Research Article

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Evaluation of diphenyl dimethyl bicarboxylate (DDB) as a probable hepato- protector in rats against whole body gamma irradiation

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Many antioxidants have been studied as hepatoprotectors against ionizing radiation injury since they reduce the oxidative effect of the reactive oxygen species to normal cells. Diphenyl dimethyl bicarboxylate (DDB) has been clinically used against chemically damaged liver since it has an antioxidant action for scavenging free radicals and inhibiting lipid peroxidation reactions. Ionizing radiation is known to induce disturbances in serum liver aspartate aminotransferase and alanine aminotransferase (AST and ALT), alkaline phosphatase (ALP) activities and proteins (total protein, albumin and globulin) levels. The aim of the present work was to evaluate if DDB pre- or/and post- treatments have a hepatoprotector effect against gamma (γ) irradiation damaged liver. DDB was administered daily with a dose of 50 mg/Kg body weight to whole body irradiated rats with 4.5 Grey (Gy). A follow up period of five weeks post-irradiation showed an improvement in the disturbed different parameters in the irradiated group. The pre-treated, post-treated and pre- and post-treated animals with DDB against irradiation showed earlier and potent improvement, since it increased total protein and albumin levels to near that of the control. On the other hand, DDB reduced the altered activity of AST, ALT and ALP earlier than the irradiated group. It was concluded that all the treatments with DDB have a hepato-radioprotective effect and the pre- and post- treatment has the most potent effect against the alterations induced by irradiation.

Key words: Gamma-Irradiation, Radioprotector, Hepatoprotector, Transaminases, ALP, Serum proteins.

The most important resources to know about the effect of radiation on man are the tens of thousands of persons who deal with radiation daily in hospitals, universities, military units, national laboratories, and government agencies (Mettler and Voelz, 2002). Radiation therapy has been used in cancer treatment for many decades; it is used to eradicate cancer and as a palliative to relieve pain associated with metastases (Borek, 2004). Low doses of ionizing radiation should not be considered insignificant with regard to increasing the incidence of somatic mutations (neoplastic and non-neoplastic diseases) and heritable mutations in humans owing to its interaction with other toxins that can enhance damage produced by irradiation. It is very prudent to continue to support the well-established radiobiological concept that "no radiation dose can be considered completely safe", and that all efforts must be made to reduce both the radiation dose and biological damage, no matter how small that damage might be, without signifying the benefits of radiation (Prasad, 2005).

Radiations can damage cells by direct effect on deoxyribonucleic acid (DNA) and other cellular targets and by indirect effect through reactive oxygen species (ROS) that radiolysis. produced through water Exposure to ionizing radiation produces oxygen-derived free radicals in the issue environment; those include hydroxyl radicals (the most damaging), super oxide anion radicals and other oxidants such as hydrogen peroxide (Borek, 2004). The generated oxygen species result in oxidative damage to cell membranes, disturbance in metabolism as well as in structure and function of body organs (Kaushala, 2004).

Antioxidants have been studied for their capacity to reduce the harmful effects of radiation on normal tissues for at least 50 years (Okunieff *et al.*, 2008). Antioxidants supplements may reduce treatment-related adverse effects during radiation therapy by reducing oxidative damage to normal cells (Lawenda *et al.*, 2008).

Results from animal experiments indicate that antioxidant nutrients are protective against lethality and other radiation effects but to a lesser degree than most synthetic protectors. Some antioxidant nutrients and phytochemicals have the advantage of low toxicity although they are generally protective when administered at pharmacological doses. A number of phytochemicals including caffeine, genistein, and melatonin, have multiple physiological effects, as well as antioxidant activity, which result in radioprotection in vivo (Weiss and Landauer, 2003).

