Synergistic Potential of Propolis and Vitamin E Against Sub-Acute Toxicity of $AlCl_3$ in Albino Mice: In Vivo Study

S. SAJJAD[1], H. MALIK[2], L. SAEED[3], I. HASHIM[4], U. FAROOQ[5], F. MANZOOR[6]

[1]Department of Zoology, Lahore College for Women University, Lahore, Pakistan, [2]Department of Zoology, Lahore College for Women University, Lahore, Pakistan, [3]Department of Zoology, Lahore College for Women University, Lahore, Pakistan, [4]Department of Zoology, Lahore College for Women University, Lahore, Pakistan, [5]The Islamia University of Bahawalpur, Pakistan, [6]Department of Zoology, Lahore College for Women University, Lahore, Pakistan

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Summary

Current study evaluated the synergistic potential of propolis and vitamin E against sub-acute toxicity of aluminum chloride on different biochemical parameters and liver histology. Swiss albino mice (n=42) were randomly divided into seven groups. Group I received 0.2 ml of 0.9 % saline solution, Group II received Propolis (50 mg/kg b.w.), Group III received vitamin E (150 mg/kg b.w.), Group IV received $AlCl_3$ 50 mg/kg b.w., Group V received $AlCl_3$ + Propolis, Group VI received $AlCl_3$ + vitamin E and Group VII received $AlCl_3$ + propolis + vitamin E. Blood and tissue samples were collected after 7 and 21 days. The body weight of the animals significantly increased in all groups except Group IV. The concentration of serum high density lipoprotein significantly decreased in Group IV and increased in Group V, VI and VII. The level of aspartate aminotransferase, alanine transferase, alkaline phosphatase, triglycerides, total cholesterol, and low density lipoprotein significantly increased in $AlCl_3$ treated group and increased in Group V, VI and VII. Tissue sections were processed and stained by hematoxylin and eosin. Group II showed cellular necrosis. Group V, VI showed decreased number of vacuolization, sinusoidal spacing and macrophage cell infiltration. Group VI showed less degenerative changes in the third week. Vitamin E and propolis in combination with Al provides more protection against $AlCl_3$ induced toxicity.

Key words

Liver • Toxicity • $AlCl_3$ • Tissue Necrosis • Propolis • Vitamin E

Corresponding author

S. Sajjad, Department of Zoology, Lahore College for Women University, Lahore, Pakistan. Fax: +924299203077. E-mail: drsumerasajjad786@gmail.com

Introduction

Human beings exposed to Al through various routes like chemicals; pollutants discharged through industries, pharmaceutical products containing phosphate binders, food additives and certain antacids causing detrimental effects (Reinke *et al.* 2003, Stojanovic and Ninkovic 2009). Pregnant women are more prone to toxicity of Al through products like food, drinking water, soil ingestion, cosmetics, dust and various medications (Roig *et al.* 2006). Al is the metal, which provoked neurotoxicity, cardiotoxicity, hepatotoxicity and nephrotoxicity by inducing oxidative stress due to its capacity to produce enormous free radicals (Sushma and Rao 2007, Turkez *et al.* 2010). It was observed that increased level of reactive oxygen species (ROS) produced by Al was attenuated by vitamin E administration (Abubakar *et al.* 2004).

Propolis or bee glue, a resinous mixture, is composed of more than 300 compounds such as phenols aldehydes, sequiterpene quinines, amino acids, steroids, polyphenols and coumarins (Khalil 2006). Propolis has variety of bioactive components including active substances of polyphenolic fractions that are responsible

Fax +420 241 062 164, e-mail: physres@fgu.cas.cz, www.biomed.cas.cz/physiolres

for its antibacterial, antiviral, antifungal, antiprotozoal, antimicrobial, analgesic, anti-inflammatory, antioxidant, locally anesthetic, cytostatic, i.e. anticancer, and immune-stimulating and immunomodulatory effects of propolis in living organisms (Eyng *et al.* 2013, Kačániová *et al.* 2013).

