H or electron and change its deep violet color into light yellow. A decrease in absorbance is measured with a spectrophotometer at 517 nm.

Initially, the tissue extract (0.1ml) was mixed with 500µl of Tris buffer and 150µl of 0.2 mM DPPH. After 30min, the variation in absorbance was recorded at 517nm and considered as DPPH radical scavenging activity due to standards. The activity was calculated by following formula:

$(1-A_{sample}/A_{control}).100$

Superoxide anion radical scavenging activity assay

Ability of Aloe-emodin and Barbaloin to scavenge superoxide anion radicals generated by methionine and riboflavin [Beauchamp and Fridovich, 1971] was studied at different concentrations (0.35-12mM). The generated superoxide radicals oxidize to reduce Nitrobluetetra zolium (NBT). The formazan dye is consequently formed. Antioxidant compounds inhibit the NBT reduction and formazan dye formation. Thus, the absorbance is decreased and is measured with a spectrophotometer at 560nm.

All solutions were prepared in 0.2 phosphate buffer (pH 7.4). Tissue extract was mixed with 3 ml of reaction buffer solution containing 1.3µM riboflavin, 0.02M methionine, and 5.1µM NBT. The reaction solution was exposed to two 30W fluorescent lamps for 20 min and the absorbance was then measured at 560nm. The reaction mixture without any tissue extract was used as control. The superoxide anion radical scavenging activity (%) was calculated as $(1-A_{sample}/A_{control}).100$

Total antioxidant activity assay

Total antioxidant capacities of Aloe-emodin and Barbaloin samples were analyzed according to the method of Prieto *et al*. (1999). The principle of the method is based on the reduction of Mo(VI) to Mo(V) by the sample and the subsequent formation of green phosphate/Mo(V) compounds with a maximum absorption at 695nm. As antioxidant compounds cause the reduction of Mo(VI) and the formation of green compounds, the absorbance is enhanced.

In brief, 0.1ml of the tissue extract was mixed with 1ml of the reagent solution (0.6M sulphuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate). The tubes were capped and then incubated at 95°C for 90min. After the samples were cooled to 25°C, the absorbance was measured at 695nm against blank. The total antioxidant activity was expressed as the absorbance of the sample and the higher absorbance value indicated the higher antioxidant activity.

Analysis of liver antioxidant enzymes activity

Superoxide dismutase activity assay

Liver superoxide dismutase (SOD) activity was determined as described by Kono (1977). The principle of the method is based on the inhibition of NBT reduction by the SOD activity. Autooxidation of hydroxylamine generates superoxide anion radicals. One unit of SOD activity was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. To evaluate the SOD activity, 200µl hydroxylamine, 100µl NBT, and 200µl Na_2CO_3 were mixed with 250µl EDTA, 400µl buffer, and 150µl of tissue extract and the absorbance was then measured at 560nm. SOD activity was also expressed as unit SOD g^{-1} of protein.

Catalase activity assay

Liver catalase (CAT) activity was evaluated by the method of Aebi (1974). According to this method, because the catalase enzyme catalyzes the H_2O_2 decomposition, a decrease in H_2O_2 absorbance, which is directly proportional to the catalase activity, can be assessed by a spectrophotometer at 240nm. At first, 250µl of phosphate buffer (pH 7.2), 200µl of stabilizer, and 250µl of H_2O_2 were mixed with 100µl of tissue extract and the absorbance was then determined at 240nm. CAT activity was also expressed as unit CAT g^{-1} of protein.

Glutathione peroxidase activity assay

Glutathione peroxidase (GPX) activity was indirectly determined by a coupled reaction with glutathione reductase (GR) [Flohe and

Gunzler, 1984]. Oxidized glutathione (GSSG), produced upon reduction of hydroperoxide by GPX, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340nm. Under conditions in which the GPX activity is rate limiting, the rate of decrease in the A_{340} is directly proportional to the GPX activity.

In brief, a mixture consisting of 500µl of 0.05M potassium phosphate, 100µl of isoascorbate, 100µl of 0.01M glutathione (GSH), 100µl of 1.5mM NADPH, 100µl of EDTA , and 500µl of $MgCl_2$ was added to 100µl of tissue extract. To start the reaction, 50µl of H_2O_2, as substrate, was added to this mixture. The absorbance was then measured at 340nm. GPX activity was also expressed as unit GPX g^{-1} of protein.