Diphenyl dimethyl bicarboxylat, or full name dimethyl -4, 4'-bimethyloxy -5, 6, 5', -dimethylene-dioxydiphenyl-2, 2'-6' bicarboxylate (DDB), is a synthesized intermediate derivative of Shizandrin C. an 1active component isolated from Fructus Schizandrae (Wang and Xu, 2008). It is a 2hepatoprotector against experimental liver injury in mice and rats. It has been also clinically used for the treatment of viral hepatitis B and C for more than 20 years in 3-China, and also in Korea and Egypt for almost ten years; where no side effects have been reported up to now (Jin et al., 4-2007). DDB is used in the treatment of chronic viral hepatitis for improving liver functions; it could reduce serum ALT activity in animal models and in humans. DDB has also antioxidant action for scavenging free radicals and inhibiting lipid 5peroxidation reaction in vitro systems (Wang and Xu, 2008).

In this respect, the present work was carried out to study hepatoprotector effect of DDB against whole body γ -irradiation- 6induced alterations in serum liver transaminases, alkaline phosphatase activities and proteins levels in rats.

MATERIALS AND METHODS

Animals: Forty Female Wistar Albino rats aging 4 - 5 month and weighing about 120-160 g were obtained from the animal house of the National Research Center, Giza, Egypt. The animals were kept in wellventilated cages at room temperature and under controlled light cycles. They were maintained on normal laboratory rats chow and water *ad libitum*. All the animal experiments were performed according to the regulations of Animal Ethics Committee, Government of Egypt.

Irradiation: Whole body gammairradiation was performed at Middle Eastern Regional Radioisotope Center for Arab Countries (MERRCAC) in Cairo, Egypt, using Cobalt 60 Gamma-3500 Noraton– Norcontrol AS irradiation unit. Animals were whole body irradiated with a single dose of 4.5 Gy gamma radiation delivered at a dose rate of 1.23 rad per second during the irradiation procedures.

Chemicals: Dimethyl- 4, 4'bimethyloxy-5, 6, 5', 6'-dimethylenedioxy diphenyl- 2, 2'- bicarboxylate (a product imported from Guang Zhou Xing Qun Pharmaceutical, China) was purchased from an Egyptian pharmacy.

Experimental Design: Animals were divided into six groups, two of them containing six rats each. The other four groups, containing seven rats each; were whole body gamma irradiated with 4.5 Gy gamma rays. These groups are:

Control group: Animals that did not receive any treatment.

DDB group: Animals were orally administered aqueous suspension of DDB by a daily single dose of 50 mg/kg body weight for five weeks.

Irradiated group: Animals were irradiated with a dose of 4.5 Gy and kept without DDB treatment for five weeks.

Pre-treated group: Animals were orally treated with aqueous suspension of DDB (a daily single dose of 50 mg/kg body weight) one week pre-irradiation with 4.5 Gy and then followed up (without treatment) for five weeks.

Post-treated group: Animals were irradiated with 4.5 Gy and then orally treated with aqueous suspension of DDB (a daily single dose of 50 mg/kg body weight) for five weeks post irradiation.

Pre- and Post- treated group: Animals were orally treated with aqueous suspension of DDB (a daily single dose of 50 mg/kg body weight) one week preirradiation with 4.5 Gy and continuously treated with the same dose of DDB for five weeks post irradiation.

Blood samples from control and irradiated rats collected by standard venipuncture with glass capillaries tubes from all rats groups weekly for five week post-irradiation (1st, 2nd, 3rd, 4th, 5th weeks). The sera were separated to individual Eppendorff vials and stored at -4°C till time of assay.

Biochemical Studies: Six biochemical parameters were chosen to be estimated in the sera since they are considered to be an indicator for the activity of liver. Five parameters AST, ALT (German Society for Clinical Chemistry, 1970), alkaline phosphatase (German Society for Clinical Chemistry, 1972), total protein (Henry, 1964) and albumin (Doumas *et al.*, 1971), were biochemically assayed using prepared commercial kits from Diamond Diagnostics. The sixth one (globulin) were calculated by subtracting the individual value of albumin from the corresponding value of total protein.

The mean values of each group were represented as curves. The results of the different parameters were statistically analyzed using Two Way Classification Analysis of Variance (F-Test) and Multiple Range Test.

