Curcumin Attenuates Hepato-, and Nephrotoxicity Induced by Cypermethrin Through Inhibition of Oxidative Stress in Male Albino Rabbits

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Abstract

The reactive oxygen species (ROS) have been involved in the toxicity of several pesticides. This study was aimed to induce oxidative stress in rabbit's liver and kidney by cypermethrin (CYP), a type II pyrethroid pesticide and to evaluate the protective effect of Curcumin (CMN). Numerous plant moieties are identified to exhibit protective potential by neutralizing the oxidative stress. In this study we evaluated the protective role of powdered dried rhizome of *curcuma longa L.* (CMN) against CYP-induced liver and renal toxicity. For this purpose, all the rabbits were divided in 4 groups, each containing 5 rabbits. The 1st group considered as control while 2^{nd,} 3rd and 4th group were treated with CYP (25mg/kg), CMN (50 mg/kg) alone and CYP (25 mg/kg) in combination with CMN (50 mg/kg), respectively. Biochemical markers were investigated in serum while antioxidant potential and histopathological analysis was performed in liver and kidney tissues. CYP administration resulted in significant reduction of antioxidant protein and enzymes (GSH, GST, catalase, SOD and GPx). Moreover, remarkable variations were observed in biochemical markers (urea, creatinine, AST, ALT, ALP and bilirubin) as well as in histology of kidney and liver in CYP-treated rabbits. The administration of CMN in combination with CYP significantly restored the level of endogenous antioxidants. Furthermore, the normal level of serum biochemical markers was observed with normal histology of liver and kidney in rabbits treated with CMN. Similarly, CMN alleviated the harmful effects of CYP on lipid profiles, hematological parameters and body weight of rabbits.

Introduction

Hazardous synthetic chemicals, for instance, pesticides have been detected in soil, air, water as well as in animal and human tissues. These pesticides have damaging impacts and even mortalities. Yearly around 3 million cases of pesticides toxicity and 220,000 fatality globally have been reported [1]. Pyrethroid pesticides were prioritize over other groups of pesticides like carbamates, organophosphates and organochlorines due to a wide range of control and low toxicity to non-targeted organism [2]. The biodegradation of these pesticides is also easy like phytoremediation and microbial degradation. But the toxicity of the pyrethroid pesticides were recently received much attention due to high exposure to the environment and mammals which results in alterations in the physiological as well as in biochemical (enzymatic and hematological) parameters of animals [3]. Cypermethrin (CYP) is a synthetic pyrethroid pesticide that has been extensively utilized against various pests and insects in numerous parts of the globe in many agriculture practices [2, 4]. It has been determined by several evidences that prolong exposure to CYP has a detrimental effect and results in the damage of brain, liver and kidney by induction of oxidative stress and lipid peroxidation [2, 5, 6]. Moreover, it has shown by *in vivo* and *in vitro* studies that CYP extremely damage DNA and initiating the imbalance between pro- and anti-oxidant level in rat lymphocytes. Furthermore, some studies described that CYP resulting in free radical-facilitated tissue damage [2]. In addition, other *in vitro and in vivo* studies revealed that pyrethroids results in hepato-, and nephrotoxicity in experimental animals. Lipid peroxidation and oxidative stress are the main contributors in toxicity of pyrethroids [5, 7, 8].

Since few years, determination of antioxidant defense and free radical generation has turned into an integral component of investigation in mammals. There is a linear connection between dietary supplementation with a number of vegetables and plant products and the decline of adverse effects of different toxicants and environmental contaminants. Phytoproducts are recognized to exhibit their defensive role by scavenging free radicals and controlling antioxidant defense system [9]. Curcumin (CMN), a yellow-colored dye derived from rhizome of *curcuma longa L*., has a domestic common use as a spice and food-coloring agent. CMN belongs to the class of anti-inflammatory and antioxidants plant products recognized to be a potent inhibitor of ROS formation [2, 10]. CMN has been determined as a strong scavenger of a range of ROS like superoxide anion radicals and hydroxyl radicals. Moreover, studies have shown that CMN administration protects arsenic, acetaminophen and gentamicin-induced oxidative stress in rats [11]. Furthermore, CMN also inhibits the production of free radicals in myocardial ischemia in rats and paraquat-induced lung injury [12]. Keeping in view the aforementioned consideration, the present study was aimed to evaluate the use of CMN in protection and reduction of oxidative stress and its associated organs damage induced by CYP-administration in animal model of male albino rats.