Propolis and caffeic acid phenethyl ester (CAPE) provide protection against oxidative stress in hypertensive rats (Salmas *et al.* 2017). Propolis alleviates concanavalin A-induced hepatitis by modulating cytokine secretion and inhibition of reactive oxygen species (Mounieb *et al.* 2017). Vitamin E (tocopherol) function as biological antioxidants to protect cellular macromolecules (DNA, protein, lipids) and other antioxidant molecules from uncontrolled oxidation by free radicals during normal metabolism (Huang and Huang 2004). Vitamin E, C and Omega-3 showed protection against aluminum chloride induced liver and kidney toxicity in female albino mice (Gorgees *et al.* 2016). Vitamin E protects biological systems by inhibiting the lipid peroxidation (LPO) (Nogueira *et al.* 2013). Recent studies have reported that vitamin E and Metallothionein alleviate Cd-induced hepatotoxicity through their antioxidative and antiapoptotic effects (Duan *et al.* 2018). According to Drigla and coworkers (2016), aqueous solution of propolis and bee venom cotreatment showed antiproliferative effects on luminal (MCF-7) and triple negative breast cancer (TNBC). The current study aimed to investigate comparative ameliorative effects of propolis and vitamin E against subacute toxicity of $AlCl_3$ on liver of albino mice as no previous study known to us investigated their combined effect in an animal model as exploited in present research.

Materials and Methods

Chemicals used

Aluminium Chloride, AnalaR. BDH, laboratory supplies Poole, BH15 1TD, England. Vitamin E, Abbott Laboratories Pakistan Ltd.

Extraction of propolis

In current study propolis (Biopropolis 1995, YS Organic Bee Farms, Sheridan, IL, USA) was extracted with 100 ml of ethanol 70 % at ambient temperature, in the absence of bright light and with moderate shaking for 1 week. Extracts were filtered and concentrated to dryness with a rotary evaporator at 50±1 °C to give solid residues (Sforcin *et al.* 2002).

Experimental protocol

In the current study forty two (42) healthy adult albino mice with 3-4 months age weighing 23-41 g were used. Animals were kept under the standard laboratory conditions (23-26 °C and 12 h light/dark cycle). Mice were fed on commercial rodent chow in pellet form. Water was available *ad libitum*. All the experimental work was performed under the guidelines given by Research Ethical Review Committee of Lahore College for Woman University (Memo number RERC/Zoo/2015/06); Lahore, Pakistan. Animals were randomly divided into 7 groups; each group contained 6 mice. All the doses were orally administered daily for three weeks. Group I (Control): 0.2 ml of 0.9 % saline solution, Group II (Propolis) Ethanolic extract of propolis 50 mg/kg body weight was given (Newairy *et al*. 2009). Group III (vitamin E) 150 mg/kg body weight (Aziz and Zabut 2011), dissolved in sunflower oil. Group IV ($AlCl_3$ treated group): 50 mg/kg body weight (b.w.) of $AlCl_3$ dissolved in 0.2 ml double distilled water (Majida *et al.* 2013). Group V ($AlCl_3$ + Propolis treated group): 50 mg/kg b.w. $AlCl_3$ + 50 mg/kg b.w. Propolis (Newairy *et al.* 2009) dissolved in 0.2 ml double distilled water. Group VI ($AlCl_3$ + vitamin E treated group): 50 mg/kg b.w. $AlCl_3$ + 150 mg/kg b.w. Vitamin E (Aziz and Zabut 2011). $AlCl_3$ dissolved in 0.2 ml double distilled water and vitamin E dissolved in sunflower oil. Group VII ($AlCl_3$ + vitamin E + Propolis treated group): 50 mg/kg b.w. + 50 mg/kg b.w. + 150 mg/kg b.w. $AlCl_3$ + Propolis dissolved in 0.2 ml double distilled water and vitamin E dissolved in sunflower oil, respectively.