Determination of liver oxidative damage biomarker levels

Measurement of malondialdehyde levels

Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation. lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues. To evaluate the MDA levels, the method of Wills (1965) was used. According to this method, the MDA- TBA (Thiobarbituric acid) adduct formed by the reaction of MDA and TBA under high temperature (90-100°C) and acidic conditions causes the formation of a pink colored product (Thiobarbituric acid reactive substances). As the proteins of liver tissue homogenate can also react with TBA and generate the chromatic product, these proteins is initially precipitated with 40% trichloroacetic acid (TCA).

In brief, 0.5µl of TCA was mixed with 100µl of tissue extract and was placed in a boiling water bath for 15min. 0.5ml of TBA was then added to this mixture. The absorbance was measured at 532nm. Measurements were expressed in terms of µmole MDA g^{-1} of protein.

Measurement of dityrosine

Dityrosine (DT) is a specific product of protein oxidation and is formed by various oxidation systems. It is a highly fluorescent molecule that is resistant to acid hydrolysis and protease activity [Dalle-donne *et al*., 2001]. DT was analyzed by reversed- phase HPLC with simultaneous UV-detection (280nm) and fluorescence-detection (ex.280nm, em.410nm). A HPLC column equipped with a guard column was used for these analyses. A gradient was formed from 10mM ammonium acetate, adjusted to pH 4.5 with acetic acid, and methanol, starting with 1% methanol and increasing to 10% over 30 min. The flow rate was 0.8 ml/min. A standard DT sample was prepared according to the method of Ref. The level of DT was expressed as µmole DT g^{-1} of protein [Amado *et al*., 1984].

Assessment of Aloe-emodin and Barbaloin antiglycation property on liver tissue

Pentosidine content assay

Pentosidine as a AGEPs was quantified using the method of Dyer *et al*. (1991). Initially, the tissue extract was dialyzed against phosphate buffer at a ratio of 1000:1 for 24h, changing the dialysate every 8h. The concentrated extract then hydrolyzed in 6M HCL for 12h at 110°C. After hydrolysis, hydrochloric acid was removed by evaporation, water was added, and the hydrolyzed extract was neutralized by the addition of NaOH. Pentosidine was analyzed by reversed- phase HPLC with simultaneous fluorescence-detection (ex. 325nm, em.385nm).

So, 100 µl of the hydrolyzed extract was injected on a Nova-pak c-11 reversed-phase column and was eluted with 0.1% trifluoroacetic acid and acetonitrile over 80 min. the flow rate was 0.8 ml/min. The pentosidine standard was detected by fluorescence at ex. 325/ em.385 nm and eluted at approximately 67 min. According to the standard curve, the pentosidine content was expressed in terms of pmole pentosidine mg^{-1} of protein.

Glyoxal content assay

Glyoxal is an organic compound with the formula OCHCHO. This yellow colored liquid is the smallest dialdehyde (two aldehyde groups) and is generated by the glucose autooxidation. Liver glyoxal content was determined as described by Wells-Knecht *et al*. (1995). In brief, a mixture consisting of 100 µl of tissue extract, 800 µl of sodium formate (pH 2.9), and 100 µl of Girard′s reagent T was incubated at 37°C for 30 min. Following incubation, the absorbance was measured at 294 nm. According to the standard curve, the gloxal content was expressed as µmole glyoxal g^{-1} of tissue.

Statistical analysis

All the experiments were performed

at different concentrations of Aloe-emodin and Barbaloin (0-12 mM) and repeated three times. The experimental results values had been presented in terms of mean± standard deviation of three parallel measurements. The data obtained were assessed by Anova test with a significance level of $P<0.05$.

RESULTS

Antioxidant activity

Inhibition of DPPH and/or diminish in superoxide radicals by Aloe-emodin increased from 0.35 to 1.5 mM treatment in which significant increase ($P<0.05$) was observed for 0.75 and 1.5 mM with respect to control. Then, increase in exposure concentration caused decrease in these parameters and reached them to about 50% and 70% of control at 12 mM for DPPH and superoxide scavenging respectively (Table 1).

