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Curcumin Phytosome Ameliorates Aluminum Chloride-Induced Nephrotoxicity in Rats

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ABSTRACT

Aim of the work: aluminum chloride ($AlCl_3$) toxicity to animals and human increased in the last decade. Thereby, this study evaluated the potential ameliorative role of curcumin phytosome (CP) on $AlCl_3$-induced nephrotoxicity.

Material and methods: rats were allocated into four groups (n=6), Control group; CP group: rats orally administered with CP (200 mg/kg b.wt.) for 21 days; $AlCl_3$ group, intraperitoneally injected with three doses of $AlCl_3$ (30 mg/kg/b.wt.) every five days; group $AlCl_3$ and CP, rats received CP for 7 days prior to $AlCl_3$ and then received CP concurrently with $AlCl_3$ for another 14 days.

Results: $AlCl_3$ acquaintance significantly increased levels of creatinine, urea, uric acid, LPO and NO as well as reduced renal GSH, SOD and GPx activity in comparison with the control group. These biochemical variations linked with histological renal atrophy and morphological lesions in the glomeruli and the different renal tubules. All these biomarkers in addition to the histopathological changes of injured kidneys were distinctly reversed after treatment with CP. CP is effective in mitigating the nephrotoxicity evoked by $AlCl_3$ through restoring the oxidant/antioxidant equilibrium. The pathway of renal ameliorative effect of curcumin phytosome may be related to its ability to decrease MDA and other ROS and increase of antioxidants activity.

Keywords: aluminum chloride, nephrotoxicity, curcumin phytosome, antioxidants.

INTRODUCTION

Aluminum is plentiful element in the earth's layer and is broadly distributed all over the environment. Aluminum recorded in the priority list of risky substances by the Agency for Toxic Substances and Disease Registry [(1)].The huge hazard effects of aluminum have resulted from its entrance in many applications including food processing such as its addition to yellow cheese as well as packaging, hemodialysis procedures, antiperspirants, phosphate binders hyperparathyroidism controlling, and using cans bottle[(2)].Unfortunately, aluminum eradication from the body is inadequate and occurs through urine; therefore kidney is susceptible to a failure after aluminum exposure[(3)].

Renal function is contributing to elimination of aluminum such as aluminum chloride ($AlCl_3$) via glomerular filtration, reabsorption of filtrated $AlCl_3$ in tubules, secretion and excretion in distal tubules[(4)]. The extreme exposure of aluminum due to dissimilar human daily lifestyle raised the threat of renal aluminum withholding due to accumulation of aluminum to renal tubules resulted in renal dysfunction[(2,5)].In this context, the kidney is a vigorous organ that is more liable to toxic insults of $AlCl_3$.

$AlCl_3$ impairs the prooxidant/antioxidant stability which motivates the pro-oxidant properties of iron or copper [(6)].This augments lipid peroxidation (LPO) process and diminishes the activities of the antioxidants accordingly over generates oxidative stress leading to renal toxicity.Therefore, antioxidant compounds that can mitigate the oxidative insult may be a nominee to relieve $AlCl_3$ toxicity[(2)].

Recently, phytotherapeutics curcumin is one of the best famous spices extracted from *Curcuma longa*. Curcumin has many therapeutic characters due to its phenolic property as it can be considered as antidiabetic, anti-inflammatory, hepatoprotective, anticarcinogenic, antioxidant and cardioprotective role[(3,7,8)]. Regardless of these therapeutic roles, curcumin is restricted in clinical application. Curcumin is a poorly water-soluble, thereby its bioavailability is very low when administered orally[(9,10)]. The universal bioavailability of curcumin is only 1.8 ng/mL from the inventive of 500 mg administered to rats[(11)]. Thereby, inserting lipids to the poor soluble promising agents enrich their bioavailability[(7)].

Phytosome is a novel technology, in which phospholipids assimilated to a natural compound to produce lipid compatible complex boosts the absorption of the candidate[(12)]. The newly formed curcumin phytosome heighten the absorption of curcumin to five-fold further than the free curcumin in rats[(13)]. Therefore, curcumin phytosome was anticipated to exhibit superior antioxidant and renal protective role due to its amended bioavailability. Accordingly, the present study aimed to probe the ameliorative effect of curcumin in contradiction of the toxicity of $AlCl_3$.

MATERIALS AND METHODS

Administrative agents and dosing

Aluminum chloride/anhydrous ($AlCl_3$) was obtained from LOBA Chemie (India). It was injected three intraperitoneally doses (30 mg/kg b.wt) every five days for 21 days[(14)]. Curcumin phytosome (CP) was an aptitude sample from Meriva® (Indena Spa, Milan, Italy). CP administered orally as a suspension to rats with a dosage of 200 mg/kg b.wt for 21 days[(10)].

