Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.25, 2014

The role of Znic in alleviating B-toxicity in plants differing in their sensitivity to boron in terms of rooting response of cuttings.

*Shaheed[1] , A . I ., Alwan[2] , A . H., and Hussein[3] , K . A
1 Babylon Univ., Coll. of Sci. Dept. of Biol.
2,3 Karbala Univ. , Coll. of Sci., Dept. of Biol.
e-mail abdullah _ shhd @yahoo.com

Absract

The role of Znic in alleviating B-toxicity in terms of adventitious root formation (ART) in cutting of plants differing in their sensitivity to boron such as Mung bean (sensitive) ,Cucumber (Moderately tolerant) and Tomato (tolerant) has been carried out . The toxic level of B in addition ,to the promontory conc. Of Zn-salt was determined for each of the above spp. Three of Zn-salts were tested (sulphate ,nitrate and chloride) and the best promontory salt is Znic sulfate in developing the higher number. of roots in addition to the best conc. of the same salt was 15,10,15 pmm for Mung bean , Cucumber and Tomato respectively ,comparing to other Zn-salt & their concentration .

The toxic levels of boron was 200,300 and 400 µg/ml for Mung bean , Cucumber and Tomato respectively . These levels reduced growth parameters in terms of rooting response to 50% or beyond (55.16% ,50.83% and 53.49%)for Mung bean , Cucumber and Tomato compared to control in addition ,to the localized toxic symptoms like necrotic spots at leaves edges of the a above 3- spp. respectively .

Boron detoxification was occurred completely by supplying Znic sulphate prior to toxic–B treatment (pre-treatment)in all spp. compared to its supply as post- treatment or simultaneously with toxic –B . The protective role of Znic – sulphate was significantly enhances the average of root number /cutting to its levels in control treatment (in absence of toxic – B)in all spp. under study .

Toxic levels of B for the above spp. were caused significant damage for plasma- membrane of leaf tissues via permeability perturbation in terms of EC% with increasing 111.05%, 50.015% and30.65 in cutting of Mung bean , Cucumber and Tomato respectively . Whereas ,in seedling (in presence of root system) the % of damage was declined to 83.74%,42.59% and 9.91% when exposed to B-toxicity .These results confirms the sequostration mechanism of boron in roots exclusively ,rather than its transport to leaves .

Key woreds: Boron-toxicity, Boron-detoxification,Cucumber,Mung bean,rooting response ,Tomato and Znic .

1- Introduction

Boron toxicity is one of the important agricultural problem which is considered nutriential imbalance that limits growth and production of plants, as it has also been mentioned in South Australia, in Mediterranean countries (Aquea, et al. 2012).However, the ability of Plants for tolerance high levels of boron depend on plant species , genetic tolerance and its ability for absorption and acculamation of different ions where the range between optimal concentration ,toxic and deficiency are extremely narrow (Gupta ,1985) . Toxicity is widely variable between species and even among varieties of the same species.Thereafter , safe B concentrations in irrigation water range from 0.3 mg L^{-1} for sensitive plants (e.g., *Phaseolus aureus*) to 1-2 mg L^{-1} for semi-tolerant plants (e.g., *Zea mays* and *Solanum tuberosum*), 2-4 mg L^{-1} for tolerant plants (e.g., *Daucus carota* and *Cuminos melo*) and 4-6 mg L^{-1} for high tolerant plants such as *Solanum lycopersicon* (Nable, *et al.*, 1997). This behavior shows the relation of species ability to exclude B at roots level by reducing the permeability of lipids membrane and/or the presence of Boron transporters (BOR) and Nodulin – like intrinsic protein (NIP) essential for B extrusion from the cytoplasm and they may be responsible for reducing B accumulation (Miwa *et al.*, 2007; Sutton *et al.*, 2007).So, in this trend stresses occurs in its maximum level among imbalances in mineral ions status , that have numerous functions in the plants including maintaining charge balance, electron carriers, structural components, enzymes activty, and providing osmoticum for cell turgor and growth(Waraich, et al., 2011a) . The metabolic function of (Zn) are based on its strong tendency to form tetrahedral complexes with N- , O- and S-donor ligands .They reported that Zn is important for its ability to influence auxin levels via its production of tryptophan , a precursor for auxin biosynthesis. It shares in signal transduction via activation ,the interaction with phosopholipids and protein-kinases (Hänsch and Mendel, 2009). In addition, Zn application reduces the activity of membrane-bound NADPH oxidase which in turn decreases the generation of ROS (Waraich, et al., 2011b) . Zn is involved in oxidative stress-induced expression of genes encoding antioxidative defence enzymes, such as H2O2-scavenging (Catalase). ROS acculaumtiom occurs under B toxicity and also in Zn deficiency in many plants to cause oxidative damage to proteins, lipids and DNA , thereafter cell death (Molassiotis, et al .,2006). Among the microelements, manganese, boron, iron and zinc are the most active in rooting of cuttings (Rejman, et al., 2002). The mechanisms of their action may be different, even within the same species . The role of znic in alleviating boron toxicity in Plants differing in their Sensitivity to boron through

