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ANTIOXIDANTS AS SUPPLEMENTS DURING DRUG-INDUCED THROMBOCYTOPENIA: A COMPARATIVE ANALYSIS OF VANILLIC ACID, L-CARNITINE AND CARIPILL™

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Drug-induced thrombocytopenia (DIT) is a disorder where platelet count declines as an adverse effect of therapeutic drugs. Plant extract of C. papaya Caripill™ is known to elevate platelet count under thrombocytopenic conditions. To evaluate the contribution of supplements with antioxidant potential to treat DIT, the comparative study of Caripill™, vanillic acid L-carnitine effect on platelet count and indices of oxidative stress in a model of rat thrombocytopenia induced through oral administration of hydroxyurea was performed. Wistar rats were grouped into four categories with five animals in each group: control (without any treatment); control + antioxidants; thrombocytopenia; thrombocytopenia + antioxidants. The above-mentioned antioxidants were supplemented orally at 50 mg/kg for 7 days. The level of lipid peroxidation products, superoxides, protein carbonyls and sulfhydryls, SOD and CAT activity in isolated platelets as oxidative stress markers, and indices of platelets aggregation and ATP secretion as functional markers were used. Vanillic acid was shown to be beneficial, similar to Caripill™, during hydroxyurea-induced thrombocytopenia by maintaining platelet functions, enhancing both the antioxidant capacity of platelets and its number. L-carnitine efficiently up-regulated the enzymatic antioxidants, maintained platelet functions and protected lipids and proteins from oxidation in thrombocytopenic rats, however, it could not improve the platelet count. These findings open new avenues for employing the studied antioxidants as supplements for therapeutic purposes.

K e y w o r d s: L-carnitine, vanillic acid, Caripill, thrombocytopenia, platelets, oxidative stress, antioxidants.

Platelets are formed by the process of thrombopoiesis from megakaryocytes [1, 2]. Platelets play a very critical role in hemostasis, as well as several inflammatory processes [3, 4]. Platelet count below 150,000 cells/µl of blood is referred to as thrombocytopenia. Low platelet count can be due to (i) decreased production of platelets, (ii) increased destruction of platelets, or (iii) increased sequestration of platelets [5]. Thrombocytopenia can either be mild (100,000-150,000 cells/µl), moderate (50,000-100,000 cells/µl) or severe (below 50,000 cells/µl) [6].

Thrombocytopenia may result from medications that interfere with platelet functions, or any underlying disease/infection [7]. Dengue, malaria, aplastic anemia, hypersplenism, and chikungunya are a few diseases that result in thrombocytopenia [8-10]. Oxidative stress (OS) might also play a role in the ethio-pathogenesis of the condition [11]. Furthermore, there are pharmacological (intravenous immunoglobulins or Rh-anti D, corticosteroids, or immunosuppressants) and surgical (splenectomy and laparoscopic splenectomy) treatments available for thrombocytopenia. However, severe cases require platelet transfusions [12].

Plant extracts of *Carica papaya* [13-16], *Euphorbia hirta* [13, 17], *Ipomea batatas*, *Althernanthera sessilis*, and *Momordica charantia* [13, 18] elevates platelet count under thrombocytopenic conditions. The leaf extract of *C. papaya* has various polyphenols that contribute to its antioxidant potential and play a key role in its platelet augmenting property [19-22]. Few studies have concluded that *C. papaya* leaf extract can increase platelet count in dengue fever.

Vanillic acid (4-hydroxyl-3-methoxy benzoic acid) is a benzoic acid derivative and an oxidized form of vanillin. It possesses free radical scavenging ability [23]. Vanillic acid is known to decrease lipid peroxidation (LPO) & restore enzymatic & non-en-

zymatic antioxidants in plasma [24, 25]. Pyo et al., have also reported that vanillic acid can inhibit mild platelet aggregation [26].

Similar to thrombocytopenia, various other pathologic conditions such as coronary heart diseases, and renal and heart failures are characterized by elevated OS. The antioxidant potential of L-carnitine has been proven effective in such conditions [27-31]. It is an organic solute and plays a key role in the β-oxidation of long-chain fatty acids by transporting them to the mitochondrial matrix [32]. Studies have also reported the benefits of using L-carnitine as an additive during platelet storage [33-35].

However, the role of L-carnitine and vanillic acid under thrombocytopenic conditions has not been reported. Therefore, this study was conducted to compare the efficacy of L-carnitine and vanillic acid with Caripill™ during drug-induced thrombocytopenia, through platelet functions and antioxidant status. Wistar rats were selected as the models for this study due to the similarity in physiology with humans and to minimize the baseline variations as they are bred in controlled conditions.