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Animals and experimental design

Twenty four male albino rats (*Rattus norvegicus*) weighing 150±20g were purchased from the animal house of the Faculty of Science, King Khalid University, KSA. Each 6 animals held in one cage and fed *ad libitum* in a 12 h light/12h dark cycle at 23±2°C room temperature. Animals were examined for health status and accustomed to the laboratory environment for one week prior to use.

All experiments were conducted in agreement with the standard animal ethics and the study protocol was reviewed and approved by the ethical committee of the Faculty of Science, King Khalid University. Following 7 days of housing time, rats were categorized into 4 groups (n=6) as follows;

- Group 1: control, rats fed normal diet and *ad libitum* for 21 days.
- Group 2: curcumin phytosome (CP), rats received CP orally (200 mg/kg b.wt.) for 21 days.
- Group 3: aluminum chloride ($AlCl_3$), rats injected with three doses of $AlCl_3$ (30 mg/kg/ b.wt.) intraperitoneally every five days; then continued to receive distilled water to complete 21 days.
- Group 4: $AlCl_3$+CP, rats received CP for 7 days before $AlCl_3$ injection, then received CP alongside with $AlCl_3$ for an additional 14 days.

Sample collection

Rats euthanized after completion of the experimental period. The blood samples were collected by cardiac puncture and prepared to obtain serum.Further, kidneys immediately excised, placed in ice-cold saline and divided into two portions. One portion was fixed in neutral buffered formalin for the histological examination and the other was cut into small pieces and homogenized with 10% 0.1 M Tris-HCl buffer (pH 7.4) to obtain supernatant and determination of oxidative/antioxidative biomarkers.

Determination of renal functions

Renal function tests determined in serum including creatinine[15], urea[16] and uric acid[17]. All parameters were estimated by using spectrophotometer as the manufacture instruction of Biodiagnostic Kit Company (Egypt).

Determination of oxidative/antioxidative biomarkers

Determination of renal lipid peroxidation (LPO)

The renal lipid peroxidation (LPO) content was measured colorimetrically according to the method described by **Ohkawa *et al.*** [18].

Determination of renal nitric oxide (NO)

Renal NO content was estimated by determination of azo dye that formed by the reaction between nitrous acid, sulphanilamide, and N-(1–naphthyl) ethylenediamine (NEDA) in acidic medium[19].

Determination of renal reduced glutathione (GSH)

The renal GSH content measured colorimetrically according to the method described by **Beutler *et al*.** [20].

Determination of renal superoxide dismutase (SOD)

Renal SOD was determined by measuring the ability of the enzyme to inhibit the phenazine methosulphate (PMS)-mediated reduction of nitroblue tetrazolium (NBT) dye [21].

Determination of renal glutathione peroxidase (GPx)

The renal GPx activity was measured according to the UV method described by **Paglia and Valentine**[22].

Statistical analysis

The current data were analyzed by using IBM Statistical Package for the Social Sciences version 20.0 (copyright by IBM SPSS software, US). The data of the present study were normally distributed according to Shaprio-Wilk and Kolmogorov-smirnov tests data, thereby parametric statistical analyses were performed[23]. To compare between the different groups and assess the efficacy of CP against $AlCl_3$ nephrotoxicity, one–way ANOVA followed by Tukey post hoc test was performed. Data expressed as the mean ± standard error of mean (SEM) and dissimilar letters of the same column of tables or histograms denote a significant difference at $P < 0.05$.

Histological Processing

Fresh portions of the kidney from each rat were rapidly cut, fixed in neutral buffered formalin (10%), then dehydrated with grades of ethanol. Dehydration was followed by clearing the samples in two changes of xylene, impregnated with two changes of molten paraffin wax, then embedded and blocked out. 5 µm thick sections were cut with a microtome (Leica RM 2025, Germany). Paraffin sections were stained and examined for alterations in the renal tissues of each rat, using an optical microscope (Olympus Microscope BP73 with Digital Camera, Japan).

RESULTS

Curcumin phytosome reduces $AlCl_3$-induced nephrotoxicity

No effect was observed in the creatinine and urea levels between CP and control groups (Table 1). On the other hand, creatinine and urea concentrations elevated significantly ($P<0.05$) upon intraperitoneal $AlCl_3$ injection relative to the control group. However, treatment with CP during $AlCl_3$ exposure showed an ameliorative effect by significantly decreasing ($P<0.05$) both creatinine and urea levels as compared to $AlCl_3$ group. While non-significant change revealed in the uric acid concentration between all groups.