Materials And Methods

Chemicals

Cypermethrin was purchased from arid agriculture center Kohat. Rhizomes were obtained from *Curcuma longa L.* plant which was purchased from the local farmers field of Bannu, Khyber Pakhtunkhwa, Pakistan. Crude ethanolic extract of *Curcuma longa rhizomes* was used as CMN. The plant material was identified by taxonomist Dr. Mushtaq Ahmad, Dr. Muhammad Zafar at Herbarium of Pakistan (ISL) Quaid-I-Azam University Islamabad and voucher specimen has not submitted in publicly available herbarium. The enzymes activities of aminotransferases (AST & ALT), alkaline phosphatase (ALP) and the level of urea and creatinine were analyzed by kits that were purchased from med-lab chemicals Islamabad. All the chemicals were of analytical grade. The entire chemicals were dissolved in DMSO and for control group only DMSO was used.

Animals and experimental design

20 white male albino rabbits were used in the present study which were purchased from National Institute of Health (NIH), Islamabad, Pakistan. The selection of male rabbits was according to already published studies. Moreover, to omit the effect of “sex as a biological variable (SABV)” on the study, only one sex is included in the whole study. The title specified the sex of animals (male) as per “Sex And Gender Equity in Research (SAGER)” guidelines [13]. Animals were provided standard environmental and food conditions accordingly. Rabbits were divided into control (received no treatment), CYP-treated, CMN-treated, and combination of both CMN + CYP-treated group. Rabbits were orally administered their respective doses every day for 45 days. There was 1 h difference between the administration of CYP and CMN. The dose selection of all compounds was based on previous published studies with some modifications [2, 3, 14].

Animal models

CYP-induced rabbit model, animals were divided into four groups and every group with five rabbits:

Group I: Normal control animals

Group II: CYP-induced group (25 mg/kg b.w)

Group III: CMN- treated group (50 mg/kg b.w)

Group IV: CYP- (25 mg/kg b.w) + CMN-treated group (50 mg/kg b.w)

Sampling and tissue preparation

Rabbits were euthanized by a trained technician through cervical dislocation. Blood samples were obtained from the sacrificed rabbits and retained instantly on ice. Heparin was used as an anticoagulant and plasma samples were gained by centrifugation at 1000 rpm for 15 min and stored at -40◦C. Biochemical parameters were investigated in serum while the activity of endogenous antioxidants was assayed in liver and kidney tissues. These tissues were instantly separated and washed with chilled saline solution. The tissues were then distinctly homogenized (10%w/v) in ice cold phosphate buffer (0.01 M, Ph. 7.4) containing 1.5% KCL using homogenizer. The homogenate was centrifuged at 10,000 rpm/20mint at $4C^0$ and the resultant supernatant was used for the enzyme assay. Histopathological examinations of kidney and liver were performed by H&E (hematoxylin and eosin) staining [2, 3, 15].

Methods

Determination of liver and kidney function

The effect of CMN on sensitive biomarkers of liver and kidney injury such as ALT, AST, ALP, bilirubin, urea and creatinine were investigated respectively by using commercially available kits as described previously [8, 15, 16]. Briefly, the blood samples collected at the end of experiment from all the animals and serum was separated after 10 min of centrifugation at 4^oc/6000 rpm for analysis as per manufacturer's protocol.

Determination of antioxidant activities

Estimations of numerous oxidative stress-related biochemical markers were carried out in homogenized liver and kidney tissues as per previous methodology [15, 16]. In brief, liver tissues were homogenized at 4^oc/12000 rpm for 30 min and the resultant supernatant was used for estimation of various antioxidant enzymes and proteins. GSH (glutathione) content was evaluated by the method of Ellman et al. [15, 17]. GST (glutathione S transferase) activity was analyzed by using a method established by Warholm et al [17, 18] . CAT (Catalase) activity was assayed by the procedure as described by Aebi et al. [15, 17]. The activity of SOD (superoxide dismutase) was measured as per the method of Sundaram et al. [16, 19]. GPx (Glutathione peroxidase) activity was determined by the method of Necheles et al. [20, 21]. The presence

of these antioxidant enzymes and proteins were identified by monitoring the changes in absorbance at specific wavelength using spectrophotometer.