RESULTS

Serum Total Protein: Figure (1) illustrates the variation in the mean values of serum total protein level in different treated groups through a follow up period of five weeks. Table (1) shows the multiple range test which illustrates the degree of significance between each pair of the groups. The standard error values of the different groups were in the range of 0.03-0.39, 0.12-0.7, 0.17-0.42, 0.24-0.62, 0.12-0.54 and 0.25-0.69 (lowest value and highest value of all week within each group) for the control group, the DDB treated group, the irradiated group, the DDB pre- treated irradiated group, the DDB post- treated irradiated group and the DDB pre and post treated irradiated group respectively. The values of SE were less than 10% of the mean value of the same group. Two way analysis of variance F-test for total protein was highly significant in case of different treatments (F1=14.5831**) and insignificant in case of five weeks follow up period (F2=3.801+). The mean values of total protein level in the irradiated group very highly significantly decreased compared to the control groups (Table 1). This decrease observed from the first week post irradiation and began to be improved at the fourth week (Fig. 1).

Figure (1): Total Protein (g/dl) 7.5 6.5 Total Protein (g/dl) 5.5 4.5 –<u>⊿</u>– DDB 3.5 -Irrad. DDB Post 2.5 DDB Pre DDB Pre&Post 1.5 2 3 5 1 4 weeks

Figure: 1. Serum total protein in different groups at 1st to 5th week post-irradiation.

Serum Albumin: Figure (2) illustrates the variation in mean values of serum Albumin level in different treated groups through a follow up period of five weeks. Table (2) shows the multiple range test which illustrates the degree of significant between each pair of the groups. The standard error values of the different Albumin groups were in the range of 0.1-0.35, 0.04-0.35, 0.15-0.21, 0.17-0.57, 0.18-0.41 and 0.07-0.26 (lowest value and highest value within each group) for the control group, the DDB treated group, the irradiated group, the DDB pre-irradiation treated group, the DDB postirradiation treated group and the DDB pre irradiation and post treated group respectively.



Figure: 2. Serum albumin level in different groups at 1st to 5th week post-irradiation.

Two way analysis of variance F-test for Albumin was significant in case of different treatments (F1=6.815*) and insignificant in case of five weeks follow up period (F2=4.872⁺). The level of serum albumin in the irradiated group decline than the control groups (Figure 2). This decline was highly significant compared to the control group (Table 2). The level of the albumin of the irradiated group started to be improved at the 3rd week but doesn't reach the control level (Figure 2). The post-treated group and the pre- and post-treated group showed significant increase in albumin level compared to the irradiated group (Table 2). The improvement in those DDB treated groups was earlier and more potent than that occur in the irradiated group (Figure 2). Both groups have insignificant change compared to the control group. The DDB pre-treated group showed no significant difference with both the irradiated group and

Groups	Irradiated	DDB Pre-treated	DDB Pre + post	DDB	DDB Post-treated
Control	2.0***	1.44***	0.77*	0.47†	0.3†
DDB Post-treated	1.7***	1.14**	0.47†	0.17†	
DDB	1.53***	0.97**	0.3†		
DDB Pre + post	1.23***	0.67†			
DDB Pre-treated	0.56†				

Table 1: Multiple range test illustrating the degree of significant difference in total protein among different groups.

*** Very highly significant P<0.001, ** Highly significant P<0.01, * Significant P<0.05, †Insignificant P>0.05.

Table 2: Multiple range test illustrating the degree of significant difference in albumin among different groups.

Groups	Irradiation	DDB Pre-treated	DDB Pre + post	DDB Post-treated	control
DDB	0.72**	0.43*	0.21†	0.13†	0.04†
control	0.68**	0.39†	0.17†	0.09†	
DDB Post-treated	0.59**	0.3†	0.08†		
DDB Pre + post	0.51*	0.22†			
DDB Pre-treated	0.29†				

*** Very highly significant P<0.001, ** Highly significant P<0.01, * Significant P<0.05, †Insignificant P>0.05.

Table 3: Multiple range test illustrating the degree of significant difference in Globulin among different groups.