different mechanisms in terms of rooting responses in Mung bean , Cucumber and Tomato stem cuttings is the aim of this study .

2- Materials &Methods

Seeds of Mung bean (*phaseolus aureus* Roxb.) ,Cucumber (*Cucumis sativus* L.). and Tomato (*Solanum lycopersicum* Mill.). were germinated in sterile sawdust supplied with Hoagland solution (half strength) after soaking in current water over night . In addition ,to seedling growth were carried out in growth cabinet at 25±1C◦,under continuous illumination supplied by warm white fluorescent tubes (1500-1800 Lux) and relative humidity of 60-70% (Binder GMBH,Germany). Stem cuttings were prepared according to (Hess,1961) from 10-day –old light grown seedlings for Mung bean & Cucumber and 20 - day –old for Tomato . The cutting had apical bud,apir of fully expanded primary leaves ,epicotyls and 3 cm of hypocotyl under cotyledonary nodes, after removal of root system.

Boric acid was prepared at concentrations (0.001-500) µg/ml and Zni – salt (ZnSO4,ZnNO3 and ZnCl2) at conc.s 0.1-100 ppm.

Basal part of the hypocotyl of fresh cuttings were treated for 24 h with tested solutions or in combination of these solutions thereafter ,cuttings were transferred to boric acid (5 µg/ml) for 6 days as rooting medium because of the role of B in development of root primordia into visible roots (Middleton, et al. ,1978).

Twelve cuttings per treatment for rooting test were placed 4 per glass vial containing 15 ml (3cm depth) of the appropriate solution under the same conditions of growing seedlings .

Membrane permeability (% of damage) was measured according to (Yan, et al ., 1996) in terms of electrical conductivity (EC%).Moreover, stress tolerance index was measured according to (Fernandez,1993) and stress intensity was measured according to (Fernandez , 1993).

All experiment were designed as complete randomized design for stasitistical analysis ,depending of L.S.D . and T –test for treatment comparison by using analysis of variance (ANOV) for all experiment as individual (Levesque, 2007).

3-Results

Boron Toxicity Determination

Boron toxicity has been determined in terms of rooting response in Mung been (Boron sensitive sp.) , Cucumber (Moderate tolerant) and Tomato (tolerant sp.) cuttings as shown in Table (1). The Table showed that toxic level of B is 200, 300, and 400 µg/ml for Mung been , Cucumber and Tomato respectively . It has been known that, at this conc. the growth parameters in terms of the average of root number per cutting must be reduced to 50%.So the no. was 4.92, 14.42 and 9.08 root/cutting for Mung bean ,Cucumber and Tomato respectively, compared to control , 10.58 , 29.33 and 20.25 roots for the above spp. and with the same order . It is noteworthy, that of the above figures were statistically significant on 0.05 level of the probability.

Table (2) showed ,that salt – type had significant effect on rooting response of cutting for each sp. specially ZnSO4 that has dominant effect over the other salts in developing adventitious roots for each sp. The higher no . of roots were developed by cuttings supplied with ZnSO4 is 16.25 whereas ,the lower no . with ZnCl2 is 10.33 roots. On the other hand the triplet interaction between sp.-type ,salt –type ,and salt-conc. has also significant effect on higher rooting response represent by Tomato cuttings with ZnSO4 at 15ppm (41.83 roots) compared to other treatments. Depending on these results , ZnSO4 at 15ppm was employed for B-detoxification for both Mung bean and Tomato and at 10 ppm for Cucumber .

