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Original Article

Colchicine and Vitamin E in the management of paracetamol-induced liver damage

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ABSTRACT

Colchicine has found its place in the management of liver damage with blood disease as side effects. This paper reports on the use of Colchicine and Vitamin E in the management of paracetamol-induced hepatoxicity. Seventy-two experimental male albino rats were divided into 6 groups, Group one served as Normal control and was not induced with paracetamol; the other group (2-6) was given intra peritoneal injection of 640 mg/kg body weight of paracetamol and was allowed to develop hepatotoxicity for 72 hours. After 72 hrs, Groups 2-6 were treated for four weeks with water, Colchicine only (0.03mg), Colchicine-Vitamin E (0.2ml/kg), Colchicine-Vitamin E (0.3ml) and Colchicine-Vitamin E (0.3ml/kg). Blood was collected at weekly intervals and liver markers were assayed using standard methods and reagent kits. The level of liver enzymes of 22.00 raised by the Paracetamol-induced was reduced by administration of Colchicine alone to 15.46 and Colchicine-Vitamin E to 14.45 at fourth week. This work shows that Colchicine at low dose of 0. 03 mg/kg body weight or in combination with Vitamin E could be used to manage effectively liver damage, presume to be the action of removal of free radicals.

Keywords: Acetaminophen, *N*-acetyl-*p*-benzoquinoneimine

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INTRODUCTION

Paracetamol (acetaminophen) has successfully been used over the ages for the treatment of pain and fever associated with cold, flu, viral infections or other disorders where pain or fever may occur. The development of liver damage (Hepatoxicity) has been associated with over dose, especially if alcohol is involved [1]. This has resulted in acute liver failure, hepatic necroses, renal tubular necrosis and hypyglycaemic coma [2]. At therapeutic levels, most of the administered dose is normally metabolized by glucuronidation and sulfonation (Phase II) to produce inactive nontoxic metabolites that are easily excreted by kidney **[**3].However, a small portion is metabolized by oxidation (Phase I) through CYP2E1, to *N*-acetyl-*p*-benzoquinoneimine (NAPQI), a highly toxic and reactive metabolite that depletes glutathione (GSH) and covalently binds to mitochondrial proteins which is efficiently detoxified by conjugation with GSH. However, at toxic doses, GSH is depleted by the conjugation reaction, and NAPQI covalently binds to proteins to produce reactive oxygen species (ROS) which induce oxidative stress leading to lipid peroxidation, mitochondrial dysfunction, disruption of calcium, nitric oxide homeostasis, and finally, cell death by apoptosis and necrosis. [4,5] This is a leading cause of liver failure in the United States [5].

Colchicine was officially approved for use in the United States in 1961 and it is still widely used. It has always been reported to be safe, but could be toxic at a prolonged use of more than seven days and at higher dose. It has antifibrotic and anti-inflammatory effects and hence proposed as a treatment for liver disease. Long-term colchicine treatment in patients with hepatic fibrosis appears to exert an anti-inflammatory, anti-fibrotic and immunomodulatory effect [6]. It demonstrates the greatest anti-mitotic activity on rapidly dividing tissues, so toxicity initially presents with gastrointestinal (GI) symptoms, but patients can develop bone marrow hypoplasia, cardiac arrhythmias, cardiovascular collapse, respiratory distress, and shock, which can lead to multisystem organ failure [6]. The report stated that its association with hepatotoxicity was with cases of overdose in which the hepatic injury has been self-limited and overshadowed by the other toxicities. It is also implicated to prevent the development of hepatocellular carcinoma in virally-related liver cirrhosis. Colchicine is an effective and safe antifibrotic drug for long-term treatment of chronic liver disease in which fibrosis progresses towards cirrhosis. It was shown not only to arrest, but even to reverse this process [7]. Protection against paracetamol-induced toxicity by Vitamin E has been reported as measured by the prevention of mortality, fall in hepatic non-protein sulphydryls (NPSH) and the decrease in elevation of serum transaminases[8]. Antioxidants such as vitamin E have been shown to play very important roles in reducing the hepatoxicity of paracetamol [9]. Several vitamins, including C, E, and B_{12}, have been recognized as antioxidants and have shown hepatoprotective effects against the hepatotoxicity caused by acetaminophen (APAP) overdose. [4,10]. This has been shown by means of histological examination, analysis of serum parameters and biochemical evaluation of collagen content [11]. In this study the use of Colchicine in combination with Vitamin E in the treatment of Paracetamol-induced liver damage is investigated.

