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Research article

Resveratrol and Vitamin E ameliorate Carbendazim-induced toxicity in Wistar rats

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ABSTRACT

Carbendazim [methyl-2-benzimidazole carbamate, MBC (CBZ)], a metabolite of benomyl, is one of the most widespread environmental contaminants of major concern to human and animal health. The protective effect of resveratrol (RSV), vitamin E (E) and a combination of both on carbendazim-induced toxicity was investigated using haematology, serum biochemistry and histopathology in male Wistar rats. Exposure to Carbendazim (CBZ) caused a significant reduction in the values of PCV and significantly increased WBC as well as platelets counts when compared to the control and other groups while treatment with each of and a combination of RSV and E ameliorated the condition. Also, exposure to CBZ resulted in a significant increase in the values of Urea, Creat, AST, ALT, GGT, Total Cholesterol, Triglycerides and Low Density Lipoproteins while it decreased HDL across the groups. However, RSV and or E ameliorated the condition. Histopathology revealed that CBZ exposure resulted in inflammation of the glomerular apparatus as well as focal areas of granulation and hepatic lesions including fatty degeneration, peri-portal inflammation, cytoplasmic vacuolation and karyorrhexis of hepatocytes in the kidney and liver, respectively. Co-treated with E, RSV or their combination improved the conditions in the kidney and liver. The study demonstrates the ameliorating effects of RSV, E as well as their combination on CBZ-induced toxicities

Keywords: Carbendazim, resveratrol, vitamin E, kidney, liver

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INTRODUCTION

Carbendazim [methyl-2-benzimidazole carbamate, MBC (CBZ)], a metabolite of benomyl, is one of the most widespread environmental contaminants of major concern to human and animal reproductive health (Aire, 2005). CBZ, a systemic broad-spectrum fungicide exerts its antifungal action inhibiting microtubule polymerization and, consequently, cell division in fungi. Microtubules are formed by heterodimers, the α and β-tubulin, which interact by non-covalent bindings and have a key role in cell division, being responsible for chromosome segregation in mitosis and meiosis (Winder *et al.* 2001). Furthermore, they are responsible for organizing, transporting and placement of organelles. The CBZ-induced inhibition of microtubule polymerization has been attributed to its interaction with b-tubulin at the tyrosine residue 167, Tyr-167 in Saccharomyces cerevisiae (Li *et al.* 1996) and to the inhibition of the binding of guanosine triphosphate (GTP) to β -tubulin in rats (Winder *et al.* 2001). A number of reports on the endocrine actions of CBZ have been documented (Barlas *et al.* 2002; Goldman *et al.* 1989; Lu *et al.* 2004; Morinaga *et al.* 2004; Rehnberg *et al.* 1989; Yu *et al.* 2009).

Resveratrol (RSV; 3,5,4′-trihydroxystilbene), a naturally occurring phytoalexin found in juice and red wines, has been reported to exert a variety of pharmacological effects (Hung, *et al.* 2004). It has been shown to possess anti-cancer (Jang, *et al.* 1997), anti-inflammation (Jang, *et al.* 1999), and anti-platelet properties (Chung, *et al.* 1992). In purified or synthetic form, RSV reduces the synthesis of lipids in rat liver (Arichi, *et al.* 1982), inhibits the synthesis of eicosanoids in rat leukocytes (Kimura, *et al.* 1985), as well as interfering with arachidonate metabolism (Ragazzi, *et al.* 1988). Furthermore, it inhibits platelet activation and aggregation (Bertelli, *et al.* 1995) and has been linked to the inhibitory activity of some protein kinases (Jayatilake, *et al.* 1993). Also, RSV exerts a strong inhibitory effect on reactive oxygen species produced by human polymorphonuclear leukocytes (Rotondo *et al.* 1998) and has been demonstrated to possess cardio-protective

effects against ischemia-reperfusion injuries in rat hearts (Hung, *et al.* 2004).