Globulin groups	Irradiated	DDB Pre-treated	DDB Pre + post	DDB	DDB Post-treated
control	1.29***	0.88***	0.65**	0.52*	0.21†
DDB Post-treated	1.08***	0.67**	0.44†	0.31†	
DDB	0.77**	0.36†	0.13†		
DDB Pre + post	0.64**	0.23†			
DDB Pre-treated	0.41†				

*** Very highly significant P<0.001, ** Highly significant P<0.01, * Significant P<0.05, †Insignificant P>0.05.

the control groups. The degrees of significant between each pair of the groups are shown in (Table 2).

Serum Globulin: The results of serum globulin are represented in figure (3) and table (3). Figure (3) illustrates the variation in mean values of serum globulin level in different treated groups through a follow up period of five weeks. Table (3) shows the multiple range test which illustrates the degree of significant between each pair of the groups. The standard error values of the globulin different groups were in the range of 0.12-0.33, 0.21-0.29, 0.12-0.3, 0.12-0.25, 0.12-0.39 and 0.06-0.75 (lowest value and highest value within each group) for the control group, the DDB treated group, the irradiated group, the DDB pre-irradiation treated group, the DDB post-irradiation treated group and the DDB pre and post irradiation treated group respectively.

Two way analysis of variance F-test for globulin was very highly significant in case of different treatments (F1=619.8***) and also very highly significant in case of different follow time intervals up (F2=745.3***). Globulin level in the irradiated animal group showed very highly significant reduction compared to the control group (Table 3). A marked improvement in the level of globulin in the irradiated group started after the 3rd week but its level still away from the control level (Figure 3).

Figure: 3. Serum globulin level in different groups at 1st to 5th week post-irradiation.



The post-treated group with DDB restored the globulin to the control level, while the pre-treated group insignificantly affects the globulin level compared to the irradiated group. The pre and post-treated group showed highly significant decrease in the globulin level compared to the control level and highly significant increase than irradiated group level. The degrees of significant between each pair of the groups are illustrated in table (3). **Serum AST:** The results of serum AST are

represented in figure (4) and table (4). Figure (4) illustrates the variation in mean values of serum AST level in different treated groups through a follow up period of

AST groups	DDB	Control	DDB Pre + post	DDB Pre-treated	DDB Post-treated
Irradiation	171.84***	171.05***	97.32***	83.94***	64.89***
DDB Post-treated	106.95***	106.16***	32.43***	19.05**	
DDB Pre-treated	87.9***	87.11***	13.38†		
DDB Pre + post	74.52***	73.73***			
Control	0 79+				

Table 4: Multiple range test illustrating the degree of significant difference in AST among different groups.

*** Very highly significant P<0.001, ** Highly significant P<0.01, * Significant P<0.05, †Insignificant P>0.05.

Table 5: Multiple range test illustrating the degree of significant difference in ALT among different groups.

ALT Groups	DDB	Control	DDB Pre + post	DDB Pre-treated	DDB Post-treated
Irradiated	19.32***	17.93***	12.07***	10.24**	9.31**
DDB Post-treated	10.01**	8.62*	2.76†	0.93†	
DDB Pre-treated	10.08**	7.69*	1.83†		
DDB Pre+post	7.25*	5.86†			
control	1 39 1				

*** Very highly significant P<0.001, ** Highly significant P<0.01, * Significant P<0.05, †Insignificant P>0.05.



Figure: 4. Serum AST level in different groups at 1st to 5th week post-irradiation.

five weeks. Table (4) shows the multiple range test which illustrates the degree of significant between each pair of the groups. The standard error values of the different AST groups were in the range of 5.09-8.05, 3.47-6.06, 9.74-20.68, 6.49-15.97, 8.72-24.4 and 3.98-18.95 (lowest value and highest value within each group) for the control group, the DDB treated group, the irradiated group, the DDB pre-irradiation treated group, the DDB post-irradiation treated group and the DDB pre and post irradiation treated group respectively. Two way analysis of variance (F-test) for AST was highly significant in case of different (F1=170.12**) treatments and highly significant in case different follow up time intervals (F2=116.19**) The AST results showed very highly significant increase in AST level of the irradiated group compared to control group (Table 4). The maximum of this increase appears within 2-3 weeks then the level of the AST began to decline after

the 3rd week (Figure 4). The three treated groups with DDB show very highly significant decrease in the AST level compared to the irradiated group although they still have very highly degree of significant increase compared to control group the different degrees of significant between each pair of the groups are illustrated in table (4).