Materials and Methods

Chemicals. Caripill™ (Micro Labs Limited, Bangalore, India) and hydroxyurea (Hydrea, Sarabhai Chemicals) were purchased from MedPlus Pharmacy (Bengaluru, India). Thiobarbituric acid (TBA), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), epinephrine, Griess reagent and bathocuproinedisulfonic acid disodium salt (BCS) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). L-carnitine and vanillic acid were purchased from HiMedia Laboratories. Cytochrome C (Cyt C), collagen and adenosine triphosphate (ATP) were purchased from SRL chemicals. All other chemicals were of reagent grade, and organic solvents were of spectral grade.

Animal care and maintenance. Ethical clearance for the present study was obtained by the Institutional Animal Ethics Committee, Nargund College of Pharmacy, Bangalore, India (IAEC/NCP/93/2015). All procedures performed in this study were in accordance with the ethical standards of Institutional Animal Ethics Committee, Nargund College of Pharmacy, Bangalore, India (IAEC/NCP/93/2015) and the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986) .

Four-month-old male Wistar rats were acclimatized to the animal house conditions for a week. Five rats were housed in each cage, at 28 ± 1°C, with a 12 h light and 12 h dark cycle. Animals were fed with lab chow and tap water ad libitum.

Experimental design. The rats were grouped into the following categories with five animals in each group: Group 1 – Controls: I – CON-N: Normal control; II – CON-CP: Caripill™ control; III – CON-VA: Vanillic acid control; IV – CON-LC: L-carnitine control. Group 2 – Thrombocytopenia: I – CON-THR: Thrombocytopenia control; II – THR-CP: Thrombocytopenia+Caripill™; III – THR-VA: Thrombocytopenia+Vanillic acid; IV – THR-LC: Thrombocytopenia+L-carnitine.

Thrombocytopenia was induced by hydroxyurea, at 400 mg/kg body weight (b.w.). Briefly, hydroxyurea was orally administered to each rat (thrombocytopenia group) for 10 days [36]. The above-mentioned antioxidants were supplemented orally at 50 mg/kg for 7 days.

Platelet count. Blood (~2 ml) was drawn from rats by retro-orbital method into EDTA vacutainer tubes and the platelet count was analyzed by hydrodynamic focusing method using an automated haematology analyzer (MINDRAY BC5380 5-part analyzer).

Blood sampling. Animals were lightly anesthetized with ether and restrained in dorsal recumbancy. The syringe needle was inserted just below the xiphoid cartilage and slightly to the left of the midline. Blood was carefully aspirated from the heart into collection tubes with CPDA-1 (citrate phosphate dextrose adenine) [37].

Isolation of platelets. Platelets were isolated from the blood collected from Wistar rats (4 months old). Blood was centrifuged at 1500 rpm for 15 min at room temperature to obtain platelet-rich plasma (PRP). The PRP was then centrifuged at 4000 rpm for 15 min at 22°C. The resulting platelet pellet was gently resuspended in Tyrode's buffer (pH 7.4) [38].

Platelet aggregation. The platelet suspension (10 µl) was added to each well of the microtitre plate and incubated [with and without collagen (2 µg/ml)] at 37°C. The absorbance was recorded at 405 nm in shaking mode for one minute [39].

Adenosine triphosphate (ATP) secretion. Platelets were incubated (37°C) with collagen (2 µg/ml). Supernatant of the centrifuged samples was treated with 1.2 M perchloric acid. The absorbance of the supernatant was read at 260 nm. The amount of ade-

nine nucleotides was calculated from ATP standard in 0.6 M perchloric acid [40].

Glucose. Glucose concentration in platelets was analyzed enzymatically by GOD-POD method as described in the Autospan Gold kit. Absorbance was read at 546 nm [41].

Lactate dehydrogenase [LDH, 1.1.1.27]. Platelet samples were treated with the mixture of reagent 1 (Tris, NaOH and pyruvate) and reagent 2 (NADH) and incubated at 37°C for 5 min. The decrease in absorbance per minute was measured for 3 min at 340 nm and expressed as U/mg protein [42].

pH. pH of the samples was checked using Fisher Scientific pH strips [43].

Superoxides. Superoxides were measured by the method of cytochrome C (Cyt C) reduction. 200 µl of Cyt C (160 µM) was added to the platelets and incubated at 37°C. The samples were centrifuged at 3500 rpm for 5 min. Absorbance was measured at 550 nm and the amount of superoxides was calculated using the extinction coefficient of 18700 $M^{-1}\cdot cm^{-1}$ and expressed as µmol/mg protein [44].