Traditionally, Vitamin E was used as a common term for four tocopherols (alpha-, beta-, 16 gamma-, delta-tocopherol) and four tocotrienols (alpha-, beta-, gamma-, delta-tocotrienol) having been shown to have varying levels of biological activity in experimental animal studies (Traber, 2006). Sources of vitamin E include egg yolk, nuts and seeds, vegetable oil, oil-based spreads as well as sunflower oil. The uptake, transport and tissue delivery of vitamin E has been demonstrated to involve molecular, biochemical and cellular processes closely related with overall lipid and lipoprotein metabolism (Rigotti, 2007).

There is paucity of research information on the protective effects of RSV and vitamin E in CBZ-induced toxicity. This study was therefore designed to investigate the protective role of RSV and vitamin E in CBZ-induced toxicity in adult male Wistar rats.

MATERIALS AND METHODS

Experimental Animals: Forty adult male Wistar rats were used for the study. They were kept in the Animal House, Faculty of Veterinary Medicine, University of Ibadan. Commercial rat feed pellets and water was given ad libitum. The rats were stabilized for five weeks before the commencement of the treatment protocol.

Treatment protocol: The rats were randomly distributed into eight groups:
I: 15 mg/ kg body weight Resveratrol alone (R).
II: Vitamin E (100 mg/ kg body weight) plus 15 mg/ kg body weight Resveratrol (V+R).
III: Vitamin E (100 mg/ kg body weight) alone (V).
IV: Vitamin E (100 mg/ kg body weight) plus 400 mg/ kg body weight Carbendazim (V+C)
V: Control (0.1 ml corn oil + 0.1ml Carboxymethyl cellulose = vehicle) CT
VI: 400 mg/ kg body weight Carbendazim alone (C).
VII: Vitamin E (100 mg/ kg body weight), 15 mg/kg body weight Resveratrol and 400 mg
Carbendazim (E+R+C)
VIII: 15 mg Resveratrol plus 400 mg/kg body weight Carbendazim (R+C)
All treatments were by oral route and lasted for 7 days. Carboxymethyl cellulose was used as the solvent for resveratrol while corn oil was the solvent for both vitamin E and Carbendazim.

Blood sampling : Blood samples were collected on Day 8 after deeply anaesthetizing each rat with a combination of xylazine and ketamine. Blood sampling was carried out as reported by Guyton and Hall (2006); Ola-Davies *et al*. (2014). Briefly, blood samples were collected from the orbital sinus of the rats into clean lithium heparinized tubes. Drops of whole blood were used to fill some heparinised microhematocrit capillary tubes to determine packed cell volume (PCV), and hemoglobin (Hb). Whole blood was also used to make three air dried blood smears. The smears were stained with Wright's stain and examined for red blood cell (RBC), white blood cell (WBC), differential WBCs (lymphocytes, neutrophils, basophils, eosinophils, monocytes) and platelet estimate. Blood samples were also collected for biochemical analysis, centrifuged at 3000 rpm for ten minutes to isolate the serum. Na^+, K^+, Cl^-, HCO^-_3 were determined by use of automated analysers as described by Meyer and Harvey (1998). Commercially available kits were used according to the respective manufacturer's protocol for the measurement of serum liver enzyme activity. Serum alkaline phosphatase (ALP) activity was determined by a kit from BioSystems SA., Spain. Serum aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alanine transaminase (ALT), acid phosphatase and prostatic acid phosphatase activities, Urea, Creatinine, Bilirubin (total and conjugated bilirubin), Proteins, high density lipoprotein (HDL), low density lipoprotein (LDL), were measured using RANDOX® laboratory reagent kits obtained from RANDOX Laboratories Ltd., Ardmore, United Kingdom. Serum Cholesterol and Triglyceride levels were determined by Ecoline CHOD-PAP and Ecoline 25 GPO-PAP assay kits (1.14856.0001, Merck KGaA, Darmstadt, Germany), respectively.

Histological study: Liver and kidney samples were collected in 10% formalin for histopathological analysis. Tissues were processed and embedded in parafin wax and sections were made of about 4–6 μm. After staining with hematoxylin and eosin, slides were examined under the microscope (Olympus, Japan) for histopathological changes and photographed.

Statistical analysis
Data generated from the treatments were analysed using mean-standard deviation and was subjected to two-way ANOVA with Dunnett's multiple comparisons test (GraphPad Prism 6).