Estimation of lipid peroxidation (malondialdehyde)

Lipid peroxidation (LPO) was estimated in terms of malondialdehyde (MDA) production by the procedure narrated by Utley et al. [16, 22]. The quantity of thiobarbituric acid reactive substances (TBARS) was determined by recording the absorbance of the supernatant at 532 nm by using a microplate reader.

Lipid profile analysis

Total lipids (TL), cholesterol, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein cholesterol (LDL), and very low-density lipid protein (VLDL) were estimated in plasma by the methods as described previously [3, 23, 24].

Hematological data

To notice any changes in total blood count associated with CYP or CMN, blood was collected in anti-coagulated EDTA blood tubes from each rabbit. The non-coagulated blood was then tested for total erythrocyte count (TEC), total leukocyte counts (TLC), packed cells volume (PCV) and hemoglobin (Hb) according to previously described procedures [3].

Histopathology

For histopathological examination, the kidney and liver of rabbits were dissected. Next, these tissues were embedded in paraffin after routine fixation (in 10 % formalin), decalcification and dehydration (in alcohol). Tissue sections of kidney and liver were prepared at 4-5 µm thickness and stained with H&E after which examined with the help of light microscope as per formerly described methodology for any histopathology appeared during the experimentation period. The histological changes were classified by using various symbols such as (-) showing no changes and (+) showing changes [8, 15].

Determination of body weight variations

Body weights of rabbits were measured initially (day 0) and at the end (day 45) of experimentation to assess any changes in weight among the groups. The changes were described as a percentage of average weight gain in all the groups as described by Navarro-Alvarez N et al. [15, 25].

Statistical analysis

Data collected was presented as mean ± SD. Groups were compared for statistical significances by one way ANOVA followed by pairwise comparison. R studio version 3.6.1 was used for analysis of data. Statistical significance was fixed at $p < 0.05$.

Results

Effect of CMN treatment on kidney and liver functions

Protective effect of CMN was assessed against CYP-induced kidney and liver damage by measuring the serum level of sensitive biochemical markers of these organs. An increased elevation was recorded in the level of urea and creatinine in CYP-challenged animals. However, treatment with CMN significantly reduced these parameters to normal (Fig. 1). Similarly, exposure to CYP caused abnormal liver function in CYP-treated rabbits. Administration of CMN for a specified period of time markedly restored the liver function by reducing the level of ALT, AST, ALP and bilirubin near to the normal values as shown in Fig. 2.

Effect of CMN treatment on antioxidant potential

Table. 1 showed significant reduction in the level of endogenous antioxidant enzymes and protein in liver and kidney tissues effected and damaged by CYP administration. While the level of these antioxidants was preserved in rabbits received CMN treatment as that of normal control showing its protective role on liver and kidney tissues. Similarly, CYP-challenged rabbits exhibited elevated level of LPO in liver and kidney tissue homogenates when compared to control animals. Treatment with CMN attained significantly reduction in LPO level when compared to CYP-challenged animals.

Effect of CMN treatment on lipid profiles

The results (Table. 2) indicated that CYP treatment elevated the plasma level of TL, TG, LDL and VLDL significantly, while the level of HDL was decreased in the animals of this group and these results agree with some former studies. The concentrations of these parameters were significantly decreased except HDL in plasma of rabbits treated with CMN as shown in Table. 2.

Effect of CMN treatment on hematological changes

The results (Table. 3) showed that CMN treatment did not significantly change any parameter of blood as compared to normal control. However, CYP treatment marginally reduced HB, TEC, PCV and TLC as compared to normal control and CMN-treated rabbits.