However, Barbaloin exhibited significant scavenging activity only at high concentrations (3-12 mM). At concentrations ranging from 0.35 to 1.5 mM, the scavenging activity by Barbaloin decreased. DPPH and superoxide scavenging parameters reached to about 80% and 90% of control at 0.35 mM. After then, increase in concentration of treatment (3-12 mM), significantly ($P<0.05$) increased these indexes as compared with the control value (Table 1).

In addition, the evaluation of total antioxidant capacity showed that there was significant ($P<0.05$) increase at low concentrations (0.75 and 1.5 mM) for Aloe-emodin and at high levels (3, 6, and 12 mM) for Barbaloin respectively (Table 1).

Assessment of liver antioxidant enzymes activity

The effects of Aloe-emodin and Barbaloin were tested on the activity of liver antioxidant

Table 1. Free radical- scavenging effects and total antioxidant capacity (TAC) of Aloe-emodin and Barbaloin at various concentrations

Concentration	Inhibition of DPPH (%)		Inhibition of $O_2^{\cdot}$(%)		TAC	
(mM)	Aloe-emodin	Barbaloin	Aloe-emodin	Barbaloin	Aloe-emodin	Barbaloin
0	9.93± 0.73	9.93± 0.73	23.3± 1.10	23.3± 1.10	0.40± 0.04	0.40± 0.04
0.35	11.36± 1.56	8.46± 0.65	23.1± 1.51	22.1± 1.61	0.42± 0.10	0.49± 0.07
0.75	17.13± 1.48*	8.46± 0.65	24.4± 0.45*	22.6± 0.88	0.63± 0.11*	0.57± 0.13
1.5	18.16± 1.59*	10.03± 1.92	29.9± 1.61*	24.03± 1.41	0.7± 0.07*	0.59± 0.09
3	12.73± 1.2	14.3± 0.91*	27.7± 1.24	26.6± 0.62*	0.4± 0.09	0.85± 0.08*
6	6.56± 0.45	15.93± 1.85*	20.7± 1.16	27.9± 1.30*	0.38± 0.09	1.04± 0.16*
12	4.76± 0.95	16.8± 1.01*	17.7± 1.10	28.2± 0.80*	0.37± 0.11	1.21± 0.21*

Data are expressed as the mean± S.D. of three parallel measurements.
Data are statistically significant at *P< 0.05 as compared with the control value.

Table 2. Inhibitory effect of various concentrations of Aloe-emodin and Barbaloin on the formation of liver pentosidine and glyoxal compounds

Concentrations	Pentosidine content (pmole mg^{-1} of protein)		Glyoxal content (µmole g^{-1} of tissue)	
(Mm)	Aloe-emodin	Barbaloin	Aloe-emodin	Barbaloin
0	25.6± 1.15	25.6± 1.15	25.06± 2.15	25.06± 2.15
0.35	30.53± 3.22	21.03± 1.68	23.33± 1.41	27.1± 2.6
0.75	21.6± 1.95*	18.23± 1.55	18.10± 1.47*	16± 1.6
1.5	16.93± 0.75*	22.23± 2.05	15.66± 1.62*	10.8± 0.88
3	26.86± 0.85	18.3± 2.05*	16.8± 1.15	9± 0.78*
6	28.56± 1.9	10.4±0.87*	17.2± 1.75	8.5± 0.62*
12	36.2± 3.1	8.5± 0.7*	34.8± 3.05	5.23± 0.76*

Data are expressed as the mean± S.D. of three parallel measurements.
Data are statistically significant at *P< 0.05 as compared with the control value.