Blood sampling

Three mice from each group were anesthetized and sacrificed for the tissue and blood collection through cardiac puncture technique after week 1 and week 3. The collected blood was then centrifuged at 4,000 rpm for 10-15 min. Serum was stored at -20 °C until estimation of biochemical parameters i.e. triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) by using commercially available kits (Human Gesellscharft fur Biochemica und Diagnostica, GmbH, Wiesbaden, Germany and Crescent Diagnostics, Jeddah Industrial City, Saudi Arabia). Percent increase or decrease in Body weight was calculated by following formula: = Final weight – Initial weight / Final weight + Initial weight x 100.

Histological study

Liver tissues were processed for microtomy by a standard protocol of fixation, embedding and staining (Srivastava and Yadav 2007, Drury and Wallington 1980). The prepared slides (5 µm thickness tissues section) were observed using microscope (Trinocular camera fitted microscope- E-200, digital microscopic camera – Nikon Japan Ei1-L2) by using 400x magnification and photographed.

Statistical analysis

Data was statistically analyzed by using SPSS software v 19. Dunnett T3 was used as a *post hoc* test. Probability level of $p<0.05$ was considered significant and $p<0.01$ was considered as highly significant.

Results

Propolis and vitamin E treated group showed significant increase in mean body weight as compared to control group (Table 2). Significant decrease in body weight of Group IV animals was observed as compared to control group in week 1 and week 3 (Table 1) while the remaining groups showed significant increase in mean body weight in week 1 and week 3 respectively. Group II and III showed non-significant change in serum levels of TC, TG, HDL, LDL, AST, ALT and ALP as compared to control group (Table 2). Group IV showed significant elevation in serum TC, TG along with highly significant increase in serum LDL levels as compared to control, Group II and Group III in 1st and 3rd week (Table 2). Although Group V, VI and VII showed highly significant increase in serum TC and TG levels as compared to control but when same groups compared with Group IV in week 3 they showed significant decreased serum TC and TG values from 1st to 3rd week respectively (Table 2). Serum HDL values reduced non-significantly in all groups as compared to control group but Group IV showed highly significant decrease in HDL value as compared to control, Group II and Group III (Table 2). Group V, VI and VII showed non-significant elevation in serum HDL level as compared to Group II from 1st to 3rd week (Table 2).

Table 1. Mean ± SEM of percent increase or decrease in initial and final body weight (g).

Group	Initial body weight	Final body weight	Percent increase or decrease	
Control	24.05 ± 0.91	30.1 ± 0.38	↑	11.17
Propolis	18.71 ± 0.32*	25.52 ± 0.33	↑	15.39
Vitamin E	17.15 ± 0.50*	23.55 ± 0.61	↑	15.72
$AlCl_3$	30.10 ± 0.46*	22.05 ± 0.55*	↓	15.43
$AlCl_3$ + Propolis	25.71 ± 0.66*	30.90 ± 0.37	↑	9.16
$AlCl_3$ + Vitamin E	23.76 ± 0.37*	27.71 ± 0.52*	↑	7.67
$AlCl_3$ + Vitamin E + Propolis	22.19 ± 0.91*	25.52 ± 0.38*	↑	6.97

* $p<0.05$; in comparison to control group.

Serum LDL level was significantly elevated in Group IV as compared to control however Group V, VI and VII displayed highly significant reduction as compared to Group IV in 3rd week (Table 2). Level of serum AST was significantly elevated in Group IV as compared to control group. Moreover, concomitant administration of propolis, vitamin E alone and together with Al (Group V, VI and VII) showed non-significant decline in AST concentration as compared to Group IV in 1st and 3rd week respectively (Table 2). Likewise, Group IV significantly raised serum ALT levels in week 3 as compared to control, Group II and III (Table 2); Group V, VI and VII indicated highly significant decreased serum ALT levels from 1st to 3rd week as compared to Group IV (week 3) (Table 2). Group IV showed significant increase in serum ALP level as compared to control in week 1 and week 2 respectively. Group VI and VII significantly decreased ALP concentration in 1st and 3rd week (Table 2) as compared to Group IV (week 3). Histological study of control group, propolis and vitamin E treated showed normal architecture of hepatocytes i.e. no vacuolations