Table 1. Effect of curcumin phytosome (CP) on aluminum chloride ($AlCl_3$)-induced renal injury in rats

Parameter / Groups	Creatinine (mg/dl)	Urea (g/dl)	Uric acid (mg/dl)
Control	0.495±0.047[a]	4.367±0.157[a]	3.026±0.125[ab]
CP	0.498±0.037[a]	4.041±0.248[a]	3.314±0.092[ab]
$AlCl_3$	0.692±0.030[b]	5.309±0.091[b]	3.707±0.167[b]
$AlCl_3$+ CP	0.444±0.014[a]	3.490±0.283[a]	3.274±0.178[ab]

Each value symbolizes mean of six rats ± SEM. Similar superscript letters of the same column are not significantly different and dissimilar superscript letters of the same column were significantly different (P < 0.05). $AlCl_3$: aluminium chloride; CP: curcumin phytosome.

Curcumin phytosome attenuated $AlCl_3$-induced oxidative insult

$AlCl_3$ provoked renal oxidative stress manifested by a significant increment (P<0.05) in LPO and NO levels accompanied by significant decrease (P<0.05) in the contents of GSH, SOD, GPx compared with control group. Conversely, a significant decrease (P<0.05) in LPO and NO levels detected in renal tissue of $AlCl_3$+CP group as compared with the untreated $AlCl_3$ group. Additionally, treatment with CP markedly replenished renal GSH, SOD, and GPx when compared to $AlCl_3$-injected rats (Fig. 1).

CP did not cause significant changes in the oxidative/antioxidative biomarkers in comparison to the corresponding control values.