Nitrites. Platelet samples were treated with Griess reagent (100 µl) and incubated in dark for 30 min at room temperature. Absorbance was read at 548 nm. Sodium nitrite standard was used to determine the amount of nitrites present in the samples [45].

Conjugate dienes. Platelets were treated with ether/ethanol, 1:3 (v/v) mixture and vortexed. The mixture was centrifuged at 4000 rpm and the absorbance was measured spectrophotometrically at 235 nm [44].

Thiobarbituric acid reactive substances (TBARS). TBARS was measured according to Olas et al. [46]. Platelets were treated with an equal volume of 20% (v/v) cold trichloroacetic acid (TCA) in 0.6 M HCl and centrifuged at 2000 rpm for 15 min. The supernatant was mixed with 0.12 M thiobarbituric acid in 0.26 M Tris (pH 7.0). The mixture was incubated in a boiling water bath for 15 min and the absorbance was read at 532 nm.

Protein carbonyls (PrC). Protein carbonyls were measured according to Reznick and Packer [47]. Platelets were mixed with 10 mM dinitrophenyl hydrazine in 2.5 M HCl and incubated at room temperature. TCA (20%) was added after 1 h and incubated in ice for 10 min. Samples were centrifuged at 3000 rpm and the supernatant was discarded. The protein pellets were washed with ethanol: ethyl acetate [1:1 (v/v)]. The final precipitate was dissolved in 6 M guanidine HCl in 133 mM Tris. The absorbance was read at 370 nm. The carbonyl content was calculated using absorption coefficient, 22,000 $M^{-1}\cdot cm^{-1}$.

Protein sulfhydryls (P-SH). Sodium phosphate buffer (0.08 mol/l Na-PO_4; 0.5 mg/ml Na_2-EDTA and 2% SDS; pH 8.0) was added to the platelet samples and the mixture was vortexed. 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was added to the mixture and incubated at room temperature for 15 min. Absorbance was read at 412 nm. Molar absorptivity of 13,600 $M^{-1}\cdot cm^{-1}$ was used to calculate the amount of protein sulfhydryls in the samples [48].

Superoxide dismutase [SOD, EC 1.15.1.1]. SOD in the platelet sample was measured according to Misra and Fridovich [49]. Platelet samples were added to carbonate buffer (0.05 M, pH 10.2, 0.1 mM EDTA). Epinephrine was added to the mixture and absorbance was measured at 480 nm. SOD activity was expressed as the amount of enzyme that inhibits oxidation of epinephrine by 50% which is equal to one unit and expressed as U/mg protein.

Catalase [CAT, EC 1.11.1.6]. Catalase was measured according to Aebi [50]. Absolute ethanol was added to the platelet samples and incubated in ice bath for 30 min. After incubation, phosphate buffer and 66 mM H_2O_2 were added and absorbance was measured at 240 nm. The molar extinction coefficient 43.6 $M\cdot cm^{-1}$ was used to determine the catalase activity and expressed as U/mg protein.

Total antioxidant capacity (TAC) - cupric ion reducing antioxidant capacity (CUPRAC). The samples were treated with 0.25 mM bathocuproinedisulfonic acid disodium salt (in 10 mM PBS) and absorbance was taken at 490 nm. 0.5 mM $CuSO_4$ was added to the samples and incubated for 3 min at room temperature. Reaction was stopped by adding 0.01 M EDTA and absorbance was read at 490 nm. Uric acid in 1 M NaOH was used as standard [51].

Protein. The protein concentration in the samples was determined according to Lowry et al. [52].

Statistical analyses. Results are represented as mean ± SE (n = 5). One-way ANOVA was performed between the groups for all the parameters and the results were considered significant at $P < 0.05$. Tukey-Kramer multiple comparison test was performed using GraphPad Prism 6 Software (GraphPad Software Inc., California, USA).

Results and Discussion

Variations in the body weight (b.w.) of all the experimental rats were similar to control rats.

Thrombocytopenia control (CON-THR). Platelet count decreased by ~80% in thrombocytopenic rats after hydroxyurea treatment (400 mg/kg b.w.). Hydroxyurea is a ribonucleotide reductase inhibitor that reduces hematopoietic cell production by killing the cells in the S-phase of cell division [53]. LDH activity elevated by one-fold in CON-THR, which indicates deterioration in platelet membrane integrity after administration of hydroxyurea (Fig. 1).

Furthermore, SOD activity (Fig. 2, *b*) and the total antioxidant capacity (Table 1) decreased by ~70% and CAT (Table 1) by 80% in the thrombocytopenic rats. This was in accordance with the studies of Zhang and Zehnder and Li et al. where antioxidant enzymes declined during thrombocytopenic situations [54, 55].