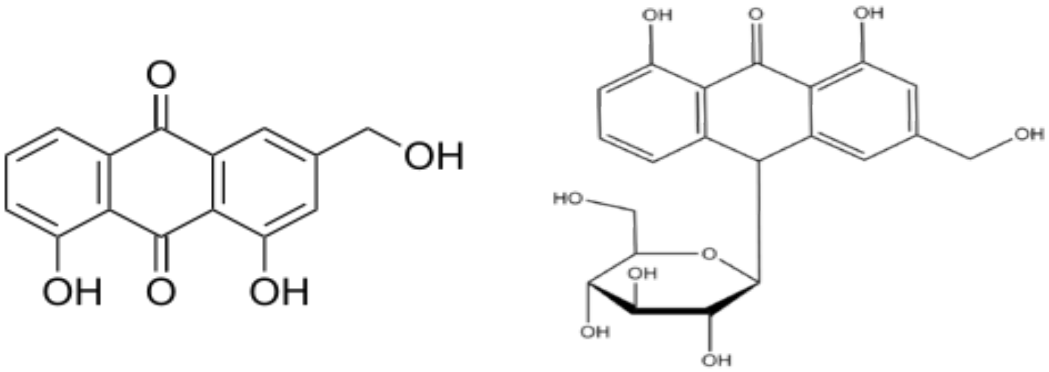

Fig. 1. Structure of Aloe-emodin, 1,8-Dihydroxy-3-(hydroxymethyl) anthraquinone (a) and Barbaloin, 10-beta-D- glucopyranosyl-1,8 dihydroxy-3-hydroxymethyl-9(10H)- anthracenone (b)

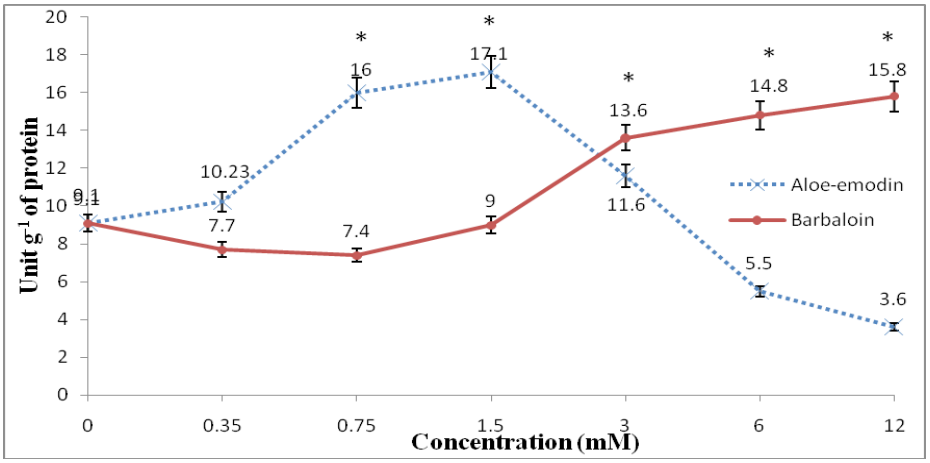

Fig. 2. Comparison of the effect of Aloe-emodin and Barbaloin on liver superoxide dismutase activity at different concentrations. The effect of (…x…) Aloe-emodin and (-•-) Barbaloin on the activity of liver superoxide dismutase enzyme at various concentrations was measured. Values are given as the mean± S.D. of three parallel measurements. Values are statistically significant at *P< 0.05 as compared with the control value.

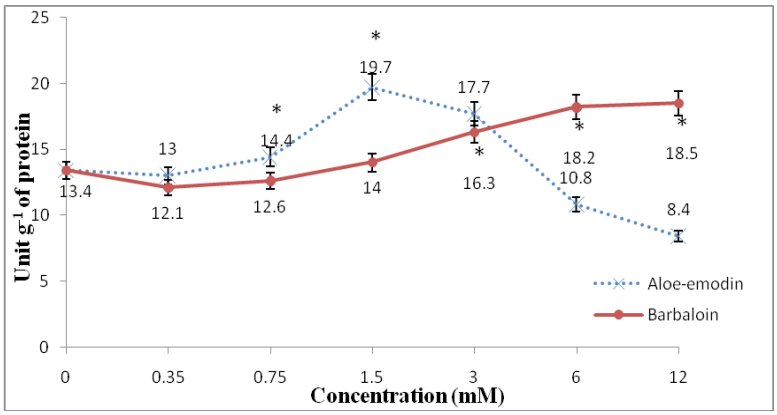

Fig. 3. Comparison of the effect of Aloe-emodin and Barbaloin on liver catalase activity at different concentrations. The effect of (…x…) Aloe-emodin and (-•-) Barbaloin on the activity of liver catalase enzyme at various concentrations was measured. Values are given as the mean± S.D. of three parallel measurements. Values are statistically significant at *P< 0.05 as compared with the control value

enzymes, including SOD, CAT, and GPX (Figs. 2-4). Aloe-emodin enhanced the antioxidant enzymes activity at concentrations from 0.35 to 1.5 mM in which significant increase (P<0.05) was observed at 0.75 and 1.5 mM as compared with control. Elevation in concentration of treatment caused decrease in the activity of these enzymes and reached to about 40%, 63%, and 85% of control at 12 mM for SOD, CAT, and GPX activity respectively (Figs. 2-4).