Serum ALT: The results of serum ALT are represented in figure (5) and table (5). Figure (5) illustrates the variation in mean values of serum ALT level in different treated groups through a follow up period of five weeks. Table (5) shows the multiple range test which illustrates the degree of significant between each pair of the groups.



Figure: 5. Serum ALT level in different groups at 1st to 5th week post-irradiation.

The standard error values of the different ALT groups were in the range of 1.44-2.89, 1.23-3.46, 3.18-7.89, 0.65-5.29, 2.02-5.33 and 2.08-5.91 (lowest value and highest

ALP groups	Control	DDB	DDB Pre + Post	DDB Post-treated	DDB Pre-treated
Irradiated	62.49***	61.08***	51.74***	38.54**	33.08**
DDB Pre-treated	29.41**	28**	18.66*	5.46†	
DDB Post-treated	23.95**	22.54*	13.2†		
DDB Pre + Post	10.75†	9.34†			
DDB	1 14+				

Table 6: Multiple range test illustrating the degree of significant difference in ALP among different groups.

*** Very highly significant P<0.001, ** Highly significant P<0.01, * Significant P<0.05, †Insignificant P>0.05.

value within each group) for the control group, the DDB treated group, the irradiated group, the DDB pre-irradiation treated group, the DDB post-irradiation treated group and the DDB pre and post irradiation treated group respectively. Two way analysis of variance F-test for ALT was highly significant in case of different treatments (F1=14.212**) and significant in case different follow up time intervals (F2=7.314*). The results revealed very highly significant increase in ALT level of the irradiated group compared to the control group (Table 5). The level of ALT started to decline just after the 3rd week in the irradiated group (Figure 5). The three treated groups with DDB show potent improvement in the level of ALT (fig. 5). Both the pre-treated and post-treated animals with DDB show highly significant decrease compared to the irradiated group and significant increase compared to control, while the pre and post-treated group shows very highly significant decrease compared to irradiated group and insignificant change compared to control group (table 5). The comparisons between each two pair of the groups are shown in the table (5).

Serum ALP: The results of serum ALP are represented in figure (6) and table (6). Figure (6) illustrates the variation in mean values of serum ALP level in different treated groups through a follow up period of five weeks. Table (6) shows the multiple range test which illustrates the degree of significant between each pair of the groups. The standard error values of the different ALP groups were in the range of 7.94-9.53, 8.19-11.15, 4.67-16.64, 2.89-16.57, 8.82-19.01 and 5.7-17.64 (lowest value and highest value within each group) for the control group, the DDB treated group, the irradiated group, the DDB pre-irradiation treated group, the DDB post-irradiation treated group and the DDB pre and post irradiation treated group respectively. Two way analysis of variance F-test for ALP was very highly significant in case of different treatments (F1=44.471***) and insignificant in case different follow up time intervals (F2=37.163***). ALP levels were very highly significantly elevated in the irradiated group compared to the control group as shown in table (6). This elevation started to decline clearly after the 2^{nd} week as shown in figure (6).



Figure: 6. Serum ALP level in different groups at 1st to 5th week post-irradiation.

Also figure (6) show potent reduction in the level of ALP of the three treated groups with DDB. The ALP level of the pre and posttreated group shows very highly significant reduction than the irradiated group while the level of pre-treated and post-treated groups show highly significant reduction compared to irradiated group (table 6). The pre-treated groups and post-treated are highly significantly elevated compared to control group while the pre and post treated group is insignificantly changed when compared with the control. The comparisons between each two pair of the groups are shown in the table (6).

DISCUSSION

lonizing radiation is known to generate reactive oxygen species (ROS) in irradiated biological systems causing damage in tissues. The majority of these damages are due to the aqueous free radicals generated by the radiolysis of water; since biological organs contain 80% water. These free radicals react with cellular macromolecules, such as DNA, ribonucleic acid (RNA), membrane, etc. causing inhibition in liver protein synthesis (Manciluae, 1978) which lead to cell dysfunction and so mortality of cell (Tominaga *et al.*, 2004).