Platelet aggregation [without collagen (Fig. 3, *a*) and with collagen (Fig. 3, *b*)] and ATP secretion (Fig. 4) were similar in CON-THR with respect to CON-N (normal rats), indicating that the platelet functions were not affected after hydroxyurea-induced thrombocytopenia, along with pH (7.1) and glucose. Superoxides (Fig. 2, *a*), nitrites (Table 2), conjugate dienes (Table 2), TBARS (Table 2), protein carbonyls (Table 2) and sulfhydryls (Table 2) levels in CON-THR were similar to CON-N, suggesting administration of hydroxyurea at a concentration of 400 mg/kg b.w. only decreased platelet count and the antioxidant defenses, but the proteins and lipids were protected during drug-induced thrombocytopenia (DIT).

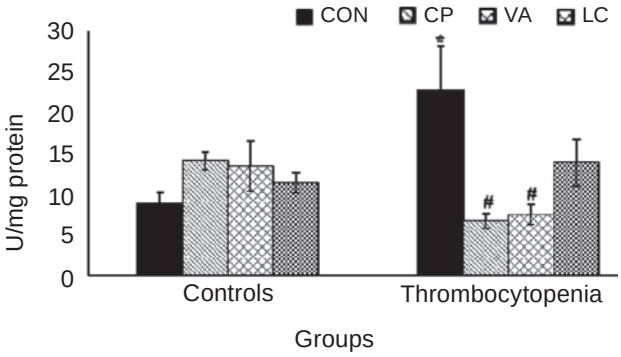

*Fig. 1. Lactate dehydrogenase in platelets during thrombocytopenia represented as mean ± SE, n = 5, P < 0.05. CON – without antioxidant; CP – Caripill™ ; VA – vanillic acid; LC – L-carnitine. *Represents significant difference against CON-N (Normal rats); #represents significant difference against CON-THR (Thrombocytopenia control)*

Caripill™. Control (CON-CP): ATP is actively secreted from platelet granules when they interact with agonists such as collagen, or free radicals [56]. ATP secretion decreased by 90% in CON-CP against CON-N which can be due to reduced levels of free radicals (Fig. 4). Furthermore, TAC decreased by 70% (Table 1), while SOD elevated by 50% (Fig. 2, *b*) in CON-CP with respect to CON-N. However, the variations in platelet aggregation [without collagen (Fig. 3, *a*) and with collagen (Fig. 3, *b*)], platelet count, LDH (Fig. 1), pH (7.1), glucose, superoxides (Fig. 2, *a*), nitrites (Table 2), lipid peroxidation and protein oxidation products (Table 2) and CAT (Table 1) were similar in CON-CP in comparison with CON-N. These results indicate that supplementation

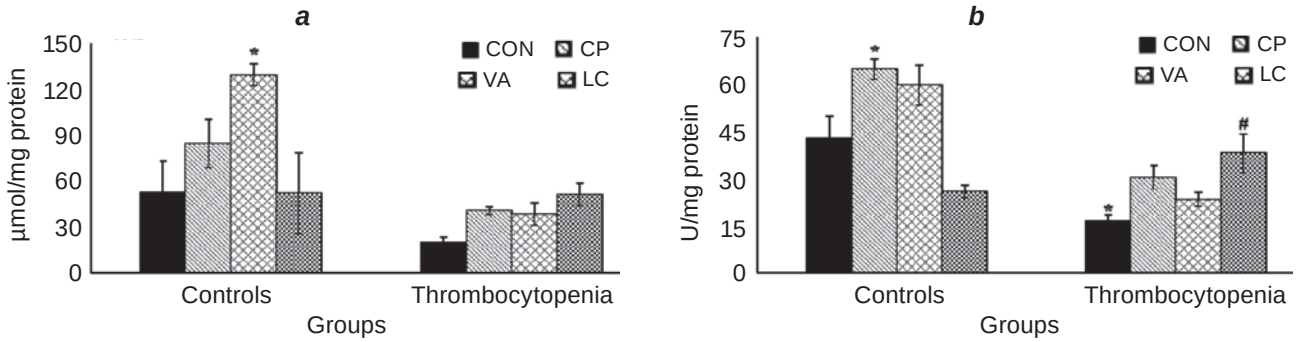

*Fig. 2. **a** – Superoxides in platelets during thrombocytopenia represented as mean ± SE, n = 5, P < 0.05. **b** – Superoxide dismutase in platelets during thrombocytopenia represented as mean ± SE, n = 5, P < 0.05. CON – without antioxidant; CP – caripill™; VA – vanillic acid; LC – L-carnitine. *Represents significant difference against CON-N (Normal rats); #represents significant difference against CON-THR (Thrombocytopenia control)*