Table (3) shows that in terms of Electrical conductivity (EC.) the damage of plasma membrane was declined significantly in all spp. to 39.21% in leaf tissues of seedling (in presence of roots system) supplied with toxic level of B compared to 47.11% in cuttings (in absence of roots).

The same table shows that toxic B caused increase of plasma membrane damage from 24.65% to 37.06% whereas , supplying ZnSO4 was caused signif. decline in % of plasma membrane damage that approaches its level in d/H2O (control). However, the same table shows that sp.-type had signif. effect in % of damage & recorded the higher degree in Tomato (31.34) compared to Mung bean and Cucumber (27.25 &27.73 respectively).

Table (1) Determintion toxic boron concentrationsby terms rootting response of Mung bean Cuvcumber and Tomato cuttings.

Plant specie * B Conc.	Plant species			B Conc . (µg/ml)
	Tamato	Cucumber	Mung bean	
20.06	20.25	29.33	10.58	0.0
20.78	20.50	31.25	10.58	0.001
20.86	20.75	31.50	10.33	0.01
22.81	24.17	32.42	11.83	0.1
23.22	24.67	34.00	11.00	1
23.19	23.75	35.17	10.67	5
21.75	20.00	35.00	10.25	10
21.42	21.08	33.00	10.17	25
19.92	21.08	29.58	9.08	50
18.97	22.42	26.50	8.00	100
17.75	23.08	22.58	7.58	125
15.81	21.42	19.58	6.42	150
14.08	18.58	17.83	5.83	175
11.53	13.17	16.50	4.92	200
9.17	8.75	14.42	4.33	300
8.97	9.08	14.08	3.75	400
7.72	7.08	12.25	3.83	500
2.68	2.68			L.S.D
	18.81	25.59	8.19	Average
	0.65			L.S.D

However , the interaction between plant status (cuttings &seedlings)&treatment was lowered the % of damage in leaves from 41.75 in case of cuttings to 32.42 in case of seedlings . Meanwhile , seedlings lowered the % of damage in ramedy treatment (ZnSO4) from 26.13 to 23.16 whereas ,in case of B-detoxification from 26.39 to22.82 which is not differ significantly compared to control .

Controlling of B detoxification in cuttings of different spp.by using ZnSO4 in terms of rooting responses was shown in table (4) . ZnSO4 was supplied prior to toxic – B as pre-treatment or simultaneously with toxic – B . Consequently , pre- application of ZnSO4 was raising the rooting response (20.56 roots) to the level of control treatment (19.64 roots) whereas ,other treatments doesn't approaches the required level of complete ramedy from boron –toxicity .

	Plant species				
Salt species *CON.	Tamato	Cucumber	Mung bean	Con.	Salt species
16.67	20.75	19.42	9.83	0.0	ZnSO4
17.89	22.83	21.08	9.75	0.1	
17.89	22.92	20.33	10.42	1	
18.53	23.5	21.67	10.42	5	
21.69	26.42	26.25	10.67	10	
24.67	41.83	21.5	12.42	15	
17.44	22.75	20.58	9	20	
13.19	13.83	17.33	9.48	25	
8.67	10.67	8.17	7.17	50	
6.14	6.83	8.08	3.5	100	
16.31	21.75	17.92	9.75	0.0	Zn(NO3)2
18.5	24.58	20.58	10.33	0.1	
19.03	25.42	20.67	11	1	
20.97	31.33	20.92	10.67	5	
21.03	33.5	19.08	10.5	10	
18.69	29.08	16.25	10.75	15	
15.17	19.5	18.5	11.5	20	
13.78	18.25	13.75	9.33	25	
10.03	14.33	9.08	6.67	50	
7.89	12	7.33	4.33	100	
15.5	18.33	19.33	8.83	0.0	ZnCL2
16.14	19.17	20.42	8.83	0.1	
15.97	16.67	21.67	9.58	1	
14.67	15.08	18.83	10.08	5	
14.36	14.58	18.17	10.33	10	
10.61	10.58	13	8.25	15	
7.81	7.83	9.5	6.08	20	
5.75	3.75	7	6.5	25	
2.44	0	4	3.33	50	
0	0	0	0	100	
2.55	4.42			L.S.D	
Salt species				Salt species	Plant species * Salt species
16.28	21.23	18.44	9.16	ZnSO4	
16.14	22.93	16.01	9.48	Zn(NO3)2	
10.33	10.60	13.19	7.18	ZnCl2	
0.81		1.40			L.S.D
Con.				Con.	Plant species * Con.
16.16	20.11	18.89	9.47	0.0	
17.51	22.19	20.69	9.64	0.1	
17.71	21.67	20.89	10.33	1	
17.91	23.31	20.47	10.39	5	
18.52	24.83	21.17	11.08	10	
18.52	27.17	16.92	9.89	15	
13.57	16.69	14.86	8.86	20	
11.27	11.94	12.69	8.08	25	
8.06	8.33	7.08	5.72	50	
4.69	6.28	5.14	2.61	100	
1.47		2.55		L.S.D	
	18.25	15.88	8.61	Plant species average	
	L.S.D			0.81	