MATERIALS AND METHODS

Animals: Seventy- two male Wistar albino rats weighing 120g-290g and about 6-8 weeks old were obtained from the department of Veterinary Science, University of Nigeria, Nsukka and kept in cages in the animal house of Madonna University, Elele Campus, where the research work was done. Colchicine, cysteine, and paracetamol were purchased from Qauli-kems, Jonkins Chemicals and Emzor Pharmacy respectively. The animals were treated according to the ethical guidelines of the University; They were kept in well ventilated room with ambient temp, with water and food *ad libitum.*

Animal Treatment and Dosage of Drug Administered: The 72 experimental rats were divided into six groups of 12 rats each. Group one served as Normal control, received normal feed (palletized Guinea Grower's mash from Bendel Feed and Flour mill Limited, Nigeria), water and was not induced with paracetamol, nor treated with Colchicine and cysteine. The other groups (2-6) were given intra peritoneal injection of 640 mg/kg body weight of paracetamol and were allowed to develop hepatotoxicity for 72 hours. After 72 hrs, Groups 2-6 were treated for four weeks with water, Colchicine only (0.03mg), Colchicine/Vitamin E (0.03/0.1ml/kg), Colchicine /Vitamin E (0,03/0.2ml/kg) and Colchicine /Vitamin E (0,03/0.3ml/kg) in mg/Kg body weight of rat respectively

Collection of Blood samples: The rats were sacrificed painlessly under chloroform anesthesia. Blood was collected at weekly intervals by cardiac puncture, centrifuged at 3000rpm for 10minutes and serum was collected for further analysis.

Determination of Liver Markers: The serum activities were determined by spectrophotometric

methods for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using the method of Reitman and Frankel [12], Alkaline phosphatase (ALP) GSCC [13], direct and total bilirubin using the Jendrassik and Grof [14] and gama-glutamyltransferase using Szasz method [15].

Statistical Analysis: The data obtained were expressed as mean + SEM. The significance of difference among the various treated groups and control group were analyzed by means of one-way analysis of variance ANOVA followed by Dunnett's multiple comparison test using Graphat instant Software (San Diego, CA.USA). The level of significance was set at $p < 0.05$.

RESULTS

Table 1 shows the effects of Colchicine and Vitamin E on Aspartate Transferase Level (U/L) in Paracetamol –Induced Liver Disease, After Four Weeks of Treatment in Albino Rats. The normal AST level was between 10.72 +.30 to 11.15+ .034 from the first to the last week which did not show any significant change. Paracetamol -induced liver disease significantly (p<,0.05) raised AST level after four days and raised the level from 10.72+ .30 to 33.90 +3.16 in the 1st week to 37,56+ .63 U/L (Mean +SEM) in the fourth week. Colchicine decreased the AST level from 33.90 +3.16 to 30.18 +0.05 after 1st week and to 28.56 +0.199 after 4th week. Vitamin E (0.3ml/kg) significantly decreased the AST level from 20.64 +.72 to 19.39 +.56 U/L in the fourth week. Vitamin E significantly decreased AST level in combined therapy than when colchicine was administered alone. The 1st week to 37,56+ .63 U/L (Mean +SEM) in the fourth week. Colchicine decreased the AST level from 33.90 +3.16 to 30.18 +0.05 after 1st week and to 28.56 +0.199 after 4th week. Vitamin E (0.3ml/kg) significantly decreased the AST level from 20.64 +.72 to 19.39 +.56 U/L in the fourth week.