RESULTS

The mean and standard deviation of haematological parameters of the rats are given in Table 1. Exposure to carbendazim caused a significant reduction in the values of PCV when compared to the control and other groups while treatment with each of and a combination of resveratrol and vitamin E ameliorated the condition (Table 1). However, carbendazim exposure significantly increased WBC and platelets counts while treatment with each of and a combination of resveratrol and vitamin E improved the condition (Table 1). Values for RBC, Hb, NEU, LYM, MON and Ba had no significant difference across the different groups. It is however, important to note that carbendazim caused a reduction in the values of NEU when compared to the other groups.

Also, no significant differences were observed for the values of Na^+, K^+, Cl^-, HCO^-_3, TP, ALB, GLO and CB across the groups. Carbendazim induced a decrease in the value of TB while treatment with either resveratrol or vitamin E ameliorated the condition (Table 2). Exposure to CBZ resulted in a significant increase in the values of UREA, CREAT, AST, ALT and GGT across the groups.

Table 1.
Effect of Resveratrol and Vitamin E on carbendazim-induced changes in the haematological parameters of rats

	R	V+R	V	V+C	CT	C	E+R+C	R+C
PCV	44.00 ±3.61^{b}	45.67 ±4.93^{b}	45.00 ±3.61^{b}	47.33 ±3.22^{b}	45.33 ±3.22^{b}	40.33 ±1.53^{a}	47.33 ±2.08^{b}	44.00 ±2.65^{b}
RBC	7120 ±1307^{a}	6796 ±395^{a}	7853 ±607^{a}	7090 ±64^{a}	7770 ±1139^{a}	7060 ±196^{a}	7526 ±1211^{a}	7358 ±1618^{a}
WBC	9763 ±3065^{b}	11936 ±2479^{b}	9700 ±953^{b}	10253 ±2426^{b}	10466 ±4895^{a}	12030 ±1462^{a}	9963 ±1978^{b}	9672 ±520^{b}
PLAT	466333 ±5089^{b}	482333 ±6882^{b}	452000 ±6009^{b}	493333 ±1258^{b}	425666 ±7356^{b}	526000 ±8685^{a}	432666 ±6007^{b}	471666 ±1197^{b}
NEU	44.00 ±8.54^{a}	44.00 ±6.08^{a}	43.00 ±6.00^{a}	46.70 ±7.00^{a}	44.33 ±17.19^{a}	37.67 ±7.64^{a}	48.00 ±6.25^{b}	47.33 ±7.02^{b}
LYM	59.00 ±8.54^{a}	54.67 ±6.80^{a}	63.00 ±11.79^{a}	63.67 ±6.43^{a}	57.33 ±17.90^{a}	60.33 ±7.64^{a}	58.33 ±7.10^{a}	54.00 ±6.56^{a}
MON	0.00 ±0.00	0.67 ±1.16	0.67 ±0.58	0.33 ±0.58	1.00 ±0.00	0.00 ±0.00	0.67 ±1.16	1.33 ±0.58
EOS	0.42 ±0.00	0.67 ±1.16	0.67 ±0.58	0.53 ±0.00	0.46 ±0.00	0.89 ±0.00	0.56 ±0.00	0.47 ±0.00
BA	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.33 ±0.58	0.33 ±0.58	0.00 ±0.00	0.00 ±0.00	0.33 ±0.58
Hb	14.20 ±1.31	14.07 ±2.72	14.57 ±0.75	14.53 ±0.46	14.00 ±0.00	14.17 ±1.04	15.67 ±0.58	15.23 ±1.17

Means with different superscripts within rows are significantly significant (P<0.05)

Table 2
Effect of Resveratrol and Vitamin E on carbendazim-induced changes in serum biochemistry and electrolytes parameters of rats