Table (2):-Interaction between Znic salts on rooting response of Mung bean Cucumber and Tomato cuttings Tomato cuttings.

Table (5) shows that stress intensity due to B- toxicity was higher in cuttings compared to seedlings in all spp.under study . Consequently , the high values of stress intensity were presented in both cuttings & seedlings (0.478 & 0.454 respectively) of sensitive sp. (Mung bean) whereas, the lower values were correlated in both cuttings & seedlings (0.123 &0.019 respectively) of tolerant sp. (Tomato).

In contrast, table (5) shows that tolerance index for B- toxicity was reduced in cuttings compared to seedlings in the above spp. . Obviously , the lower values of tolerance index were represented in both cuttings & seedlings (0.520 & 0.548 respectively) of sensitive sp.(mung bean) whereas, the higher values were correlated in both cuttings & seedlings (0.876 & 0.980 respectively) of tolerant sp.(Tomato). In other words , increasing of stress intensity on Mung bean (sensitive sp.) and declining of tolerance index in the same sp. and vice versa for Tomato . The latter shows decreasing of stress intensity & increasing of index tolerance in cuttings & seedlings (tolerant sp.) .

Table (3) Relative damage of plasma membrane caused by toxic boron in terms of electrical conductivity of Mung bean ,Cucumber and Tomato cuttings and seedlings

	Plant species				
Plant Status *Teratment	Tamato	Cucumber	Mung bean	Plant Status	Teratment 24 h in
26.13	29.62	27.07	21.70	Cutting	d(H2O)
23.18	29.14	21.81	18.58	Seeding	
41.70	38.70	40.61	45.80	Cutting	toxic Boron
32.42	32.03	31.10	34.14	Seeding	
26.38	29.25	23.27	26.63	Cutting	Recovery
22.82	29.32	19.62	19.52	Seeding	
2.08	3.61				L.S.D
Plant Status				Plant Status	Plant species * Plant Status
47.11	32.52	30.32	31.38	Cutting	
39.21	30.16	24.18	24.08	Seeding	
1.20	2.08				L.S.D
Teratment				Teratment	
24.65	29.38	24.44	20.14	d(H2O)	Plant species * Teratment
37.06	35.36	35.85	39.97	toxic Boron	
24.60	29.28	21.45	23.08	recovery	
1.47	2.55				L.S.D
	31.34	27.25	27.73	Plant species average	
	1.47			L.S.D	

Table (4) :- Boron defoxitication by using ZnSO4 in terms of rooting response of Mung bean, Cucumber and Tomato cuttings .

	Mung bean	Cucumber	Tomato	Average
d.w for 24h	11.83	21.67	25.42	19.64
ZnSO4 for 24h	13.00	23.58	33.17	23.25
Toxic B for 24h	6.42	11.33	11.33	9.69
ZnSO4 for 12h→Toxic B for 12h	12.42	22.25	27.00	20.56
Toxic B for 12h→ ZnSO4 for 12h	8.17	13.42	14.25	11.94
ZnSO4 +B 40 for 24h	8.67	19.08	16.83	14.86
Average	10.08	18.56	21.33	

L.S.D for plant species = 2.18 for treatment = 5.34 for treatment * plant species =3.0

Table(5) :- Stress intensity and tolerance index for toxic- B in cuttings & seedlings of Mung bean , Cucumber and Tomato .