Table 2 shows the effects of Colchicine and Vitamin E on Alanine Transferase Level(U/L) in Paracetamol–Induced Liver Disease, After Four Weeks of Treatment in Albino Rats. The normal ALT level was between 6.8+ .115 and 6.5 +0.34 from the first to the last week which did not show any significant change. Paracetamol -induced liver disease significantly (p<,0.05) raised ALT level after four days and raised the level from 6.8+ .115 to 27.1 $\pm$0.57 in the 1st week to 29.1 $\pm$0.115 U/L (Mean +SEM) in the fourth week. Colchicine decreased the ALT level from 27.1 +057 to 22.00 +.23 after 1st week and to 15.46 +.37 after 4th week. Vitamin E (0.3ml/kg) significantly decreased the ALT level from 17.05 +.49 to 14.45 +.37 U/L in the fourth week.

Table 3 shows the effects of Colchicine and Vitamin E on Alkaline Phosphatase (U/L) in Paracetamol –Induced Liver Disease, After Four Weeks of Treatment in Albino Rats. The normal ALP level was between 11.43+ .08 and 11.126 +0.03 from the first to the last week which did not show any significant change I ALP level. Paracetamol induced liver disease significantly (p<,0.05) after four days and raised ALP level from 11.1267 +0.003 to 39.00 +0.57735 U/L (Mean +SEM). Colchicine decreased the ALP level from 39.00 +0.57735 U/L to 35.15 +0.044 after 1st week and to 34.176 +0.089 after 4th week. When colchicine and Vitamin E were given together, ALP level was significantly decreased to 33.7+ .03 in the first week to 31.8 +0.026 in the fourth week. Vitamin E (0.3ml/kg) significantly decreased the ALP level more than colchicine alone. The decrease was 28.77 +0.17 to 26.16 +0.176 in the 1st week and 26.16 +0.114 in the fourth week which is highly significant (p<.05).

Table 4 shows the effects of Colchicine and Vitamin E on Gama- GT Level (U/L) in Paracetamol –Induced Liver disease, After Four Weeks of Treatment in Albino Rats. The normal GT level was between 7.38 +0.223 U/L to 6.84 +0.21 U/L from the first to the last week which did not show any significant change. Paracetamol -induced liver disease significantly (p<, 0.05) raised GT level after four days and raised the level from 29.75 +0.16U/L in the 1st week to 32.00 +1.15 U/L (Mean +SEM) in the fourth week. Colchicine decreased the GT level from 29.75 +0.16U/L to 29.61 +0.98 after 1st week and to 27.96 +0.10 after 4th week. When colchicine and Vitamin E were given together, GT level was significantly decreased to 25.64 +0.086 in the first week and to 24.4 +0.129 in the fourth week. Vitamin E (0.3ml/kg) significantly decreased the GT level from 23.60 +0.0057 to 22.59 +0.123 U/L in the fourth week.

Table 5 and 6 showed the effects of Colchicine and Vitamin E on Total and Direct Bilirubin Level in Paracetamol – induced Liver Disease after four weeks of Treatment in Albino Rats.The normal level for total bilirubin was between 0.34$\pm$0 .035mg/dl to 0.3367 $\pm$0.031 from the first to the last week which did not show any significant change. Paracetamol -induced liver disease significantly (p< 0.05) raised total bilirubin level after four days and raised the level from 0.34 $\pm$0.035 to 1.256 $\pm$0.008 in the 1st week to 1.16 $\pm$0.0033 (Mean $\pm$SEM) in the fourth week. Colchicine decreased the total bilirubin level from 1.256 $\pm$0.008 to 1.19 v0.011 after 1st week and to 1.143 $\pm$0.02 after 4th week. When colchicine and Vitamin E were given together, bilirubin level was significantly decreased to 1.0167 $\pm$0.21 in the first

week and to 0.966 ±0.0038 in the fourth week. Vitamin E (0.3ml/kg) significantly decreased the total bilirubin level from 0.85 ±0.01 to 0.69 ±0.028 in the fourth week.