and homogenous cytoplasm blood cells with clearly visible sinusoidal spacing and no hemorrhages (Fig. 1a, b, c). Group IV showed the distorted central duct, filled with hemorrhagic debris, lipid, along with the derangement of sinusoidal spacing and infiltration of lymphocytes. The hepatic acini lost their hexagonal shape and dilated sinusoidal spacing, vascular congestion, and vacuolar degeneration were observed in 3rd week (Fig. 1g) as compared to Group I (Fig. 1a) and Group IV of 1st week (Fig. 1f). Group V, VI and VII revealed normal hepatic lobules and hepatocytes aligned around central vein, normal sinusoidal spaces in 3rd week (Fig. 1h, i, k) as compared to Group IV in 3rd week (Fig. 1g) and Group VII (week 1) (Fig. 1j).

Table 2. Alterations in serum TC, TG, HDL, LDL (mg/dl) and AST, ALT, ALP (IU/l) from 1st to 3rd week of $AlCl_3$, vitamin E and propolis exposure.

Parameters	Weeks	Control	Propolis	Vitamin E	$AlCl_3$	$AlCl_3$ + Propolis	$AlCl_3$ + Vit. E	$AlCl_3$ + Vit. E + Propolis
TC	Week 1	80.01 ± 0.87^{bbc}	84.33 ± 0.88^{bbcc}	77.6 ± 0.88^{bbcc}	$125.6\pm1.45^{*c}$	$108.0\pm0.57^{**bc}$	$114.0\pm1.53^{*c}$	$120.66\pm0.88^{**c}$
	Week 3	83.89 ± 2.74^{bc}	94.04 ± 1.77^{bbcc}	85.11 ± 0.92^{bbcc}	$158.39\pm2.65^{*b}$	$116.01\pm0.69^{**bc}$	$124.2\pm3.37^{*c}$	$126.66\pm1.12^{**c}$
TG	Week 1	80.7 ± 0.87^{bc}	87.25 ± 2.24^{bbcc}	87.33 ± 2.12^{bbcc}	$131.39\pm1.63^{*c}$	$110.93\pm1.01^{*c}$	$110.87\pm1.14^{**c}$	$116.42\pm0.91^{**c}$
	Week 3	82.19 ± 0.94^{bc}	96.79 ± 1.80^{bbcc}	85.34 ± 0.66^{bbcc}	$176.06\pm6.23^{*}$	$127.72\pm3.18^{*c}$	$120.60\pm0.84^{**c}$	$123.33\pm1.45^{**c}$
HDL	Week 1	69.81 ± 0.44^{bc}	62.25 ± 1.49^{bbcc}	58.44 ± 2.83^{bbcc}	$21.84\pm1.87^{**}$	29.15 ± 4.90	$31.22\pm1.49^{*}$	48.40 ± 4.07
	Week 3	72.18 ± 0.73^{bc}	47.96 ± 2.9	69.48 ± 0.55^{bbcc}	$19.51\pm2.69^{**}$	$43.17\pm1.63^{**}$	$44.99\pm1.40^{*}$	54.14 ± 3.31
LDL	Week 1	25.92 ± 1.69^{bcc}	32.70 ± 0.86^{bbcc}	15.93 ± 0.89^{bbcc}	$98.04\pm0.80^{**cc}$	$85.11\pm0.83^{**bcc}$	$80.31\pm0.91^{**bcc}$	$81.52\pm0.78^{**bcc}$
	Week 3	34.73 ± 0.86^{bcc}	45.56 ± 1.40^{bbcc}	46.14 ± 1.40^{bbcc}	$126.3\pm1.72^{**b}$	$90.03\pm0.87^{**cc}$	$97.7\pm1.15^{**bcc}$	$87.04\pm1.11^{**bcc}$
AST	Week 1	187.70 ± 8.52^{bc}	132.58 ± 8.66^{cc}	165.79 ± 4.55^{bbcc}	$277.20\pm2.41^{*}$	$279.93\pm7.68^{*}$	$310.33\pm3.64^{*}$	$270.33\pm3.48^{*}$
	Week 3	240.39 ± 2.86^{bc}	102.52 ± 5.13^{bbcc}	168.80 ± 7.62^{bbcc}	$342.62\pm9.61^{*}$	$293.33\pm4.68^{*}$	$288.00\pm5.79^{*}$	$296.30\pm5.24^{*}$
ALT	Week 1	51.8 ± 2.77^{cc}	66.9 ± 2.89^{cc}	58.3 ± 3.50^{bcc}	92.4 ± 6.22^{cc}	51.8 ± 1.45^{cc}	89.6 ± 4.43^{cc}	80.36 ± 2.06^{cc}
	Week 3	72.68 ± 1.14^{bcc}	43.6 ± 3.74^{cc}	58.6 ± 2.62^{bcc}	134 ± 2.71^{b}	86.60 ± 0.24^{cc}	90.92 ± 2.97^{cc}	108.6 ± 1.99^{cc}
ALP	Week 1	147 ± 8.91^{bc}	159.98 ± 4.48^{cc}	161.26 ± 4.22^{bbcc}	$279.2\pm4.52^{*}$	237.81 ± 6.55	230.55 ± 4.65^{bc}	$209.40\pm3.48^{*bc}$
	Week 3	197.1 ± 5.66^{c}	155.50 ± 3.59^{bbcc}	162.85 ± 3.13	317.82 ± 6.35	252.96 ± 5.99	265.28 ± 4.35^{c}	248.52 ± 3.67^{c}