In this study, whole body gammairradiation of rats with 4.5 Gy induced a very highly significant decrease in the total protein level compared to control group. Irradiation pre-treatment with DDB didn't improve the total protein level. Mean while, the post-treated group and pre-and posttreated group with DDB showed very highly significant increase in total protein level compared to irradiated group and reach near to control level. The decrease of total protein caused by irradiation in our results is in harmony with Kafafy and Ashry, (2001), Ali et al. (2007a) and Ramadan (2007). Reddy and Babu (1980), whose results showed an increase in protease activity and a decrease in protein content during the post-irradiation period of frog due to lysosomal damage. Roushdy et al. (1989) suggested that the decrease in protein in irradiated rats might be the result of changes in the permeability of the liver cells.

The results of the present work demonstrated that serum albumin in rats was highly significantly decreased by whole body irradiation. Treatment with DDB before irradiation showed no significant difference with the irradiated group. The posttreatment with DDB and pre-and posttreatment with DDB revealed certain degree of significant increase compared to the group. Gamma irradiated irradiation stimulates acute phase response essential for generation and limitation of inflammation. This response is usually orchestrated cytokines through and hepatocyte stimulatory factors associated with characteristic metabolic changes in protein synthesis (Fujioke et al., 2001). The rats exposed to whole body irradiation show a significantly decline of serum total protein and albumin when followed up to 14 days (Ali et al., 2007b). On the contrary to Abou-Seif et al. (2003), who reported that whole body gamma irradiated rats revealed significant elevation of protein and globulin, the present results show a very highly significant reduction of globulin in irradiated animals compared to control. The posttreated group with DDB restored the globulin level to be close to the control group, while the pre-treated group affected nonsignificantly compared to the irradiated group.

The pre-and post-treatment with DDB showed highly significant increase of total protein and albumin levels compared to irradiated group. DDB has been utilized for its antioxidative action (Toda et al., 1988) and as liver protector against different effects (Maeda et al., 1981&1982; Hikino et al., 1984; Kiso et al., 1985; Takeda et al., 1985). Ketoconazole-treated mice showed lower serum albumin level which were restored to normal value by the co-treatment with DDB (Kim et al., 1999). The protective effect of post-treatment and pre-and posttreatment with DDB against reduction of serum total protein and albumin caused by irradiation, was in agreement with Ali et al. (2007b) who reported that administration of anserine and, or zinc either pre or post irradiation dose induced a significant improvement in serum total protein and albumin, specially when it is administered post irradiation.

Radiation-induced hepatic injury represents clinical findings ranging from asymptomatic biochemical changes including elevations of serum transaminases to fulminant hepatic failure (Cromheeke et al., 2000). The observed effect of gamma the levels radiation on serum of transaminases (AST and ALT) was very highly significant elevation compared to normal control. All irradiated animals groups that treated with DDB show very highly significant decrease in AST activity compared to irradiated group. In respect of ALT, DDB pretreatment and post treatment show highly significant decline while preand post treatment with DDB show very highly significant decrease of ALT compared to irradiated animals that reach close to the control value, this decrease was obvious at the 4^{th} and 5^{th} week of the follow up period. The marked elevation in the levels of serum AST and ALT as a result of exposure to whole body irradiation might be due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage (Sallie et al., 1991; Kafafy, 2000). A similar increase in the levels of AST and ALT has been observed in rats exposed to whole body y-irradiation (Kuzin et al., 1983; Nagiev and Karpovich, 1994; Cromheecke et al., 2000; Ramadan et al., 2002; Abou-Seif et al., 2003; Ramadan, 2007).

On the contrary, Katanyatanon *et al.* (2008) found that serum ALT was within normal range suggesting no measurable radiation-induced hepatic injury of general tissue at dose 5Gy whole body irradiation when followed up 8 days.