Table 1 Catalase and total antioxidant capacity in rats during thrombocytopenia represented as mean ± SE, n = 5, P < 0.05

Groups	Catalase, U/mg protein	Total antioxidant capacity, µmol Uric acid equivalents/L
	Control	
CON-N	49.96 ± 7.70	258.30 ± 47.50
CON-CP	27.35 ± 5.00	83.91 ± 12.10*
CON-VA	41.57 ± 10.80	75.39 ± 4.80*
CON-LC	11.33 ± 2.60*	169.64 ± 15.20
	Thrombocytopenia	
CON-THR	9.76 ± 3.50	67.49 ± 34.70
THR-CP	42.38 ± 3.40#	269.97 ± 46.20#
THR-VA	45.05 ± 3.60#	229.11 ± 42.20
THR-LC	44.38 ± 10.6#	126.47 ± 50.20

Note. CON-N – Normal control; CON-CP – Caripill™ control; CON-VA – Vanillic acid control; CON-LC – L-carnitine control; CON-THR – Thrombocytopenia control; THR-CP – Thrombocytopenia+Caripill™; THR-VA – Thrombocytopenia+Vanillic acid; THR-LC – Thrombocytopenia+L-carnitine; CAT – Catalase; TAC – Total antioxidant capacity. *Represents significant difference against CON-N (Normal rats); #represents significant difference against CON-THR (Thrombocytopenia control)

of Caripill™ in the control group (CON-CP) has maintained the platelet count, along with primary platelet functions and antioxidant capacity.

Thrombocytopenia (THR-CP): administration of Caripill™ elevated platelet count by 5 folds in thrombocytopenic rats (THR-CP) with respect to CON-THR. Elevations in platelet numbers in THR-CP can be due to the megakaryopoietic stimulatory activity of Caripill™ [57]. *C. papaya* leaf extract increases the expression of PTAFR gene responsible for the formation of platelets, and stabilizes the platelet membrane [16, 58]. Furthermore, LDH significantly declined by 70% in THR-CP against CON-THR suggesting Caripill™ supplementation to the thrombocytopenic rats restored membrane integrity (Fig. 1). Conjugate dienes in THR-CP were similar to CON-THR, suggesting that Caripill™ could maintain the levels of primary products of LPO (Table 2). However, TBARS elevated by 2 folds in THR-CP with respect to CON-THR, indicating that the conjugate dienes formed have been converted to TBARS, and Caripill™ could not inhibit the formation of the

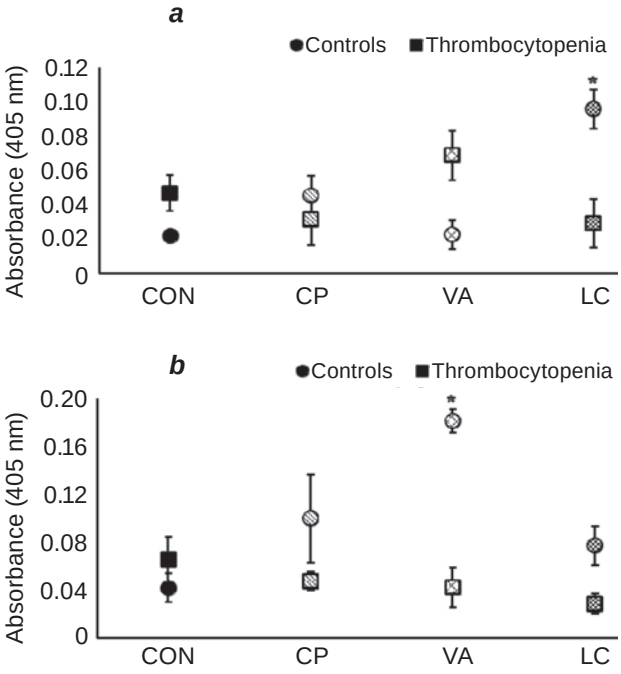

*Fig. 3. **a** – Aggregation (without collagen) of platelets during thrombocytopenia represented as mean ± SE, n = 5, P < 0.05. **b** – Aggregation (with collagen) of platelets during thrombocytopenia represented as mean ± SE, n = 5, P < 0.05. CON – without antioxidant; CP – Caripill™ ; VA – vanillic acid; LC – L-carnitine. *Represents significant difference against CON-N (Normal rats); #represents significant difference against CON-THR (Thrombocytopenia control)*