On the other hand, Barbaloin caused decrease in the activity of these enzymes at concentrations ranging from 0.35 to 1.5 mM. Increase in exposure concentration enhanced the activity of these enzymes and significant raise at their levels (P<0.05) was observed particularly in treatments with 3, 6, and 12 mM with respect to control (Figs.2-4).

Assessment of liver oxidative damage biomarker levels

Figures 5 and 6 showed the effects of Aloe-emodin and Barbaloin on the levels of liver oxidative damage biomarkers, including MDA and DT. According to these Figures, Aloe-emodin had remarkable ability to reduce the levels of these biomarkers at concentration range from 0.75 to 1.5 mM that with respect to control. With increase in treated concentrations, the levels of

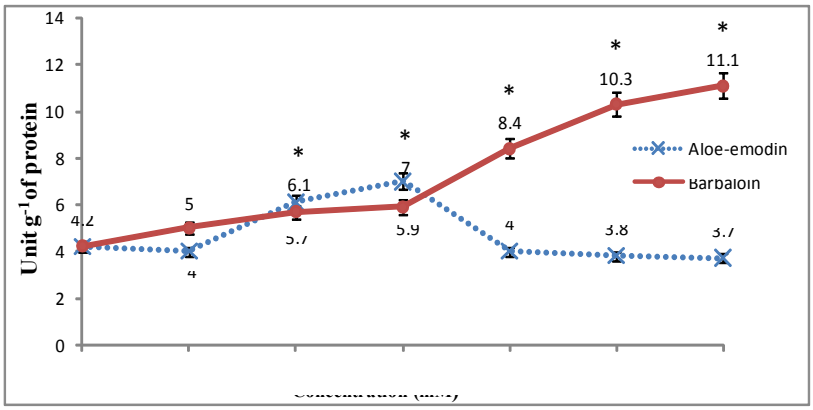

Fig. 4. Comparison of the effect of Aloe-emodin and Barbaloin on liver glutathione peroxidase activity at different concentrations. The effect of (…x…) Aloe-emodin and (-•-) Barbaloin on the activity of liver glutathione peroxidase enzyme at various concentrations was measured. Values are given as the mean± S.D. of three parallel measurements. Values are statistically significant at *P< 0.05 as compared with the control value

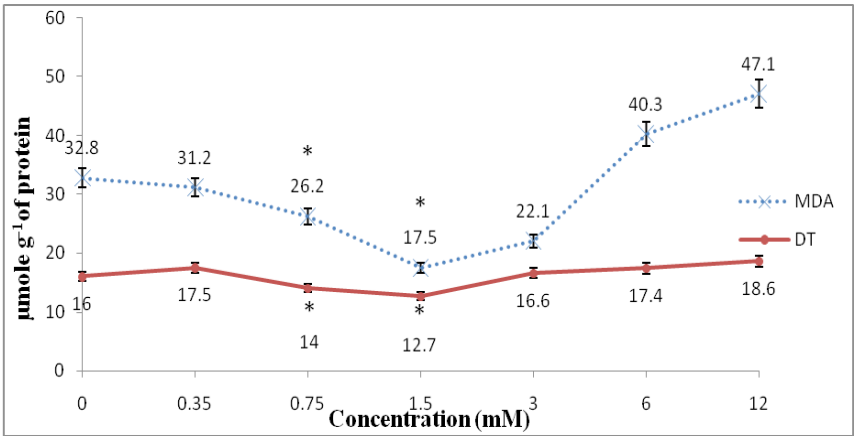

Fig. 5. The effect of various concentrations of Aloe- emodin on the levels of liver malondialdehyde and dityrosine. The effect of Aloe-emodin on the levels of liver (…x…) malondialdehyde and (-•-) dityrosine at various concentrations was measured. Values are given as the mean± S.D. of three parallel measurements. Values are statistically significant at *P< 0.05 as compared with the control value

these biomarkers increased and reached to about 1.5 and 1.2 times of control at 12 mM for MDA and DT levels respectively (Figs. 5 and 6).