* in comparison to control; [b] in comparison to $AlCl_3$ week 1; [c] in comparison to $AlCl_3$ week 3; $^{*, b, c}$ $p<0.05$; $^{**, bb, cc}$ $p<0.01$.

Discussion

Findings of current study revealed that $AlCl_3$ induced toxicity in liver of albino mice and caused significant decrease in body weight (Mahmoud and Elsoadaa 2013). El-Kenawy and coworkers (2014) observed that $AlCl_3$ caused loss of appetite with depression among rats while propolis improved the weight of animal further supporting the results of present study in which propolis and vitamin E showed increased body weight. Increase cholesterol level in current study might be due to mobilization of free fatty acids from the adipose tissue to blood stream and increased level of acetyl CoA, resulting in increased synthesis of cholesterol (Rubins *et al.* 1999). Current study revealed that concomitant administration of $AlCl_3$ with propolis and vitamin E revealed reduced levels of cholesterol, triglyceride, and LDL parameters as studied by Kalender *et al.* (2010). Mani and coworkers in 2006 studied that propolis maintains the level of cholesterol, triglyceride, LDL, HDL near to control group in rats. In the current study decrease in cholesterol, triglycerides and LDL might be due to the ability of alpha- tocopherol present in vitamin E that is responsible for down regulating CD36 protein in liver, which increased the lipid uptake in vitamin E that is responsible for down regulating CD36 protein in liver which increased the lipid uptake in hepatocytes (Podszun *et al.* 2014). In present study increased serum levels of AST, ALT and ALP in Group IV occurred which might be due to ability of $AlCl_3$ to increase vascular dysfunction, free radical formation and increased lipid peroxidation (Martinez *et al.* 2017). Contrary to $AlCl_3$, propolis and vitamin E reduced serum levels of liver enzymes, which might be due to their ability to reduce oxidative stress by quenching free radical production (Nogueira *et al.* 2013, Newairy *et al.* 2009). Moreover, it is evident from the results of present study that propolis and vitamin E acting synergistically