Effect of CMN treatment on kidney and liver histology

For the purpose to assess the protective effect of CMN against CYP-induced renal and hepatic damage, histopathological examination was performed. In case of treatment with CYP, the rabbit's kidney exhibited degenerated glomerulus, proximal tubule and distal tabule as well as the infiltration of leukocytes was high. However, leukocytes infiltration and renal tissue damage has been significantly attenuated in animals treated with CMN (Fig. 3). Similarly, CYP-induced a significant hepatic damage. As shown in Fig. 4, the hepatic tissue revealed an intense centrilobular necrosis (n), cellular hypertrophy (h), vacuolization (v), steotosis (st) as well as inflammation. Hepatic histology of rabbits treated with CMN showed a normal histology by restoring inflammatory processes towards normal that indicates protective effect of CMN.

Effect of CMN treatment on body weight variations

At the end of the study, animals in control group got approximately 37 % weight gain which was 14 % in animals received CYP treatment. However, the animals received Curcumin got nearly 34 and 30 % of weight gain, respectively, which is close to normal control group (Table. 4). This shows a normal positive impact of CMN on the general health of animals.

Discussion

Various types of drugs and chemicals that causes injuries to liver accounts for approximately 5% of overall hospital admissions and 50% of all cases of acute liver failure [26]. Similarly, various drugs and chemicals like pesticides have the ability to cause nephrotoxicity. Sufficient study by diverse workers has already been done on different animal (mice, rats, bird, fish and hamsters etc.) about the injurious effect of this culprit chemical through inhalation, dermal and also oral administration and results indicated irritation of the eyes, nose, and skin, abnormal facial sensations, dizziness, nausea, headache, vomiting, fatigue and anorexia [27]. Recently, 30% of synthetic pyrethroids insecticides used globally are highly active [28]. The potential risk and exposure to pyrethroid insecticides are increasing with growing production and application and of serious concern [29]. Pyrethroids including Cytochrome P are metabolized within liver through hydrolytic ester cleavage and oxidative pathways by Cytochrome P450 enzymes resulting in ROS generation which is the ultimate cause for oxidative stress in mammals [30]. The ROS are directly interacting with cellular mechanism of different biomolecules results in lipids damage, proteins and genetic material in cells and that can finally cause cell death [2].

However, it is considered that treatment of acute liver failure and renal dysfunction is important [31, 32], few preventive medicines or plant products have been recognized. Since time long-established, plant based natural products, which may be crude extracts or pure compounds have become the major contributors on the way to cure and prevent numerous anomalies [33]. CMN, the pure active component of *Curcuma longa L.* [34], has been investigated as anti-inflammatory [35], immunoregulatory [36] and other beneficial properties in models of kidney and liver dysfunction. Scientists have accomplished a range of studies to develop natural products that ameliorate renal and hepatic function, including studies of CMN [23]. The suggested protective mechanism of CMN against renal and hepatic dysfunction is the inhibition of tumor necrosis factor (TNF)-induced apoptosis [37, 38] and ROS formation. As a polyphenolic antioxidant, CMN has thought to suppress the activation of fibrosis in vitro by diminishing cell proliferation and inducing apoptosis [38]. Moreover, CMN's antioxidant effect has been evaluated in in a rat model of carbon tetrachloride (CCL4)-induced liver injury [39, 40]. Therefore, the designed study was proceed to evaluate the use of one of the natural products, CMN, as hepato-, and nephroprotective agent in CYP-induced animal model.

Elevation in the serum levels of hepatotoxicity markers such as AST, ALT and ALP reflecting hepatic damage as these enzymes are cytosolic by nature and leak into the plasma once cellular membrane integrity is compromised in liver injury. Further, this can be confirmed by increased serum bilirubin level

which indicates obvious obstruction in bile excretion because of hepatic injury. Therefore the estimation of these enzymes and presence of bilirubin in serum can quantify the type and extent of hepatocellular injury [41, 42]. Similarly, the indication markers of renal dysfunction are considered to be the elevated level of plasma urea and creatinine [43]. There is a correlation between increased blood urea with an elevated protein catabolism in mammalian body or with a proficient conversion of ammonia to urea because of highly synthesized enzymes involved in urea production [44]. In this study, elevation in urea level may be due to pesticides effect on liver as urea in the final-product of protein catabolism [44]. In this study, the results showed that treatment with CMN counteracted the toxic effects of CYP on kidney and liver and restoring the hepatic enzymes and renal biomarkers towards normal indicating its hepatoprotective and nephroprotective effect. Additionally, histopathological investigation justified protective effects of CMN against CYP-induced toxicity.