However, Barbaloin increased the levels of these biomarkers at low concentrations (0.35-1.5 mM). At treated concentrations from 3 to 12 mM, the levels of these parameters by Barbaloin significantly decreased (P<0.05) with respect to control (Figs 5 and 6).

Effects of Aloe-emodin and Barbaloin on liver AGEPs content

The results of antiglycation tests showed that Aloe-emodin (P<0.05) reduced significantly the liver AGEPs content, including pentosidine and

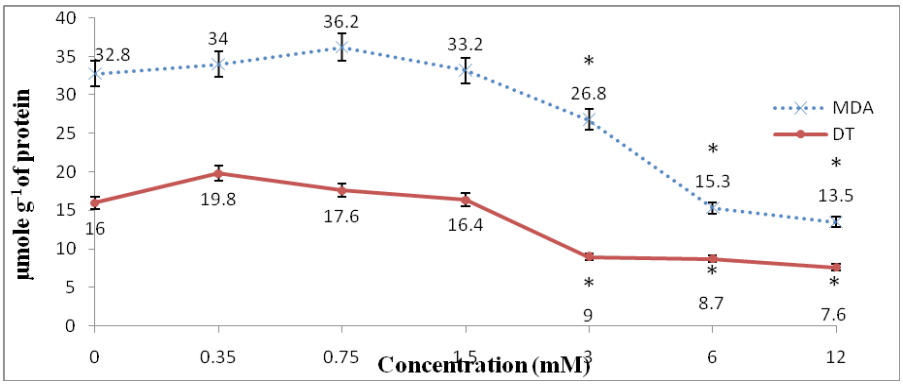

Fig. 6. The effect of various concentrations of Barbaloin on the levels of liver malondialdehyde and dityrosine. The effect of Barbaloin on the levels of liver (…x…) malondialdehyde and (-•-) dityrosine at various concentrations was measured. Values are given as the mean± S.D. of three parallel measurements. Values are statistically significant at *P< 0.05 as compared with the control value

glyoxal, at the concentrations of 0.75 and 1.5 mM as compared with control. Increase of treatment concentration caused elevation in content of these biomarkers and reached them to about 1.5 and 1.4 times of control at 12 mM for pentosidine and glyoxal content respectively (Table 2).

The content of these parameters by Barbaloin decreased from 0.75 to 12 mM treatment in which significant decrease (P<0.05) was observed for 3, 6, and 12 mM, as compared with the control value (Table 2).

DISCUSSION

Aloe vera has been widely used for medicine, food supplements and cosmetic purposes in recent years [Tian and Hua, 2004]. The therapeutic properties of this plant include antibiotic, analgesic, anti-inflammatory, antiseptic, anti-tumoral, antiviral, antioxidant, immuno-modulating and wound healing [Bassetti and Sala, 2005]. This plant is mainly consumed in the form of nutritive products. Following the consumption of these products, many of it′s constitutives are absorbed and entered into the blood and can influence on different tissues such as liver.

Since liver is the most important organ for metabolism of food and drug, the possible effect of Aloe vera on liver can be considered as a big challenge. As it has not been investigated so far, if Aloe-emodin and Barbaloin, the two main active compounds of Aloe vera, have any effect on liver, this issue has been evaluated in this research. According to our results, Aloe-emodin and Barbaloin have opposed anti-oxidative activity in liver tissue at low and high concentrations respectively.

These results are correlated with the report of Tian and Hua (2004) in which, antioxidant and prooxidant effects of Aloin and Aloe-emodin on free radical-induced DNA breaks were studied in a wide range of concentrations. They found that these compounds can have distinct effects on DNA breaks at different concentrations. At low concentrations, Aloin increases DNA damage by about 24-74%, indicating it′s prooxidant effect. With increase of concentration, however, free radical-scavenging activity increases through this compound and it can prevent ·OH-induced DNA breaks by 5-30% over control values. But Aloe-emodin at these

concentrations range has a prooxidant effect on DNA (about 29-35%). Therefore, choosing the right dose is a significant step in pharmaceutical applications of these antioxidants.