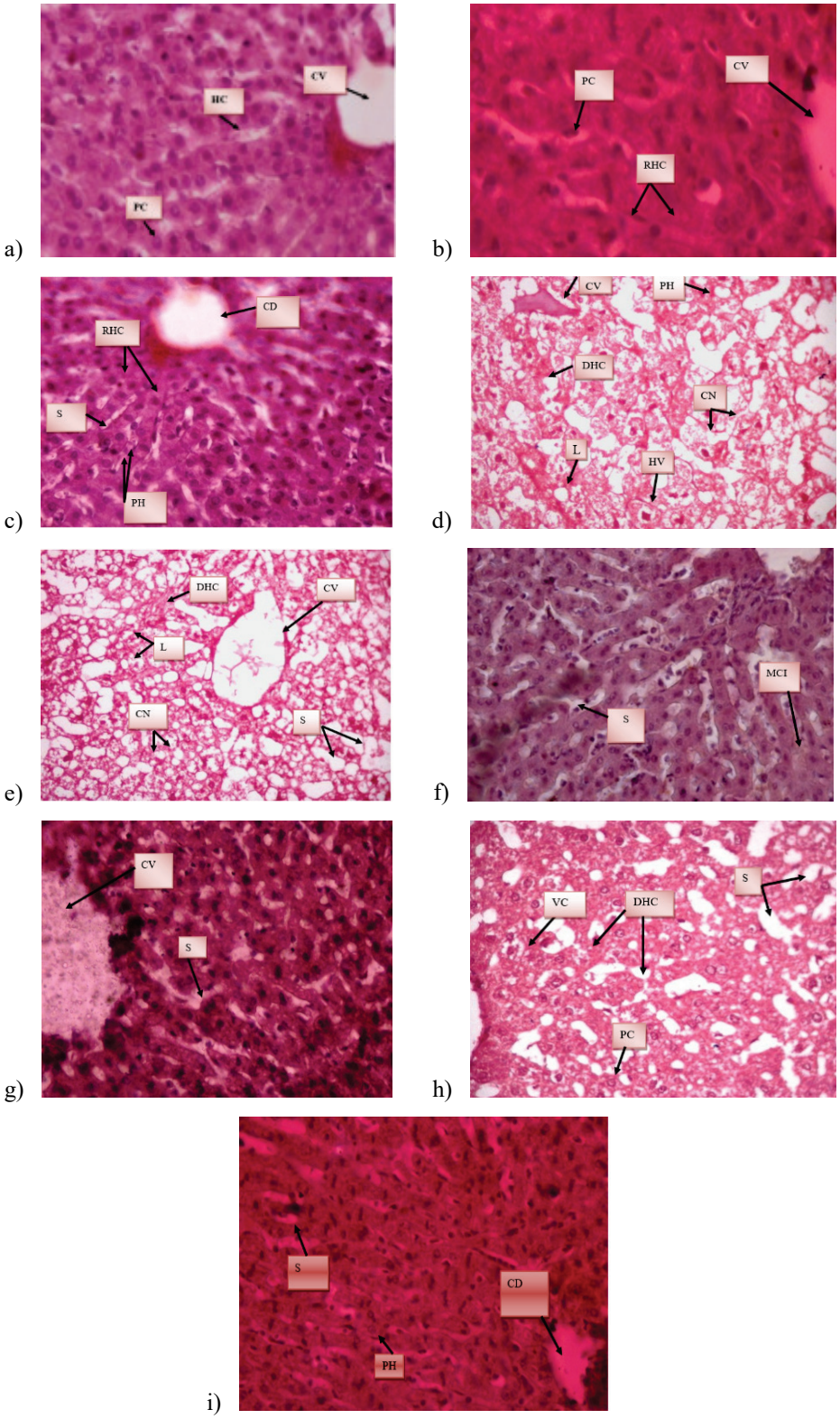

Fig. 1. (**a**) Cross section of liver in control group (H&E) at (400x) (**b**) Cross section of liver of propolis treated albino mice showing the normal histological structure during three weeks of treatment (H&E) at (400x). (**c**) Cross section of liver of Vit E treated Albino mice showing the normal histological pattern during three weeks of treatment (H&E) at (400x).(**d**) Liver of $AlCl_3$ treated group showing normal structure (H&E) at (400x) in 1st week (**e**) Liver of $AlCl_3$ treated group showing irregular structure (H&E) at (400x) in 3rd week (**f**) $AlCl_3$ + propolis treated group (H&E) at 400x in 3rd week (**g**) $AlCl_3$ + vitamin E treated group (H&E) at 400x in 3rd week (**h**) $AlCl_3$ + propolis + vitamin E treated group (H&E) at 400x in 1st week. (**i**) $AlCl_3$ + propolis + vitamin E treated group (H&E) at 400x in 3rd week. **DHC**: disrupted hepatic cords; **PH**: loss polygonal cells; **S**: sinusoidal spacing; **VC**: vacuolated cell; **CV**: central vein; **HC**: hepatic cords; **PC**: polygonal cells; **CN**: cellular necrosis; **HV**: hepatic cell vacuolization; **DHC**: disrupted hepatic cords; **L**: lipid accumulation; **S**: sinusoidal spacing; **RHC**: Radial hepatic cords. **S**: Sinusoids; **CD**: Central duct; **MCI**: macrophage cell infiltration.

showed more decrease in LDL and ALP levels and increased HDL levels compared to their individual administration along with $AlCl_3$ further supporting the findings of previous study with honey and propolis increase the activity of wound healing in rats (Takzaree *et al.* 2015).

In the current study, propolis and vitamin E showed normal architecture of hepatocytes which might be due to free radical quenching and metal chelating property of propolis as reported in the previous study in which propolis improved the histological alterations in kidney tissue by increasing the production of glutathione which in return decreased free radical productions which were initially inducing tissue damage (Garoui *et al.* 2012). On the other hand Group IV showed damaged hepatocytes which might be due to ability of $AlCl_3$ to increase vascular dysfunction (Martinez *et al.