	R	V+R	V	V+C	CT	C	E+R+C	R+C
Na^{+}	139.67±0.58	138.33±2.31	139.00±3.61	139.67±1.53	139.67±2.31	138.00±3.61	138.67±2.31	136.33±3.06
K^{+}	3.87±0.21	4.67±0.48	3.73±0.15	3.93±0.25	3.83±0.15	3.40±0.27	3.80±0.20	3.70±0.17
Cl^{-}	106.67±5.77	105.00±0.00	109.67±2.89	116.67±2.89	105.00±5.00	101.00±5.00	103.67±2.89	107.00±0.00
HCO_3^{-}	22.67±2.08	23.33±1.16	22.68±1.53	21.63±1.53	22.33±2.08	20.17±3.06	23.67±1.53	25.00±1.00
TP	7.13±0.15	7.07±0.25	6.97±0.51	6.43±0.25	7.17±0.25	5.77±0.06	6.87±0.15	6.73±0.25
ALB	4.00±0.27	4.00±0.17	3.93±0.29	3.97±0.06	4.10±0.27	3.27±0.12	3.87±0.21	3.73±0.35
GLO	3.13±0.12	3.07±0.06	3.03±0.25	3.00±0.27	3.07±0.06	2.57±0.51	3.00±0.20	3.00±0.10
TB	0.53±0.06^{b}	0.47±0.15^{b}	0.50±0.17^{b}	0.60±0.00^{b}	0.63±0.21^{b}	0.38±0.12^{a}	0.60±0.17^{b}	0.63±0.15^{b}
CB	0.30±0.10	0.36±0.00	0.39±0.06	0.26±0.10	0.23±0.06	0.20±0.06	0.27±0.06	0.25±0.12

Means with different superscripts within rows are significantly significant (P<0.05)

Table 3.
Effect of Resveratrol and Vitamin E on carbendazim-induced changes in the liver, kidney and lipid profile parameters of rats

	R	V+R	V	V+C	CT	C	E+R+C	R+C
UREA	27.00±1.73^{b}	29.67±3.51^{b}	25.67±5.03^{b}	27.33±0.58^{b}	28.00±3.61^{b}	39.33±1.53^{a}	28.33±1.16^{b}	30.33±0.58^{b}
CREAT	0.60±0.10^{b}	0.57±0.15^{a}	0.57±0.21^{a}	0.53±0.06^{a}	0.47±0.15^{a}	0.70±0.12^{b}	0.57±0.06^{a}	0.53±0.06^{a}
AST	11.33±1.16^{a}	12.33±0.58^{a}	13.67±4.04^{a}	13.33±2.08^{a}	12.33±1.53^{a}	18.33±1.53^{b}	11.67±3.79^{a}	12.33±1.16^{a}
ALT	8.27±4.84^{a}	9.67±0.58^{a}	10.00±2.00^{a}	8.00±2.65^{a}	9.67±1.16^{a}	15.00±0.00^{b}	9.33±3.22^{a}	9.83±2.52^{a}
GGT	7.00±1.73^{a}	7.00±2.00^{a}	7.67±1.53^{a}	6.90±4.53^{a}	7.33±1.53^{a}	11.33±1.16^{b}	6.67±2.89^{a}	6.67±3.06^{a}
ALP	55.33±9.07^{a}	51.33±3.06^{a}	51.67±3.51^{a}	53.33±8.93^{a}	56.33±0.58^{a}	62.33±7.57^{b}	55.00±6.08^{a}	59.67±6.66^{a}
TC	115.00±5.00^{a}	118.67±7.57^{a}	115.67±25.03^{a}	113.00±9.85^{a}	120.67±6.81^{a}	138.33±12.58^{b}	113.33±15.28^{a}	119.67±5.03^{a}
TG	63.00±5.57^{a}	61.33±5.51^{a}	62.00±9.54^{a}	68.00±5.29^{a}	63.00±19.47^{a}	81.67±11.72^{b}	67.00±9.54^{a}	62.67±4.93^{a}
HDL	44.33±7.57^{b}	35.33±3.79^{a}	42.00±7.81^{b}	33.67±5.0^{a}	37.33±3.79^{b}	30.33±6.03^{a}	34.33±6.66^{a}	32.67±3.79^{a}
LDL	75.67±10.69^{b}	75.00±1.00^{b}	75.00±18.52^{b}	75.33±6.35^{b}	73.33±9.02^{b}	99.33±17.24^{a}	73.33±15.04^{b}	77.00±7.81^{b}