Since free radicals play a major role in injury. supplementation radiation of antioxidants will be of great significance to patients undergoing radiotherapy to prevent damage to normal tissues and also to astronauts who travel deep into space and are in contrast threat of radiation exposure et al., 2008). Experimental (Pradeep evidence suggests that DDB has hepatoprotective abilities since it functions as a potent antioxidant. It has also clinically proven in the treatment of chronic viral infection and chemically damaged liver (Liu. 1989; Fu and Liu, 1992; lp et al., 2000). DDB administration resulted in a reduction of the high AST and ALT activities induced by mancozeb (Sakr et al., 2005), carbon tetrachloride (CCl₄) (Abdel-Salam et al., 2007), erythromycin stearate (Abdel-Hameid, 2007). It was found that DDB at a dose of 200 mg/kg body weight resulted in a significant decrements in AST and ALT activities caused by oxidative stress status of tamoxifen-intoxicated liver injury in rats (El-Beshbishy, 2005).

The effectiveness of DDB to improve the transaminases in irradiated animals is in accordance with the administration of silymarin (Ramadan et al., 2002) copper (II), manganese (IV) or vanadium (|V|)complexes (Abou-Seif et al., 2003), Boerhaavia diffusa extract (Manu et al., 2007), hesperidin (Pradeep et al., 2008) and melatonin (Abduo and Osman, 2008) as radioprotectors. Mansour (2006) used acetyl-L-carnitine (ALC) as antioxidants against gamma-irradiation induced transaminases increase. Treatment with ALC ameliorated the effect of radiation exposure. Although a significant protection was observed, the enzyme activities were still significantly higher than the control value. This result is in agreement with our results of AST and ALT after followed up period of five weeks except for ALT that show restoration to normal value in animals pre- and post treated with DDB.

In the present study, ALP levels were very highly significantly elevated in the irradiated group compared to the control group. Pre-treated and post-treated groups with DDB show a highly significant decrease of ALP compared to irradiated animals. Preand pos-treatment with DDB protect rats from the irradiation-inducing elevation of ALP activity which restored to the level of the control and DDB groups. In accordance with our results, Comheecke *et al.* (2000)

and Pradeep et al. (2008), found that serum ALP activity was elevated by gamma ray irradiation. ALP is a clear marker of cell proliferation. The high level of this enzyme on day 2 in irradiated animals is due to the high proliferation rate in the liver after tissue damage by toxic effect of radiation. It was found that serum and liver ALP activities were much elevated by gamma ray radiation on days 2 - 7 after irradiation, and then declined in 11th day (Manu et al., 2007). Another data of following biliary secretion of ALP mentioned an increase on 2nd day but decreased on 4th day (Abdel-Salam et al., 2006). Although the elevated level of ALP of the irradiated group in this study started to decrease but it remains higher than the control group at the end of the experiment period. The hepatic cells are relatively radioresistant and there is a marked for capacity regeneration following destruction of a large portion of the liver (Comheecke et al., 2000). One of the interesting observations, of a recent report, is that serum ALP level was within normal range at a dose 5 Gy whole body irradiation when followed up to 8 days after irradiation (Katanyatanon et al., 2008).

The protective effect of DDB treatment on ALP in our study was coincident with Manu et al. (2007), who reported that the elevated level of serum and liver ALP after radiation exposure was, reduced in the Boerhaavia diffusa-treated mice. Oral administration or intravenous injection of a single dose of silymarin caused significant protection against ALP elevation induced by whole body gamma irradiation. The protective effect of silymarin was attributed to its antioxidant and free radicals scavenging properties (Ramadan et al., 2002). It was recently reported that melatonin has the hepatoprotective effect against irradiation in rats due to the same roles (Abduo and Osman, 2008).

Further studied are needed to support provisional conclusion that dietary а antioxidants do not conflict with the use of radiotherapy in the treatment of a wide variety of cancers and may significantly mitigate the adverse effects of the radiotherapy (Moss, 2007). The statistical analysis of the present work result provide a significant evidence that pre- and posttreatment of rats with DDB has the potent effect against reduction of total protein and albumin levels and against the elevation of AST, ALT and ALP activities, as a hepatoradioprotector. There are still too much unknowns about the hepatoprotective

effect of DDB against irradiation so more investigations randomized controlled trials should be carried out to ascertain its effects. Further studies are required to evaluate the protective effect of DDB on cellular macromolecules like DNA and also on other tissues.

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