Plant sp.	Stress intensity		Tolerance index	
	Cutting	Seedling	Cutting	Seedling
Mung bean	0.478	0.454	0.520	0.548
Cucumber	0.428	0.327	0.570	0.672
Tomato	0.123	0.019	0.876	0.980
L.S.D	0.078	0.052	0.101	0.054

5- Discussion

Results of the current study (Table 5&6) revealed an increase of stress intensity particularly in Mung bean plants (cuttings &seedlings) in addition to decrease of stress index on the other side . Obviously, such results are in agreement with (CCEM,1999) of considering Mung bean as B –sensitive species . In contrast, Tomato was described by decreasing its stress intensity and increasing the stress index (B- tolerant sp.). Meanwhile , the cucumber was represented as intermediate case between the above spp.(B- moderately tolerant sp.) .However, evidence that confirms the above situation is the ascending gradient in toxic level of B (200,300 and 400µg/ml) between the above three spp. in the same order (table-1).

However, the root number was reduced to 50% in toxic levels of B, 200,300 ,400 µg/ml into 4.92 roots in Mung bean ,14.42 in cucumber and 9.08 roots in Tomato compared to control treatment for each sp. , with reduction percent 53.49%, 55.10% and 50.33% respectively. In addition ,to the morphological symptoms represented by reduction of chlorophylls (chlorosis) and brown necrotic spots on the primary leaves edges of cuttings that already supplied with toxic B (Data not presented) .These results are in agreement with Shaheed & Muhammed (2010) on Mung bean cutting ,Cervilla, et al ., (2012) on Tomato and with Wang et al,(2010) on Cucumber.

The appearance of necrotic spots on leaf edges of the three spp. treated with the toxic – B may related to the membrane damage of thylakoids (table-3). Consequently, it was suggested that one possible reason for reduction of photosynthesis by excess of B,is the damage of thylakoids (Pereira, et al.,2000). It also limited the reduction of NADPH and utilizing of ATP (Guidi, et al.,2011). The latter mentioned that reduction in electron transport create oxidative stress that regenerate ROS in chloroplast which caused lipid-peroxidation , that probably leads to death of cells (localized spots). As a results for such evidences, the morphological symptoms that occurred in leaves edges when B accumulates in large amount (Guidi, et al., 2011).

However, ZnSO4 has the dominant effect compared to other Zn-salts (Nitrate and chloride) in the number of adventitious roots that developed in cuttings of the above spp.(table,2). The average number of roots were developed in cuttings supplied with ZnSO4 was 16.3 whereas, in ZnCl2 was 10.3 roots. Moreover, the same table shows that the kind of plant sp. had significant effect on rooting response. The latter was in its maximum value (18.3 roots) in Tomato, then 15.9 in cucumber and 6.8 in Mung bean. In other words rooting response was positively proportional with increasing the values of tolerance index and vice versa with stress intensity (table 5) .

On the other hand, salt conc. had significant influence on development of rooting response. So its maximum numbers are at 15 &10 ppm for ZnSO4 whereas, 100 ppm of ZnCl2 caused complete inhibition for the three spp. Obviously, sulphate has particular role in sulfer containing amino acid biosynthesis such as cysteine and methionine. Consequently their role in biosynthesis of protein& DNA and its reflection on pre-requisite of cell division and elongation in both phases (initiation & growth and development) of adventitious root formation in cuttings (Wirtz&Hell ,2006). The latter has been reported that cysteine biosynthesis has important role in absorption of reduced sulpher& its conversion to anti-oxidant compounds such as glutathione (GSH) .

Zn- deficiency caused interruption of auxin metabolism because of its role in tryptophan biosynthesis, that acts as precursor for IAA (Blazich,1988).Data reported by Szydlo &Pacholczak (2007) confirms the role of Zn in induction of rooting in stems of ornamentals trees. It is well know that Zn ion acts as strong inhibitor for

NADPH- oxidase in root cells of Phaseolus &Cotton. Meanwhile, Zn- deficiency caused asignificant increase of the above enzyme activity & generation of superoxide anion ($O2^{-}$). Consequently supplying of Zn for the above plants for 12-24 h caused a marked reduction of enzyme activity & generation of ($O2^{-}$) (Pinton,1994). Moreover, high production of H2O2 during oxidative stress inhibits root formation in Mung bean cutting (Pal Singh et al.,2009) . This indicates that, salt (excess of B) induced generation of $O2^{-}$ by NADPH –oxidase, which strongly inhibited by Zn.