To prove the antioxidant property of these compounds, DPPH radical-scavenging method was also used. Arun *et al*. (2012) also utilized this method to evaluate the antioxidant activity of Aloin at different doses (100 and 250 µg/ml). The result of their study showed that Aloin has a considerable antioxidant activity (68% at 100µg/ml) compared to ascorbic acid as the control group. In addition, it's radical scavenging activity has been indicated to be 73% at 250µg/ml. Thus, the antioxidant activity of Aloin rises in a dose dependent manner.

Besides, Aloe-emodin had significant antioxidant property in liver tissue at low concentrations. However, increasing the concentration of treatment ended to lowering this property. This finding can be contributed to it's different structure characteristics. Aloe-emodin has a ketone group in C-10 position. Since the ketone group is replaced by a glycoside group in Barbaloin structure, this compound only showed significant antioxidant property at high concentrations. Antioxidant property of Barbaloin might be partially due to the separation of the anomeric hydrogen from the C-10 glucoside group. Accordingly, when the concentration increases, its antioxidant property also soars because the anomeric hydrogen can be easily separated from C-10 glucoside group [Tian and Hua, 2004].

Recently, there has been widespread use of Aloe vera particularly in form of gel and juice among people. Aloe vera can have different effects on different tissues of body as xenobiotics. Liver is a vital organ with central role in detoxifying of xenobiotics and chemicals. Drugs and chemicals (xenobiotics) may also affect liver function and cause the acute liver failure with the risk of liver transplantation or even death.

In response to these effects, liver antioxidant defence system may be triggered. This system plays a crucial role in the health of the liver and the reduction of it could contribute to the liver injury development through the increased production of oxidative damage biomarkers. In the present study, the effects of Aloe-emodin and Barbaloin on liver antioxidant enzymes activity and oxidative damage biomarker levels have been evaluated.

Our study indicated that Aloe-emodin can significantly increase liver antioxidant enzymes activity and reduce oxidative damage biomarker levels at low concentrations (0.75 to 1.5 mM). On the contrary, Barbaloin can do the same process at higher concentrations (3 to 12 mM), compared to control group.

These results are in accordance with the findings of studies carried out by Anilakumar *et al*. (2010); Amouoghli and Mohajeri (2012) and Ramachandraiahgari *et al*. (2012). The first study reviewed the effect of oral feeding of Aloe vera gel extract in a period of 30 days on rats that their oxidative stress was induced by azoxymethane. It was observed that administration of azoxymethane reduced the hepatic glutation (GSH) content and the antioxidant enzymes activity such as catalase, superoxide dismutase and glucose-6-phosphate dehydrogenase. They revealed that feeding of Aloe vera gel extract caused the GSH content to be increased which leaded to protection against azoxymethane-induced lipid peroxidation. Additionally, this extract caused a significant raise in hepatic antioxidant enzymes activity. Hence, they suggested that Aloe vera gel extract had the ability to reduce azoxymethane-induced oxidative stress and toxicity in liver.

Moreover, Amouoghli and Mohajeri (2012) investigated the effect of Aloe vera leaves extract on oxidative stress of hepatic tissue in streptozotocin-induced diabetic rats. They found that daily treatment of this extract in dosage of 50mg/kg body weight for 8 weeks can cause a significant decrease and increase in hepatic MDA and GSH content respectively. In addition, this extract caused a substantial enhancement of antioxidant enzymes activity of liver tissue. On this basis, they concluded that the extract alleviates oxidative stress of hepatic tissue in streptozotocin-induced diabetic rats and ameliorates diabetic hepatopathy through it′s antioxidant activity.

Also, Ramachandraiahgari *et al*. (2012) studied the antioxidant effects of Aloe vera ethanolic extract on liver and kidney of streptozotocin-induced diabetic rats. The extract administered orally to diabetic rats with a dosage of 300 mg/kg body weight for 30 days. According to results, there were significant increase in lipid peroxidation, and decrease in activity of liver and kidney antioxidant

enzymes including superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase as compared with control group. However, when the rats were treated with Aloe vera extract, lipid peroxidation level decreased significantly and the antioxidant enzymes activity increased in liver and kidney compared to diabetic rats.