Naturally, liver is abundant with a powerful defense system comprising of “antioxidants” which neutralizes the ROS [45]. The endogenous antioxidant enzymes and proteins (CAT, SOD, GST, GSH and GPX) show first-line defense against the ROS. Once ROS generates a stress condition, these antioxidants provide a first-line defense and protection against invading free radicals. Taking any source of antioxidants (like CMN) suppresses these free radicals and helps in restoring the endogenous antioxidants. It has been strongly believed that the protective ability of plant extract may be assigned to its antioxidant potential [16, 45]. In the current study, kidney and liver activity of these antioxidants were decreased markedly in CYP-treated group. However, treatment with CMN ameliorated the diminished kidney and liver activity by restoring the level of these antioxidants to nearly normal values that strongly confirms CMN potential of Hepato-, and nephroprotection against CYP-induced damage. Similarly, the by-product of membrane lipid peroxidation, i.e., malondialdehyde (MDA), was increased significantly due to ROS in CYP-challenged rabbits which was reduced remarkably after CMN treatment.

The presence of pesticides in liver was described to be related with the disruption of lipid metabolism and a rise of serum cholesterol. Hence, pesticides-induced elevation in serum cholesterol (Table. 3) may be accredited to the influence of pesticides on the permeability of liver cell membrane [44, 46]. The results obtained show that treatment with CMN significantly lowered the plasma level of TL, cholesterol, TG, LDL and VLDL while increased the level of HDL. HDL may accelerate the elimination of cholesterol from peripheral tissues towards liver to catabolize and excrete. Also, elevated levels of HDL may compete with LDL receptor sites on arterial smooth muscle cells and thus partially inhibit uptake and degradation of LDL [3, 47]. Moreover, the histological abnormalities mediated by CYP-administration was restored significantly by treatment with CMN to normal levels. Furthermore, the final body weight gain in CYP-induced rabbits was lower than that of normal and CMN-treated rabbits, suggesting overall positive effect of CMN on general health of animals.

Conclusion

In conclusion, this study indicates that CYP has injurious effect on public health especially on liver and kidney tissues. However, CMN, a potent inhibitor of ROS formation has a strong hepato-, and

nephroprotective effect against CYP-induced liver and renal injury. CMN treatment restored the normal level of biochemical and hematological parameters, ameliorates histopathological injuries, preserved the activity of endogenous antioxidants and these all properties justifies its protective effect. However, further study needed to elaborate the detailed mechanism.

List Of Abbreviations

ROS, Reactive Oxygen Species; CYP, Cypermethrin; CMN, Curcumin; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase; DMSO, Dimethyl Sulfoxide; NIH, National Institute of Health; RPM, Revolution Per Minute; KCl; Potassium Chloride; H&E, Hematoxylin and Eosin; GSH, Glutathione; GST, glutathione S transferase; CAT, Catalase; SOD, Superoxide Dismutase; GPx, Glutathione Peroxidase; LPO, Lipid Peroxidation; MDA, Malondialdehyde; TBARS, Thiobarbituric Acid Reactive Substances; TL, Total lipids; TG, Triglycerides; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein Cholesterol; VLDL, Very Low-Density Lipoprotein Cholesterol; TEC, Total Erythrocyte Count; TLC, Total Leukocyte Counts; PCV, Packed Cells Volume; HB, Hemoglobin; ANOVA, Analysis of Variance;

Declarations

Ethics approval and consent to participate

The departmental “Department of Biotechnology & Genetic Engineering, Kohat University of Science and Technology” ethical committee approved the experimental design and protocols conform to the guidelines of the NIH. It was permitted by NIH, Islamabad to use the obtained animals in *in vivo* experimentations.

Consent for publication

Not applicable

Availability of data and materials

The datasets may be available from the corresponding author on reasonable request.

Competing interests

All the authors declared no conflict of interest.