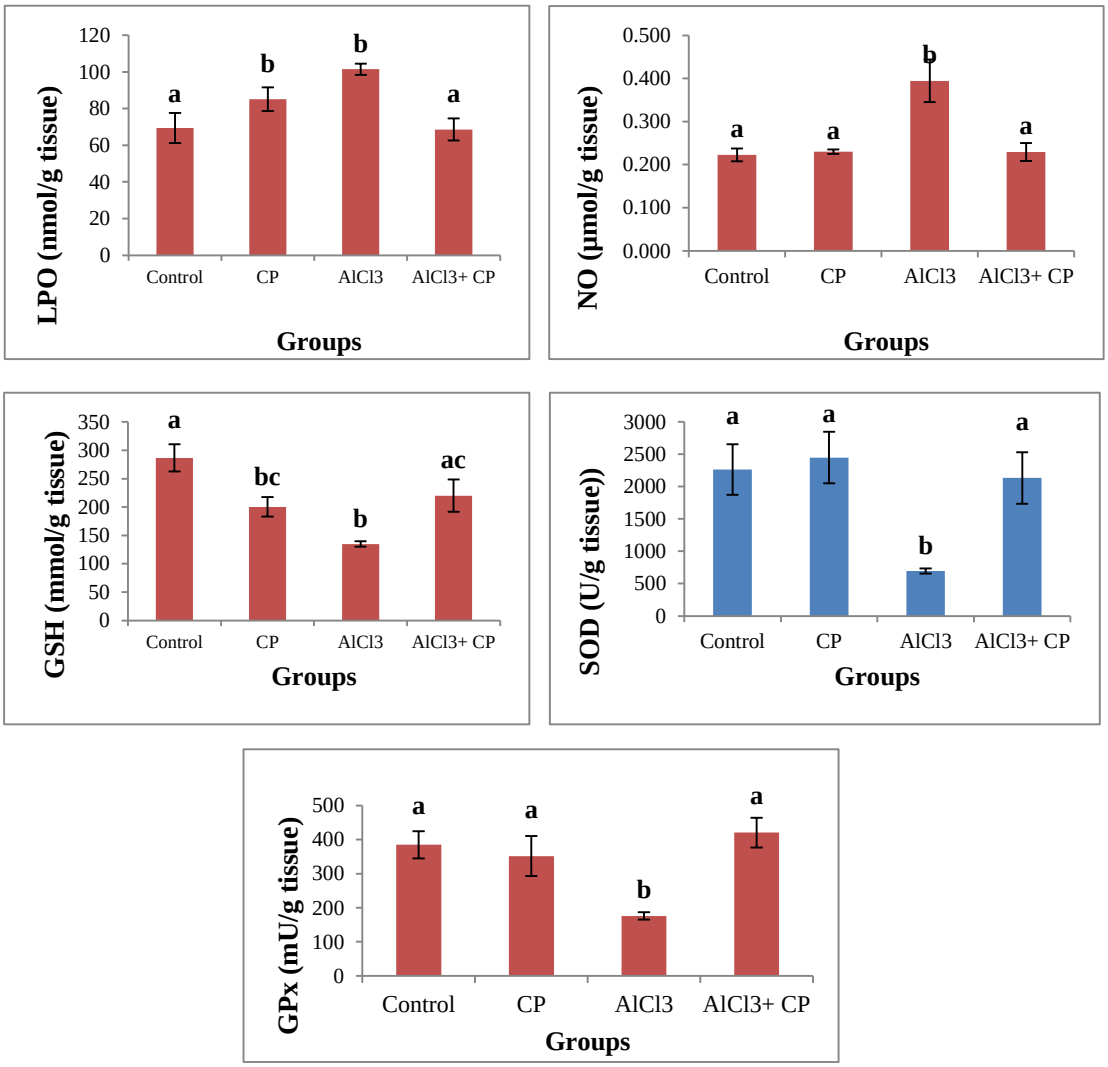

Figure 1. Effect of curcumin phytosome on aluminium chloride-induced nephrooxidative insult in rats. Each value symbolizes mean of six rats ± SEM. Similar letters of the same column are not significantly different and dissimilar letters of the same column were significantly different (P < 0.05). $AlCl_3$: aluminium chloride; CP: curcumin phytosome.

Histological examination

Histological sections of the control and CP rats showed normal renal structure (Fig. 2A&B). Kidney section of the $AlCl_3$ intoxicated group showed mild thickening of the basement membrane along with degeneration and tubular necrosis (Fig.2C). However, $AlCl_3$ rats treated with CP showed an improvement in their histological architectures (Fig. 2D).

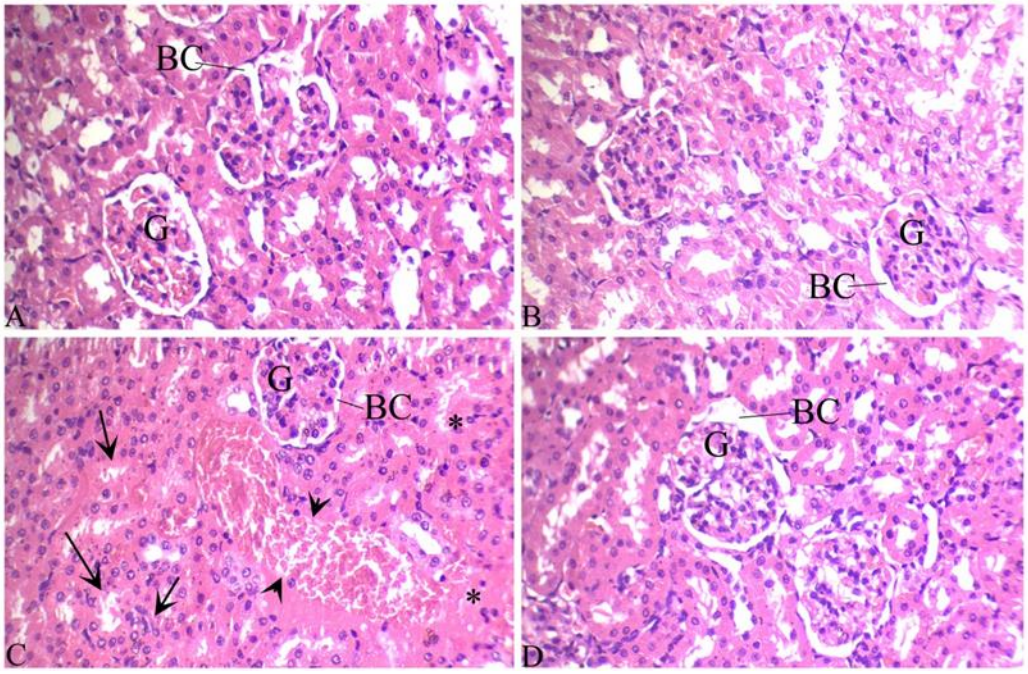

Figure 2. Photomicrographs of hematoxylin and eosin stained sections of kidneys of all studied groups. A: control group showed normal renal tissue architecture; B: curcumin phytosome group (CP) showed normal renal tissue appearance with normal Bowman's capsule and renal tubules; C: aluminium chloride group ($AlCl_3$) showing thickening of renal tubules basement membrane, pyknosis, inflammatory infiltration, degenerated and necrotic tubules (arrows), congested blood vessel (arrowheads) large hemmorhagic area; hypertrophied glomerulus(G) with atrophied Bowman's space(BC) D: aluminium chloride-curcumin phytosome treated group ($AlCl_3$+CP) displayed more or less normal feature of renal tissue. BC: Bowman's capsule; G: glomerulus (X40).