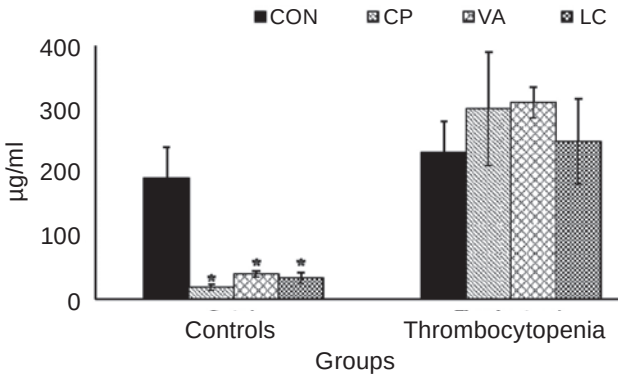

*Fig. 4. ATP Secretion in platelets during thrombocytopenia represented as mean ± SE, n = 5, P < 0.05. CON – without antioxidant; CP – Caripill™; VA – vanillic acid; LC – L-carnitine. *Represents significant difference against CON-N (Normal rats); #represents significant difference against CON-THR (Thrombocytopenia control)*

Table 2. Nitrites, lipid peroxidation and protein oxidation during thrombocytopenia represented as Mean ± SE, n = 5, P < 0.05

Groups	Nitrites, mmol/mg protein	Conjugate dienes, µmol/mg protein	TBARS, µmol/ mg protein	Protein carbonyls, µmol/mg protein	Protein sulfhydryls, µmol
			Control		
CON-N	1.95 ± 0.30	225.11 ± 45.60	7.71 ± 1.50	29.69 ± 5.20	82.50 ± 17.50
CON-CP	1.08 ± 0.10	168.50 ± 17.10	5.02 ± 2.10	30.14 ± 2.50	126.76 ± 14.30
CON-VA	0.97 ± 0.10	180.12 ± 54.20	1.76 ± 0.70	33.59 ± 5.00	97.05 ± 3.90
CON-LC	1.30 ± 0.10	157.57 ± 39.70	4.48 ± 1.80	12.34 ± 4.00*	92.20 ± 3.40
			Thrombocytopenia		
CON-THR	1.93 ± 0.30	72.66 ± 23.40	2.77 ± 0.50	32.85 ± 11.20	88.08 ± 6.10
THR-CP	1.96 ± 0.30	159.90 ± 35.70	10.42 ± 2.00#	26.30 ± 9.90	91.54 ± 4.40
THR-VA	2.62 ± 0.40	174.47 ± 19.60	9.82 ± 2.70	32.01 ± 5.20	97.79 ± 12.90
THR-LC	1.51 ± 0.40	187.22 ± 35.20	9.03 ± 1.00	32.65 ± 4.60	91.91 ± 4.50

Note. CON-N – Normal control; CON-CP – Caripill™ control; CON-VA – Vanillic acid control; CON-LC – L-carnitine control; CON-THR – Thrombocytopenia control; THR-CP – Thrombocytopenia+Caripill™; THR-VA – Thrombocytopenia+Vanillic acid; THR-LC – Thrombocytopenia+L-carnitine; TBARS – Thiobarbituric acid reactive substances. *Represents significant difference against CON-N (Normal rats), #represents significant difference against CON-THR (Thrombocytopenia control)

secondary products of LPO under thrombocytopenic condition (Table 2). SOD activity in THR-CP (Fig. 2, *b*), was in accordance with the levels of superoxides (Fig. 2, *a*) and nitrites (Table 2), which were similar to that of CON-THR. CAT and TAC (Table 1) elevated by 3 folds in Caripill™ supplemented thrombocytopenic rats (THR-CP) when compared with thrombocytopenia control (CON-THR). The up-regulation of antioxidant capacity can be attributed to the active compounds in *C. papaya* leaves such as natural phenols and polyphenols that are known to enhance endogenous antioxidant defenses [16, 59].

Platelet aggregation [without collagen (Fig. 3, *a*) and with collagen (Fig. 3, *b*)] and ATP secretion (Fig. 4) were similar in THR-CP when compared with CON-THR, indicating the negligible influence of Caripill™ on platelet functions. Similar changes were also observed in pH (7.1) and glucose in Caripill™ administered thrombocytopenic group (THR-CP) and thrombocytopenia control (CON-THR). Furthermore, the levels of protein carbonyls and sulfhydryls (Table 2) were similar in THR-CP and CON-CP, suggesting that Caripill™ could protect proteins from oxidative damage under thrombocytopenic conditions.