DISCUSSION

There is significant induction of Liver damage in the experimental animals from the analysis of the levels of liver maker enzymes ALT, ASP and confirmed with ALP and GM [16-17].The administration of Colchicine reduced the level of enzyme by the first week and further reduction observed by the fourth week , confirming the effect of Colchicine on the liver damage as reported [18].This reduction was also observed in the parameter measurement of in the administration of Colchicine with Vitamin E especially as the dose of Vitamin Eincreases with increasing duration of administration up to the fourth week[19]. There was also significant reduction of Direct and total bilirubin in confirmation of reported literature [20]. Vitamin E significantly decreased AST level in combined therapy than when colchicine was administered alone. This is attributed to the to the ability of the antioxidant supplement to balance off free radicals generated hence preventing peroxidation of the lipid components of the cell membrane. Disruption of membrane integrity is a common causative factor attributed to increase release or leakage of cellular contents [21]. This finding is was consistent with the reports of Li et al. [22]. Colchicine decreased ALT level than in combination with Vitamin E especially after four weeks. This is attributed to the to the ability of the antioxidant supplement to balance off free radicals generated hence preventing peroxidation of the lipid components of the cell membrane. Disruption of membrane integrity is a common causative factor attributed to increase release or leakage of cellular contents [21]. This finding is was consistent with the reports of Li et al. [22] This consistent to the report of Videla [18] reporting that a rapid mobilization of liver-ALP in blood, resulting increase serum levels at early stages of liver damage.

Vitamin E significantly decreased GT level in combined therapy than when colchicine was administered alone. This study is consistent with the report on the gamma-glutamyltransferase elevation [19] and the antioxidant effect on hepatotoxicity [5]. Biluribin is a product of the breakdown of the heme component of the heamoglobin. Its elevation is a function of the rate of red cell destruction and the capacity of the liver to excrete the newly formed biluribin[20].

Vitamin E significantly decreased total bilirubin level in combined therapy than when colchicine was administered alone. The observations could be attributed to theincreased presence of the various treatments over the duration of the study, which is consistent the report of Tripathi [20]

CONCLUSION

This research confirms the beneficial therapy of combine therapy of Colchicine and Vitamin E in the treatment of hepatotoxicity. Treatment with Colchicine should be supplemented with Vitamin E. From the results in the above tables. It can be concluded that the combined therapy of Colchicine and Vitamin E will benefit hepatotoxic patients management.

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Table 1 Effects of Colchicine, Cysteine and Vitamin E on Alanine Transferase Level (U/L) in Paracetamol –induced Liver Disease, After Four Weeks of Treatment in Albino Rats.

TREATMENT	Wk1	WK 2	Wk 3	Wk4
NOMAL	6.8000	6.3500	5.8500	6.5000
	±0.1154 [a]	±0.0866 [a]	±0.2020 [a]	±0.3464 [a]
PARA-INDUCED	27.1000	28.3500	29.1000	28.1667
	±0.0577[ba]	±0.0288[ba]	±0.1154[ba]	±0.3844[ba]
COLCH-ALONE	22.0000	21.3500	18.9500	15.4667
	±0.2309[ba]	±0.3752 [a]	±0.43301	±0.3711[ba]
COLCH-VIT E(0.1ml/kg)	18.5500	17.6500	16.0500	14.6667
	±0.20207	±0.2598[cba]	±0.08660	±0.35277
COLCH-VIT E(0.2ml/kg)	17.9500	17.0500	16.0500	15.7500
	.3175[cba]	±0.60622	±0.54848	±0.43301
COLCH-VIT E(0.3ml/kg)	17.0500	16.2500	15.7000	14.4500

	±0.4907^{cba}	±0.4907^{cba}	±0.4618	±0.3752^{cba}

Result Represents Mean ±SEM of Triplicate Sample. Least Significant Difference (LSD) was used to compare the means. Values were considered significant at p<0.05 and superscripts in the same column with the same letters are not significant.

[a] = Significant Difference when each Concentration is considered with Normal Level first control

[b] = Significant Difference when each Concentration is considered with Normal Level first control

[c] = Significant increase when the concentrations are compared with each other.

Table 2: Effects of Colchicine and Vitamin E on Aspartate TransferaseLevel(U/L) in Paracetamol –induced Liver Disease, After Four Weeks of Treatment in Albino Rats.