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Not applicable

ARRIVE Guidelines

Not applicable

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Tables

Table 1
Effect of CYP and CMN on antioxidant level in liver and kidney tissues

Parameters	Normal Control	CYP	CMN	CYP + CMN
GSH (µmol/g tissue) [Liver]	2.69 ± 0.05	0.77 ± 0.07###	3.22 ± 0.29	2.28 ± 0.09***
GSH (µmol/g tissue) [Kidney]	1.16 ± 0.06	0.36 ± 0.02###	1.18 ± 0.02	1.09 ± 0.03***
GST (µmol/min/mg protein) [Liver]	1.65 ± 0.03	0.32 ± 0.01###	1.88 ± 0.07	1.44 ± 0.12***
GST (µmol/min/mg protein) [Kidney]	0.57 ± 0.04	0.17 ± 0.01###	0.73 ± 0.03	0.55 ± 0.02***
CAT (µmol/min/mg protein) [Liver]	43.76 ± 1.40	23 ± 0.91###	44.08 ± 1.73	39.0 ± 1.20***
CAT (µmol/min/mg protein) [Kidney]	13.02 ± 0.53	3.61 ± 0.14###	13.73 ± 0.70	11.66 ± 0.59***
GPX (nmol/min/mg protein) [Liver]	7.69 ± 0.23	3.35 ± 0.04###	8.22 ± 0.29	7.28 ± 0.49***
GPX (nmol/min/mg protein) [Kidney]	3.16 ± 0.06	0.86 ± 0.02###	3.87 ± 0.22	2.67 ± 0.12***
SOD (µmol/min/mg protein) [Liver]	11.48 ± 0.41	2.32 ± 0.11###	13.28 ± 0.97	10.84 ± 0.62***
SOD (µmol/min/mg protein) [Kidney]	4.37 ± 0.18	1.17 ± 0.08###	4.83 ± 0.09	3.95 ± 0.19***
LPO (µmol/min/mg protein) [Liver]	18.19 ± 0.43	37.0 ± 1.11###	16.32 ± 0.73	21.57 ± 1.20***
LPO (µmol/min/mg protein) [Kidney]	3.02 ± 0.03	9.61 ± 0.24###	2.73 ± 0.05	4.66 ± 0.08***
The data is presented as the mean (n = 5) ± SD.				
(###) represents comparison to normal control group.				
****p* represents comparison to CYP-induced group.				

Table. 2: Effect of CYP and CMN on lipid profile

Parameters	Normal Control	CYP	CMN	CYP + CMN
HB (g/dl)	12.73 ± 0.18	9.25 ± 0.56$^{###}$	12.67 ± 0.24***	12.04 ± 0.15**
TEC (×10^6)/μL	5.97 ± 0.07	5.03 ± 0.14$^{###}$	5.88 ± 0.08***	5.67 ± 0.07***
PCV (%)	45.43 ± 0.32	40.8 ± 0.31$^{###}$	44.97 ± 0.09***	43.3 ± 0.39***
TLC (×10^3)/μL	4.7 ± 0.14	7.93 ± 0.26$^{###}$	4.83 ± 0.07***	5.24 ± 0.12***
The data is presented as the mean (n = 5) ± SD.				
(###) represents comparison to normal control group.				
$^{**}p$ and $^{***}p$ represents comparison to CYP-induced group.				

Table 3
Effect of CYP and CMN on hematological changes

Parameters	Normal Control	CYP	CMN	CYP + CMN
Total lipids (mg/dl)	567 ± 7.6	720 ± 10.1$^{###}$	553 ± 9.3***	613 ± 12.5***
Triglycerides (mg/dl)	121.7 ± 3.5	157.2 ± 7.5$^{###}$	112.6 ± 06.4***	135.9 ± 2.5**
Cholesterol (mg/dl)	93.5 ± 1.1	137.2 ± 8.1$^{###}$	99.7 ± 3.3***	105.2 ± 5.7***
HDL (mg/dl)	47.4 ± 0.93	35.5 ± 1.7$^{###}$	51.1 ± 2.99***	45.7 ± 3.80**
LDL (mg/dl)	78.8 ± 4.5	112.1 ± 6.5$^{###}$	74.9 ± 5.1***	87.2 ± 3.4***
VLDL (mg/dl)	25.4 ± 0.8	37.2 ± 2.9$^{###}$	23.3 ± 1.7***	28.4 ± 1.8**

The data is presented as the mean (n=5) ± SD.