Means with different superscripts within rows are significantly significant (P<0.05)

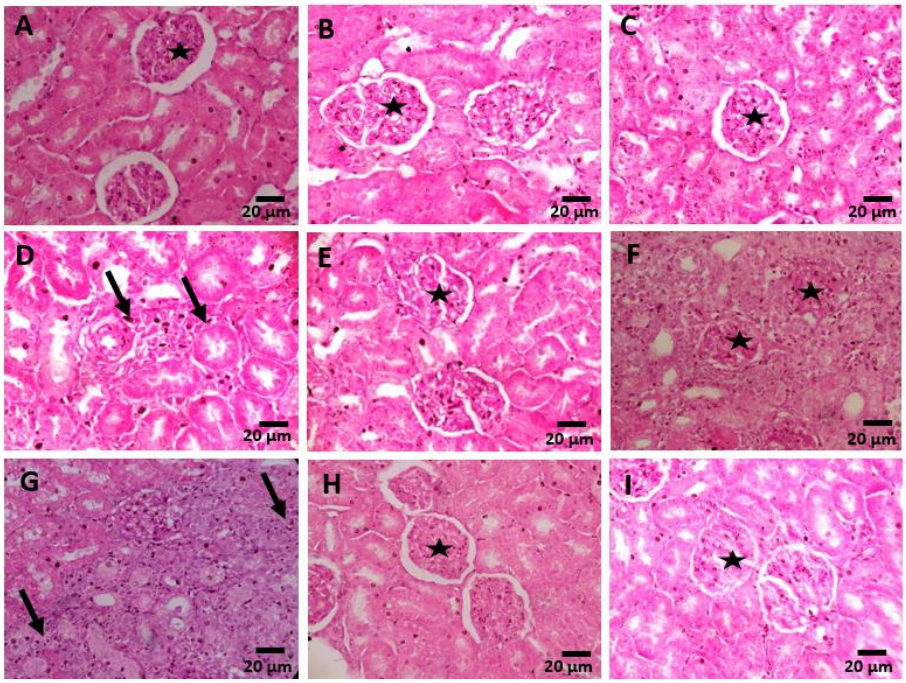

Figure 1.
Cross section of the kidney of rats (H&E). A. Resveratrol alone group showing normal tissue with normal glomerulus (asterisk). B. Vitamin E + resveratrol group showing normal tissue with normal glomerulus (asterisk). C. Vitamin E alone group showing normal tissue with normal glomerulus (asterisk). D. Vitamin E + carbendazim group showing mild peritubular inflammation (arrows). E. Control group showing normal tissue with normal glomerulus (asterisk). F. Carbendazim alone group showing inflamed glomerular tissue (asterisk). G. Carbendazim alone group showing focal area of granulation tissue (arrows). H. Resveratrol + vitamin E + carbendazim group showing normal tissue with normal glomerulus (asterisk). I. Resveratrol + carbendazim group showing normal tissue with normal glomerulus (asterisk).