TREATMENT	Wk1	WK 2	Wk 3	Wk4
NOMAL	10.7233	11.1100	9.8600	11.1500
	±0.300^{a}	±0.6524^{a}	±0.5658^{a}	±0.0346^{a}
PARA-INDUCED	33.9300	36.3100	37.7500	37.5667
	±0.1616^{ba}	±0.2367^{ba}	±1.125^{ba}	±0.6359^{ba}
COLCH-ALONE	30.1800	29.6867	29.3967	28.5667
	±0.0519^{ba}	±0.2396^{ba}	±0.2280^{ba}	±0.19953
COLCH-VIT E(.1ml/kg)	22.5800	21.9500	21.5067	20.2767
	±0.8140^{cba}	±0.6697^{cba}	±0.7592^{cba}	±0.40168
COLCH-VIT E(0.2ml/kg)	21.2767	20.5400	20.4267	19.8800
	±0.47632	±0.34064	±0.34930	±0.48497
COLCH-VIT E(0.3ml/kg)	20.6467	20.2667	19.5700	19.3967
	±0.7245^{cba}	. ±05225^{cba}	±0.5196^{cba}	±0.5629^{cba}

Result Represents Mean ±SEM of Triplicate Sample. Least Significant Difference (LSD) was used to compare the means. Values were considered significant at p<0.05 and superscripts in the same column with the same letters are not significant.

[a] = Significant Difference when each Concentration is considered with Normal Level first control

[b] = Significant Difference when each Concentration is considered with Normal Level first control

[c] = Significant increase when the concentrations are compared with each other.

Table 3: Effects of Colchicine and Vitamin E on Alkaline Phosphatase (U/L) in Paracetamol –induced Liver Disease, After Four Weeks of Treatment in Albino Rats.

TREATMENT	Wk1	WK 2	Wk 3	Wk4
NOMAL	11.4300	12.0367	10.4167	11.1267
	±0.080^{a}	±0.0166 ^{a}	±0.1010 ^{a}	±0.0033 ^{a}
PARA-INDUCED	36.0867	36.5500	37.7400	39.0000
	±0.0895^{ba}	±0.2136 ^{a}	±0.6458 ^{a}	±0.5773 ^{a}
COLCH-ALONE	35.1533	34.8900	34.8367	34.1767
	±0.044^{ca}	±0.0692^{ca}	±0.0606^{cba}	±0.0895^{cba}
COLCH-VIT E(0.1ml/kg)	29.8267	29.1500	28.4867	27.8767
	±0.14723	±0.10263	±0.02603	±0.20086
COLCH-VIT E(0.2ml/kg)	29.3267	28.5867	28.1500	27.4333
	±0.02603	±0.28521	±0.06928	±0.35244
COLCH-VIT E(0.3ml/kg)	28.7767	27.6267	27.0900	26.1600
	±0.1761^{cba}	±0.0895^{cba}	±0.0519^{cba}	±0.1113^{cba}

Result Represents Mean ±SEM of Triplicate Sample. Least Significant Difference (LSD) was used to compare the means. Values were considered significant at p<0.05 and superscripts in the same row with the same letters are significant.

[a] = Significant Difference when each Concentration is considered with Normal Level first control

[b] = Significant Difference when each Concentration is considered with Normal Level first control

[c] = Significant increase when the concentrations are compared with each other.

Table 4: Effects of Colchicine and Vitamin E on Gama- GT Level (U/L) in Paracetamol –induced Liver Disease, After Four Weeks of Treatment in Albino Rats.

TREATMENT	Wk1	WK 2	Wk 3	Wk4
NOMAL	7.3800	7.9500	8.7133	6.8467
	±.2251 [a]	±.1616 [a]	±.5565 [a]	±.2107 [a]
PARA-INDUCED	29.7533	30.3600	30.5667	32.0000
	±.1637[ba]	±.3348[ba]	±.3897[ba]	±1.1547[ba]
COLCH-ALONE	29.6100	29.1167	28.5567	27.9667
	±.0981[ba]	±.0606[ba]	±.1934[ba]	±.1010[ba]
COLCH-VIT E(0.1ml/kg)	24.8233	24.1900	23.6100	23.1367
	±.0352[bac]	±.10392	±.2193[bac]	±.0033[bac]
COLCH-VIT E(0.2ml/kg)	24.0233	23.8900	23.6267	23.3500
	±.07265	±.06351	±.00882	±.08083
COLCH-VIT E(0.3ml/kg)	23.6000	23.3567	23.0500	22.5900
	±.0057[bac]	±.0664[ba]	±.1124[ba]	±.1732[bac]

Result Represents Mean ±SEM of Triplicate Sample. Least Significant Difference (LSD) was used to compare the means. Values were considered significant at p<0.05 and superscripts in the same column with the same letters are not significant.