Since Aloe-emodin and Barbaloin had significant antioxidant property in liver tissue at low (<2.5 mMol) and high (>2.5 mMol) concentrations respectively, they could improve the antioxidant status in liver at these concentrations [Rajasekaran *et al*. 2005].

Aloe vera, used internally and/or topically, can provide great benefit. It′s constituents provide direct effect or stimulate body′s natural defenses, including those against glycation. Glycation is a non-enzymatic process which it′s progress is dependent on blood glucose concentration. So, this reaction is done at a constant slow rate in normal body.

In diabetes disease it is noticeably accelerated because of the high availability of glucose. Glycation of proteins and enzymes can clearly alter their structure, enzymatic activity and biological half-life. In the present study, the antiglycation property of Aloe-emodin and Barbaloin was evaluated in liver tissue.

Our observation confirms that Aloe-emodin has significant antiglycation property in liver tissue at low concentrations, but increasing Barbaloin concentration leads to enhancement of it′s antiglycation property.

Our results were in accordance to other reports such as Can *et al*. (2004); Rajasekaran *et al*. (2005) and Yagi *et al*. (2009). The first study investigated the effect of Aloe vera gel extract on the liver tissue of streptozotocin-induced type II diabetic rats. An increase in Non-enzymatic glycosylation (NEG) of liver proteins is a well-known sign of diabetic damage. In the liver of diabetic rats, NEG values were increased significantly in comparison to healthy rats. They found that high NEG level of liver tissue provoked by diabetes was reduced significantly by Aloe vera gel extract. Therefore, the Aloe vera gel could have a beneficial effect on liver when it is used as a hypoglycemic agent in treatment of type-II diabetes.

Furthermore, Rajasekaran *et al*. (2005) studied the effect of Aloe vera gel extract on glycosylated hemoglobin levels in streptozotocin-induced diabetic rats. They found that Oral administration of Aloe vera gel extract at concentration of 300 mg/kgb.w. to diabetic rats significantly decreases the levels of glycosylated hemoglobin. This finding can be due to the improvement in glycemic status. Hence, they recommended the usage of Aloe vera as an antidiabetic agent to diabetic cases.

In other study, the hypoglycemic effect of Aloe vera high molecular weight fractions (AHM) on type II diabetic patients was studied by Yagi *et al*. (2009). They found that continuously administration of AHM for three times a day in 12 weeks leading to significant decrease of blood glucose level. Also, HbA1c value was reduced which is a global standard for long range level of the blood glucose. This result confirms the hypoglycemic effect of AHM.

In diabetes disease, liver tissue could damage by enhanced oxidative stress [Koruk *et al*., 2004] and elevated AGEs level [Hyogo *et al*., 2007] that may be are associated with chronic liver disease, particularly NASH. Therefore, it can be expected that compounds such as Aloe-emodin and Barbaloin with antioxidant and antiglycation properties on liver tissue at a certain dose range can neutralize the effective factors, oxidative stress and AGEPs, in liver disease development that is causing by diabetes such as NASH. Consequently, they can remarkably protect liver tissue against this disease.

Although the results of this study are promising, further researches seem essential for *in vivo* investigation of the antioxidation and antiglycation properties of Aloe-emodin and Barbaloin in the liver tissue of streptozotocin-induced diabetic rats.

CONCLUSION

In vitro investigation of antioxidation and antiglycation properties of Aloe-emodin and Barbaloin, the two main active compounds in Aloe vera, were performed in mice liver tissue. we observed that Aloe-emodin and Barbaloin had significant antioxidant property in liver tissue at low (<2.5 mM) and high (>2.5 mM) concentrations respectively. Therefore, at these concentrations

range, these compounds can increase liver antioxidant enzymes activity and decrease oxidative damage biomarker levels, compared to control value.

Besides, as these compounds had considerable antiglycation property in liver tissue at a certain dose range, it can be suggested that using them can neutralize the effective factors, oxidative stress and AGEPs, in NASH disease development and can protect the liver tissue against this disease.

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