DISCUSSION

The kidney may be exposed to a high rate to aluminum due to its normal excretion function; therefore it is a sensitive organ to $AlCl_3$ toxicity[4]. The poor bioavailability of curcumin limits its use despite its high therapeutic role; thereby the development of new formula such as curcumin phytosome (CP) may boost its therapeutic effect. Thus, the present study assessed the vital ameliorative effect of CP against $AlCl_3$-induced nephrotoxicity in male rats.Exposure to $AlCl_3$ provokes nephrotoxicity due to increased levels of creatinine and urea in serum that are dependable markers of renal dysfunction. The present findings are in agreement with results of **Yakubu and Musa**[24] and **Vijayaprakash *et al.*** [25]. Creatinine and urea are metabolic wastes that are normally excreted and only small amount remain in the blood. $AlCl_3$ disrupt renal function resulted in the elevation of creatinine and urea in blood. Indeed, the histological examination confirmed the abnormalities in kidneys of $AlCl_3$ group which reflect glomerular/tubular damage and verify their nephrotoxicity. The observed changes caused by $AlCl_3$ may be mediated by several mechanisms. Prooxidant produced by $AlCl_3$ is one of the main mechanisms of $AlCl_3$ toxicity[126]. In this context, the present study revealed the onset of oxidative stress in kidney of $AlCl_3$ rats due to increased LPO, NO, as well as diminishing levels of GSH, SOD, and GPx. The main cytotoxic effect of $AlCl_3$ is due to its cellular damage that targets the cell membrane, thereby affects its fluidity and permeability causing lipid peroxidation to the plasma membrane[4]. Thus, the renal injury observed in this study may be due to increased LPO which act as autocatalysis to produce other free radicals in a propagating cascade manner ends by renal tissue degeneration. Further, $AlCl_3$ decreased GSH content and elevated the severity of renal oxidation damage. This decrease may be due to the ability of $AlCl_3$ to either inhibit NADPH-generating enzymes resulting in decreasing NADPH and subsequently diminish GSH regeneration, or due to direct inhibition to glutathione synthase or both[4]. The present study revealed that $AlCl_3$ also reduced the antioxidant enzymes including SOD and GPx significantly. The reducing activity of these defense enzymes reflects their reduced synthesis due to higher accumulation of $AlCl_3$ and/or higher accumulation of superoxide anion ($O_2^{\cdot-}$) and H_2O_2 in renal tissue[4]. Further, the interaction of $O_2^{\cdot-}$ and NO manifested in $AlCl_3$ produce peroxynitrite ($ONOO^-$) which exaggerate renal injury[27]. The formation of RONS (reactive oxygen and nitrogen species) triggers some of the vasoactive mediators that promote renal vasoconstriction and reduce the glomerular capillary filtration[28]. The above events overstress the nephrooxidative insult and this may be the possible mechanistic pathway that responsible for the increased creatinine and urea levels.

Interestingly, the present results showed that treatment of $AlCl_3$ group with CP significantly ameliorate kidney function markers by decreasing creatinine and urea levels, decreased renal LPO and NO contents as well as increased all anti-oxidative parameters rather than its ameliorating effect to renal tissue architecture compared to in the $AlCl_3$ group. These results supporting that CP is a promising agent that can defense the adverse effects of $AlCl_3$. CP may ameliorate $AlCl_3$ induced nephrotoxicity by repairing the prooxidant/antioxidant balance favoring the endogenous antioxidant molecules replenishment. This manifested by its stimulation to increase GSH, SOD, and GPx in renal tissue. The defensive effect of CP may be due to its polyphenolic groups that reduce LPO production and scavenge $O_2^{\cdot-}$ and H_2O_2 which manifested by increasing SOD and GPx in $AlCl_3$-CP treated group; this expectation is in agreement with **Farzaei *et al***. [(29)]. This study supposed that CP may stabilize the renal membrane and preserve its integrity by interaction of CP with renal membrane that trigger membrane-protective ability against peroxidative insult eventually inhibits LPO continuity. Thereby, the antioxidant activity of CP may mitigate nephrooxidative susceptibility through neutralization of RONS subsequently diminish nephrotoxicity mediated by $AlCl_3$.

CONCLUSION

Curcumin phytosome (CP) is a promising formula that suppresses the nephrotoxicity of $AlCl_3$. The ameliorative role of CP may be due to its ability to scavenge RONS and boosting the endogenous antioxidant molecules causing modulation of renal blood flow and glomerular filtration rate subsequently regulate the renal function.

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