[a] = *Significant Difference when each Concentration is considered with Normal Level first control*

[b] = *Significant Difference when each Concentration is considered with Normal Level first control*

[c] = *Significant increase when the concentrations are compared with each other*

Table 5: Effects of Colchicine and Vitamin E on Total BiliruinLevel(mg/dl) in Paracetamol –induced Liver Disease, After Four Weeks of Treatment in Albino Rats.

TREATMENT	Wk1	WK 2	Wk 3	Wk4
NOMAL	.3400	.2967	.1900	.3367
	±.0346[ba]	±.1010[ba]	±.0230[ba]	±.0318[ba]
PARA-INDUCED	1.2567	1.3100	1.3267	1.1633
	±.0088[ba]	±.0173[ba]	±.0935[ba]	±.0033[ba]
COLCH-ALONE	1.1900	1.1467	1.1233	1.1433
	±.0115[ba]	±.0033[ba]	±.0166[ba]	±.0240[ba]
COLCH-VIT E(80mg/kg)	.9600	.9567	.9267	.8800
	±.01155	±.00667	±.00882	±.00577
COLCH-VIT E(90mg/kg)	.9067	.8700	.8067	.7700
	±.00667[ba]	±.0115[ba]	±.0202[ba]	±.0346[ba]
COLCH-VIT E(100mg/kg)	.8500	.7700	.7467	.6900
	±.0152[cba]	±.0230[ba]	±.0260[bac]	±.0288[cba]

Result Represents Mean ±SEM of Triplicate Sample. Least Significant Difference (LSD) was used to compare the means. Values were considered significant at p<0.05 and superscripts in the same column with the same letters are not significant.

[a] = *Significant Difference when each Concentration is considered with Normal Level first control*

[b] = *Significant Difference when each Concentration is considered with Normal Level first control*

[c] = *Significant increase when the concentrations are compared with each other.*

Table 6: Effects of Colchicine and Vitamin E on Direct Bilirubin(mg/dl) in Paracetamol –induced Liver Disease, After Four Weeks of Treatment in Albino Rats.

TREATMENT		Wk1	WK 2	Wk 3	Wk4
NOMAL		.1800	.1600	.1500	.1267
		±.0230^{ba}	±.0100^{ba}	±.0230^{ba}	±.0033^{ba}
PARA-INDUCED		1.1200	1.1567	1.1900	1.1800
		±.0173^{ba}	±.0088^{ba}	±.0057^{ba}	±.0057^{ba}
COLCH-ALONE		1.0867	1.0600	1.0567	1.0400
		±.0033^{ba}	±.0057^{ba}	±.0088^{ba}	±.0057^{ba}
COLCH-VIT E(0.1ml/kg)		.6067	.6733	.6200	.5700
		±.0088^{ba}	±.1344^{ba}	±.14012	±.1150^{ba}
COLCH-VIT E(0.2ml/kg)		.5967	.5167	.4600	.4400
		±.0371^{ba}	±.01453	±.01528	±.0230^{ba}
COLCH-VIT E(0.3ml/kg)		.4633	.4300	.4000	.3467
		±.0176^{bac}	±.01732	±.0173^{ba}	±.0240^{bac}

Result Represents Mean ±SEM of Triplicate Sample. Least Significant Difference (LSD) was used to compare the means. Values were considered significant at $p<0.05$ and superscripts in the same row with the same letters are not significant.

[a] = Significant Difference when each Concentration is considered with Normal Level first control

[b] = Significant Difference when each Concentration is considered with Normal Level first control

[c] = Significant increase when the concentrations are compared with each other.

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