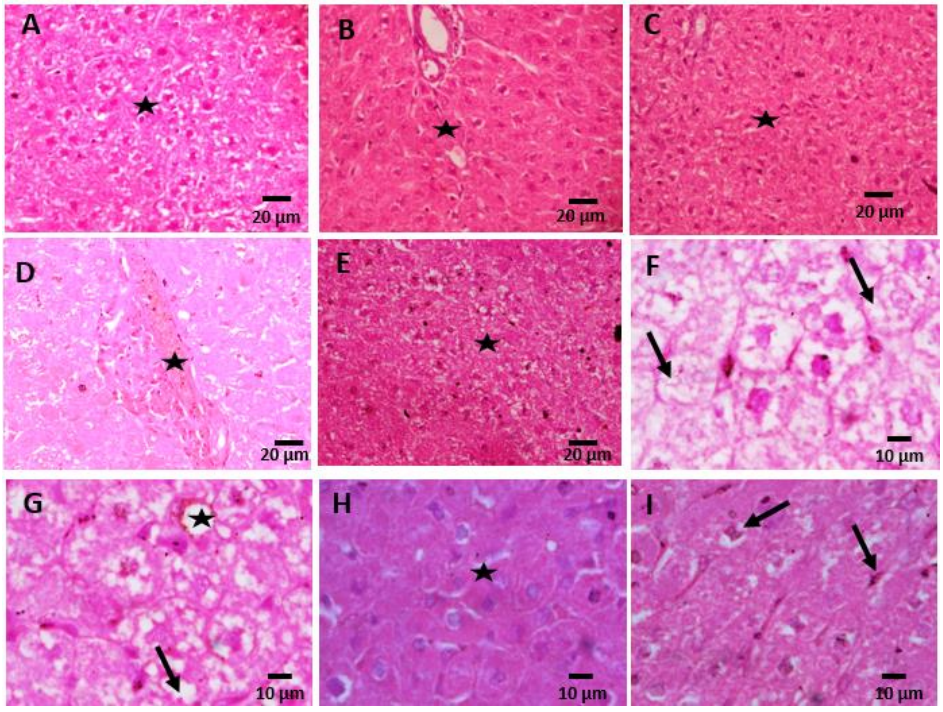

Figure 2.
Cross section of the liver of rats (H&E). A. Resveratrol alone group showing normal hepatic tissue (asterisk). B. Vitamin E + resveratrol group showing normal hepatic tissue (asterisk). C. Vitamin E alone group showing normal hepatic tissue (asterisk). D. Vitamin E + carbendazim group showing mild peri-portal inflammation (asterisk). E. Control group showing normal hepatic tissue (asterisk). F. Carbendazim alone group showing fatty degeneration (arrows). G. Carbendazim alone group showing fatty degeneration (arrow) and cytoplasmic vacuolation (asterisk). H. Resveratrol + vitamin E + carbendazim group showing normal hepatic (asterisk). I. Resveratrol + carbendazim group showing karyorrhexis of hepatocytes (arrows).

However, RSV and or Vitamin E improved the condition (Table 3). Contrarily, no significant difference was observed in the values of ALP across the groups. Also, CBZ exposure significantly increased the values for TC, TG and LDL while it decreased HDL.

Histopathology revealed that exposure to carbendazim resulted in inflammation of the glomerular apparatus as well as focal areas of granulation in the kidney (Figure1F & G). However, co-treatment with vitamin E (Figure 1D), resveratrol (Figure 1I) or their combination (Figure 1H) improved the condition. All treatments with either resveratrol (Figure 1A) or vitamin E alone (Figure 1C) or both (Figure 1B) as well as the control group (Figure 1E) revealed no lesion in the kidney. Also, carbendazim exposure resulted in hepatic lesions including fatty degeneration, peri-portal inflammation, cytoplasmic vacuolation and karyorrhexis of hepatocytes (Figure 2F &G). As observed in the kidney, co-treated with vitamin E, resveratrol or their combination improved the condition (Figure 2A, B, C, D, E, H &I).

DISCUSSION

Exposure to carbendazim has been demonstrated to be mainly through occupational or food consumption with primary exposure in humans being residues of benomyl and carbendazim used in food crops (Veerappan *et al.* 2011). This study was designed to determine the ameliorating effects of resveratrol and vitamin E on carbendazim-induced toxicities in male Wistar rats. The carbendazim-induced reduction in PCV values in the rats used for this study is indicative of its negative effect on erythropoiesis in animals. Also, the significant increase in WBC as well as platelet counts caused by carbendazim further shows it as a toxicant and further indicates an enhanced immune capacity. These anomalies were however ameliorated by the concomitant treatment with resveratrol or vitamin E.

Our observation on RBC and lymphocyte counts is contrary to the report by Muthuviveganandavel *et al.* (2008), that carbendazim caused reduction in the red blood cell counts and increase in the lymphocyte counts with carbendazim administration. This disparity may be due to difference in concentration as well as routes of exposure and duration of administration. However, findings from the present study are similar to reports on the toxic effects of carbendazim in animals (Cummings *et al.* 1990; Dalvi, 1992; Veerappan *et al.* 2011). Specifically, the observed carbendazim-induced decreased in neutrophil concentration in the rats might have resulted from the suppression of leucopoiesis in the bone marrow which may have consequential effects on the immune and phagocytic activity of the blood cells of the rats (Afolayan and Yakubu, 2009, Ola-Davies *et al.* 2014). Findings from the work therefore suggest that resveratrol or vitamin E can be used to enhance leucopoiesis in the bone marrow.