Therefore, administration of Caripill™ (50 mg/kg b.w.) was beneficial in terms of elevating platelet count, maintaining platelet functions and enhancing antioxidant defenses, thus protecting platelets from OS during DIT.

Vanillic acid. Control (CON-VA): Platelet aggregation (without collagen) in CON-VA was similar to CON-N (Fig. 3, *a*), while collagen-induced platelet aggregation (Fig. 3, *b*) and ATP secretion (Fig. 4) decreased by 3 folds and 1 fold respectively in CON-VA when compared with CON-N. Pignatelli et al., [60] have reported that flavonoids inhibit agonist-induced platelet aggregation due to their antioxidant potential. Yasuda et al. [61] also showed platelet aggregation decreased due to the antioxidant property of vanillic acid. This was evident in platelet aggregation in the presence of collagen. Superoxides elevated by one-fold in CON-VA when compared with CON-N, suggesting that vanillic acid at 50 mg/kg b.w. could not effectively scavenge these free radicals (Fig. 2, *a*), however, it could maintain the nitrite levels (1.0 mmol/mg protein) (Table 2). TAC decreased by 70% in CON-VA, which can be due to the synergistic effects of endogenous antioxidants and vanillic acid (Table 1). However, similar levels of SOD (Fig. 2, *b*), CAT (Table 1), lipid peroxidation, and protein oxidation levels (Table 2) in CON-VA when compared with CON-N suggest that vanillic acid could maintain the antioxidant status and protect the lipids and proteins from oxidative damage. Additionally, platelet count, LDH (Fig. 1), pH (7.1),

and glucose were also similar in CON-VA and CON-N, which indicates vanillic acid had minimal influence on platelet functions under control conditions.

Thrombocytopenia (THR-VA): Platelet count showed an elevation of 5 folds in thrombocytopenic rats supplemented with vanillic acid (50 mg/kg b.w.), which can be attributed to the anti-apoptotic property of vanillic acid. Polyphenols such as vanillic acid, have been shown to attenuate apoptosis [62, 63]. LDH activity was 70% lower in THR-VA when compared with CON-THR, suggesting that vanillic acid could maintain membrane integrity under thrombocytopenic conditions (Fig. 1). Furthermore, similar variations in platelet aggregation [without collagen (Fig. 3, *a*) and with collagen (Fig. 3, *b*)] and ATP secretion (Fig. 4) in THR-VA with respect to CON-THR, along with pH (7.1) and glucose, signifies the role of vanillic acid in maintaining platelet functions during DIT.

The levels of superoxides (Fig. 2, *a*) and nitrites (Table 2) were similar between THR-VA and CON-THR, along with SOD (Fig. 2, *b*) and TAC (Table 1). CAT activity increased by 3 folds in THR-VA in comparison with CON-THR (Table 1). These results indicate the influence of vanillic acid on antioxidant status, which is also reported by Kumar et al. [24] and Wang et al. [64]. Furthermore, conjugate diene levels increased by 2 folds (Table 2), but levels of TBARS (6.7 µmol/mg protein) (Table 2) were similar in THR-VA when compared with CON-THR. Although vanillic acid is a potent inhibitor of LPO, it could effectively prevent only the formation of secondary products of LPO in thrombocytopenic rats. Nevertheless, vanillic acid protected proteins from oxidative damage as demonstrated by the levels of protein carbonyls and sulfhydryls (Table 2) in THR-VA and CON-THR groups.

Vanillic acid (50 mg/kg b.w.) administration proved effective in terms of improved platelet count, platelet functions, and antioxidant status in thrombocytopenic rats.

L-Carnitine. Control (CON-LC): CON-LC showed decrements in platelet aggregation (without collagen) by 3 folds with respect to CON-N, which can be attributed to the interaction of L-carnitine with arachidonic acid to inhibit platelet aggregation [65] (Fig. 3, *a*). The results suggest that LC could prevent spontaneous platelet aggregation in the absence of an agonist. ATP secretion (Fig. 4) and catalase (Table 1) activity decreased by ~85%, and protein carbonyls (Table 2) decremented by 60% in CON-LC when compared with CON-N, which is due to the free radical scavenging ability of L-carnitine [66]. L-carnitine (50 mg/kg b.w.) supplementation in controls maintained basic platelet function and membrane integrity as observed in aggregation (Fig. 3, *a*, *b*), platelet count, LDH (Fig. 1), pH and glucose. The levels of superoxides (Fig. 2, *a*) and nitrites (Table 2) in CON-LC were comparable to that of CON-N, suggesting L-carnitine could regulate the levels of free radicals. Consequently, conjugate dienes (Table 2), TBARS (Table 2), P-SH (Table 2), SOD (Fig. 2, *b*) and TAC (Table 1) were also similar between CON-LC and CON-N. The results suggest that L-carnitine had the potential to maintain the antioxidant status of platelets.