For controlling B- toxicity, Zn (as ZnSO4) was supplied at the optimal conc. for rooting (table-2) . The relative damage of cytoplasmic membranes in terms of EC in cutting leaves was significant and approaches its maximum (47.11%) whereas , reduced to (39.2%) in seedling leaves , that supplied with toxic –B (in presence of root system). The latter case having the same trend in cuttings of all spp. under current study compared to seedling (table-3).It was raising a mechanism of B- tolerance which is the sequestration of excess B in roots & retarding its transport to leave thereafter ,lowering their damage (Schnurbuseh, et al.,2010) The negative effect of B-toxicity on membrane permeability of leaf cells of Mung bean, Cucumber and Tomato was showed in (Table -3). However, membrane permeability in terms of EC in Mung bean cuttings was 45.8 (with increasing percent 111.05%) whereas in Cucumber and tomato was 40.61 & 38.7 respectively. Such results has been verified that Mung bean is sensitive to B. Obviously ,the high % of damage in terms of EC was coincided with the lower conc. of toxic –B in Mung bean which is 200µg/ml of B compared to 300 &400 µg/ml for Cucumber &Tomato respectively.

Seemingly tolerance of B- toxicity in the above spp. depend on the differences in B- uptake, its transport through the plants or B exclusion .Meantime, plants that exclude B is more resistant for B – toxicity & by exclusion mechanism those plants which were controlling the uptake of excess B ,although B translocation was controlled by transpiration . However, under excess –B conditions, B absorption was passive & diffuse through leaf cells causing plasma – membrane damage .

On the other hand, in presence of roots (seedlings) the % of damage was reduced compared to cuttings and it was 83.74% ,42.59% and 9.91% for seedling of Mung bean ,Cucumber and Tomato respectively after exposing to B- toxicity. Consequently, it was attributed to increasing of ROS because of B-toxicity as oxidative stress (Scandalios,1993). These ROS having a high affinity for lipid peroxidation on plasma membrane then lossing its selective properties and, raising EC%. The decrease in stability of membranes reflects lipid peroxidation level caused by ROS (Zlatev & Lidon ,2012). In addition, lipid peroxidation of membranes as a result of excess-B was occurred in Tomato & cucumber (Alpaslan& Gunes,2001)and Barley (Karabal et al,2003 ; Nasim, 2010).

For controlling B- toxicity depending on the application of ZnSO4 prior to supplying the of toxic –B has a complete protective role in B –detoxification by developing a no. of adventitious roots equal to that of cuttings were treated with d/H2O (un-stressful cuttings).

It is noteworthy, that interaction between plant sp. and the ramedy treatment (ZnSO4)was revealed that ZnSO4 application as ramedy treatment for both seedlings & cuttings caused significant decline in % of damage from 39.97 in Mung bean cuttings supplied with toxic –B to 23.08 in Mung bean cuttings supplied with ZnSO4 whereas, Tomato recorded the lowest % of damage ,that is not differ from the control treatment (29.38 &29.28) respectively . In other words, EC values in Tomato that already pre-treated with ZnSO4 were equal to control treatment (d/H2O) and statistically without significant difference .These results interprets the tiny damage caused by toxic-B in Tomato because Tomato was related to group of tolerant spp. for B . However, Gunes (2009) found that Zn- application was ameliorates B- toxicity symptoms through preventing the oxidative damage of membranes via declining of lipoxygenase activity.

On the other hand , Zn reduces B accumulation and reflect antagonistic relationship in controlling B-absorption & its toxicity by plants in soils that characterized by Zn- deficiency & B- toxicity (Rajaie,2009) .Amelioration of B- toxicity by supplying Zn was reported in different spp. Like, barley (Graham et al., 1987) ,Wheat (Singh,1990) ,Aurantium (Swietlik,1995) ,Tomato (Gunes and Alpaslan,2000) and Maize (Hosseini,et al.,2007).

Finally, as a conclusion raised from the current study it's possible to use Zn in regulation & improving plant growth and development in stressful plants . The role of Zn was resides in membrane repairing at the 1-st place & controlling permeability perturbation via its participation in cell metabolism .In addition, alleviating oxidative damage of phospholipid ,Protein and DNA through its effects in may physiological & biochemical processes in terms of B- detoxification .

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