* 2017). According to Bhadauria (2012), aluminum nitrate treatment alone among rats showed loss of hepatocyte arrangement, sinusoidal spaces with plasma membrane and nuclei degeneration. Moreover, present study demonstrates that vitamin E and propolis showed protective effects by offering recovery to the damage which might be due to their healing properties as reported in another study that vitamin E promotes membrane repair by preventing the formation of oxidized phospholipids (Takzaree *et al.* 2015). In the current study, vitamin E and propolis concomitant administration along with $AlCl_3$ exhibited more pronounced protection against liver damage as evident from the serum HDL, LDL, triglycerides, AST, ALT, ALP levels. Previous study showed that propolis and bee pollen successfully protect liver tissue from various forms of regressive liver lesions, such as degeneration, vacuolar degeneration, steatosis, and necrosis of the liver parenchyma (Klaric *et al.* 2018). Presently, In addition to the improvement in biochemical parameters, hepatocytes showed marked recovery as a result of synergistic protective effects of both vitamin E and propolis which is similar to the findings of Mondal and coworkers (2016), who demonstrated that co-administration of vitamin C and vitamin E resulted in significant reduction of serum HDL, LDL and triglycerides levels and a remarkable protection against hematotoxicity and hepatotoxicity in adult male rats caused by chronic arsenic exposure. Kalender and his colleagues (2010) observed vitamin C and E, producing recovery in calcification, cell degeneration and necrosis caused by malathion further strengthening the findings of current study.

Conclusions

Significant reduction in the body weight of $AlCl_3$ treated group confirmed toxicity related changes among the albino mice. Evident changes in biochemical parameters (triglycerides, cholesterol, HDL, LDL, AST, ALT and ALP) and liver tissue showed the hepatotoxic effects of $AlCl_3$. From the results of current study, it is concluded that propolis and vitamin E exhibit protection against the liver damage, which might be due to their anti-inflammatory and antioxidant potential. Both propolis and vitamin E acting synergistically to ameliorate the toxic effects of $AlCl_3$ on mice liver.

Conflict of Interest

There is no conflict of interest.

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