Thrombocytopenia (THR-LC): Administration of L-carnitine (50 mg/kg b.w.) up-regulated the antioxidant enzymes SOD (one-fold) (Fig. 2, *b*) and CAT (3 folds) (Table 1), and maintained the TAC (Table 2) in THR-LC with respect to CON-THR. Results of THR-LC substantiate the findings of Abdul and Butterfield [67], Gomez-Amores et al. [68] and Tastekin et al. [69] that L-carnitine up-regulates the endogenous antioxidant enzymes under the situations of oxidative stress. L-carnitine maintained the platelet functions of thrombocytopenic rats as observed in platelet aggregation (Fig. 3, *a*, *b*) and ATP secretion (Fig. 4) in THR-LC and CON-THR. Furthermore, pH (7.1) and glucose were maintained in THR-LC with respect to CON-THR, suggesting L-carnitine supplementation did not affect the quality or metabolism of platelets. The levels of superoxides (Fig. 2, *a*) and nitrites (Table 2) were similar in THR-LC and CON-THR, indicating L-carnitine could maintain the levels of free radicals during thrombocytopenia. Furthermore, similar changes in conjugate dienes, TBARS, protein carbonyls, and sulfhydryls (Table 2) in THR-LC with respect to CON-THR indicate that L-carnitine enhanced the endogenous antioxidant system.

L-carnitine efficiently up-regulated the enzymatic antioxidants, maintained platelet functions, and protected lipids and proteins from oxidation in thrombocytopenic rats however, it could not improve the platelet count.

Conclusion. Vanillic acid was beneficial during hydroxyurea-induced thrombocytopenia by improving platelet numbers and enhancing the antioxidant capacity, similar to Caripill™. L-carnitine, however, could only up-regulate the antioxidant en-

zymes, but could not elevate the platelet numbers. Vanillic acid was more potent than L-carnitine in exhibiting anti-thrombocytopenic activity and had a similar influence as Caripill™ during DIT. These findings open new avenues for employing these antioxidants as supplements during DIT.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

The authors have published a Patent on Vanillic acid (Indian Patent; Application No. 201941020419) based on this study.

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АНТИОКСИДАНТИ ЯК ДОБАВКИ ЗА МЕДИКАМЕНТОЗНОЇ ТРОМБОЦИТОПЕНІЇ: ПОРІВНЯЛЬНИЙ АНАЛІЗ ВАНІЛОВОЇ КИСЛОТИ, L-КАРНІТИНУ ТА ПРЕПАРАТУ CARIPILL™

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Медикаментозна тромбоцитопенія (МТ) – це стан, при якому кількість тромбоцитів знижується через несприятливий вплив терапевтичних препаратів. Відомо, що рослинний екстракт *C. papaya* Caripill™ підвищує кількість тромбоцитів при тромбоцитопенічних станах. Антиоксидантний потенціал у лікування МТ оцінювали дослідженням впливу Caripill™, L-карнітину і ванілової кислоти на кількість тромбоцитів та показники оксидативного стресу у щурів з тромбоцитопенією, спричиненою пероральним введенням гідроксисечовини. Щури Wistar були поділені на чотири групи по п’ять тварин у кожній: контроль (без лікування); контроль + антиоксиданти; тромбоцитопенія; тромбоцитопенія + антиоксиданти. Вищезазначені антиоксиданти додавали перорально в дозі 50 мг/кг протягом 7 днів. Оксидативний стрес оцінювали за рівнем продуктів пероксидного окислення ліпідів, супероксидів, карбонільних та сульфгідрильних груп протеїнів, вимірювали активність СОД і КАТ в ізольованих тромбоцитах, крім того враховували показники агрегації тромбоцитів і секрецію АТР як функціональні маркери. Показано, що у щурів із тромбоцитопенією, ванілова кислота подібно до Caripill™ підтримувала функції тромбоцитів, підвищуючи їх антиоксидантну здатність і кількість. L-карнітин ефективно регулював ензимні антиоксиданти, підтримував функції тромбоцитів і захищав ліпіди та протеїни від окислення у щурів із тромбоцитопенією, однак не впливав на кількість тромбоцитів. Зроблено висновок, щодо використання досліджених антиоксидантів як добавок у терапевтичних цілях.

Ключові слова: L-карнітин, ванілова кислота, Caripill™, тромбоцитопенія, тромбоцити, оксидативний стрес, антиоксиданти.

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