The observed upward regulation of UREA, CREAT, AST, ALT and GGT by carbendazim across the groups, compared to the control is consistent with previous findings (Selmanoglu *et al.* 2001; Seda and Aktac, 2005; Veerappan *et al.* 2011). The ability of resveratrol and vitamin E to significantly reduce elevated serum biochemical parameters caused by endocrine disrupting substances has also been reported by different authors (Brian *et al.* 2001; Park *et al.* 2004; Hung, *et al.* 2004; Traber, 2006; Rigotti, 2007; Zhu *et al.* 2008). Serum biochemistry is of clinical relevance in identifying the target organs of toxic effects as well as the general health status of animals, and it is advocated to provide early warning of potentially deleterious changes in stressed or diseased animals (Sacher and Mcpherson, 2000).

In the present study, each of resveratrol and vitamin as well as their combination were able to significantly reduce carbendazim-induced elevated TC, TG and LDL. This finding is similar to those of previous authors (Hidiroglou *et al.* 2004; Zhu *et al.* 2008; Castrol *et al.* 2009). Also, resveratrol and vitamin E as well as their combination significantly increased carbendazim-induced reduced TB as reported by some authors (Hung, *et al.* 2004; Traber, 2006; Rigotti, 2007). Reports have shown that resveratrol has strong antioxidant activity *in vitro* (Fauconneau *et al.* 1997) with great anti-inflammatory activities (Kawada *et al.* 1998; Rotondo *et al.* 1998; Jang *et al.* 1997; Zhu *et al.* 2008). Also, resveratrol exhibits cancer chemo-preventive activity (Jang *et al.* 1997) and is able to modulate low-density lipoprotein oxidation (Frankel *et al.* 1993). In rats, dietary resveratrol has been demonstrated to dose-dependently suppresses both serum triglyceride and very-low-density as well as low-density lipoprotein-cholesterol levels in hepatoma-bearing rats (Daiki *et al.* 2003; Zhu *et al.* 2008).

Histopathological findings in the present study have further supported that carbendazim exposure is injurious to the kidney and liver of rats. The observed carbendazim-induced lesions are in consonant with those of previous authors (Igbediogh and Akinyele, 1992; Balkan and Aktac, 2005). However, resveratrol and vitamin E as well as their combination were able to ameliorate carbendazim-induced lesions of the kidney and liver. The protective effects of both resveratrol and vitamin E can be attributed to their antioxidant, anti-inflammatory and anti-proliferative activities. The cytoplasmic vacuolation of hepatocyte observed in the carbendazim treated groups is a non-specific response of rats due to toxic conditions (Zhu *et al.* 2008). Such vacuolizations might indicate an imbalance between the rate of synthesis of substances in the parenchyma cells and the rate of their release into the circulation (Gingerich, 1982; Zhu *et al.* 2008). In portal circulation, the flow of blood is from hepatic portal vein and hepatic artery into the central vein, hence, any congestion or stenosis in the central vein would result in reduced flow of blood (Faheem *et al.* 2016). The inflammation of the glomerulus and focal area granulation of the kidney tissue observed in the carbendazim-treated rats are capable of inducing decreased glomerular filtration rate and as such predispose to haemodialysis. Findings from the study suggest that carbendazim has direct nephrotoxic action. Hence its effects are expected to be excreted principally on the proximal convoluted tubules and thereby modify the structure and function of the kidney (Rahimi *et al.* 2015).

In conclusion, findings from the present study have shown that carbendazim elicits toxic effects in the kidney and liver of rats affecting biochemical and hematological parameters thereby causing histopathological changes in tissues. The study has also demonstrated the ameliorating effects of

resveratrol, vitamin E as well as their combination on carbendazim-induced toxicities. It is therefore recommended that resveratrol and vitamin E be administered as a protection to carbendazim-induced toxicity while the use of carbendazim as pesticide should be regulated due to its adverse effects in animals and by extension, humans.

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