(###) represents comparison to normal control group.

$^{**}p$ and $^{***}p$ represents comparison to CYP-induced group.

Table 4
Effect of CYP and CMN on body weight variations

Parameters	Initial weight (g) (Day 0)	Final weight (g) (Day 45)	Percentage of weight gain
Normal Control	1875 ± 65.76	2567 ± 70.19	37 %
CYP	1857 ± 81.3	2116 ± 51.4	14 % ###
CMN	1902 ± 85.25	2550 ± 99.57	34 % ***
CYP + CMN	1826 ± 61.02	2386 ± 66.93	30 % ***
The data is presented as the mean (n = 5) ± SD.			
(###) represents comparison to normal control group.			
** *p* and *** *p* represents comparison to CYP-induced group.			

Figures

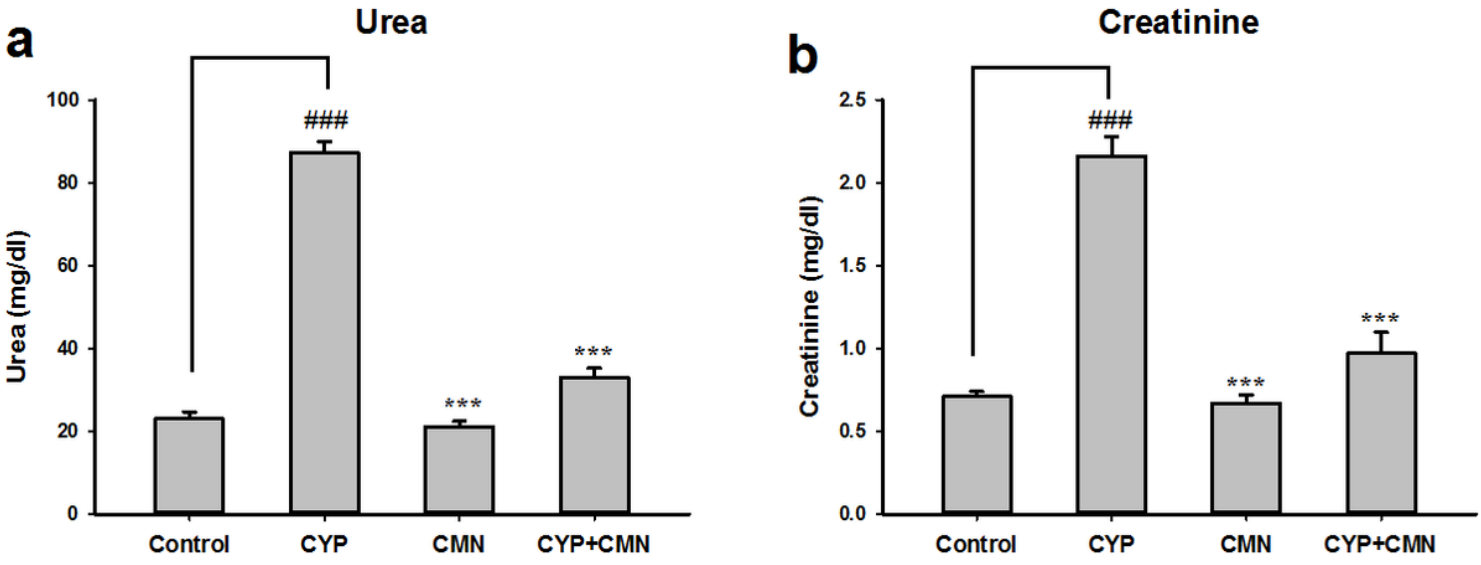


Figure 1

Effect of on CMN on kidney function. CMN reduced CYP-induced elevation in (a) urea level, and (b) creatinine level. Analysis were performed at the end of experiment in blood plasma of rabbits from each group. Data collected was presented as mean ± SD. Groups were compared for statistical significances by one way ANOVA followed by pairwise comparison. *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 represents a statistically significant difference from CYP-induced group. (###) indicates comparison to normal control group.

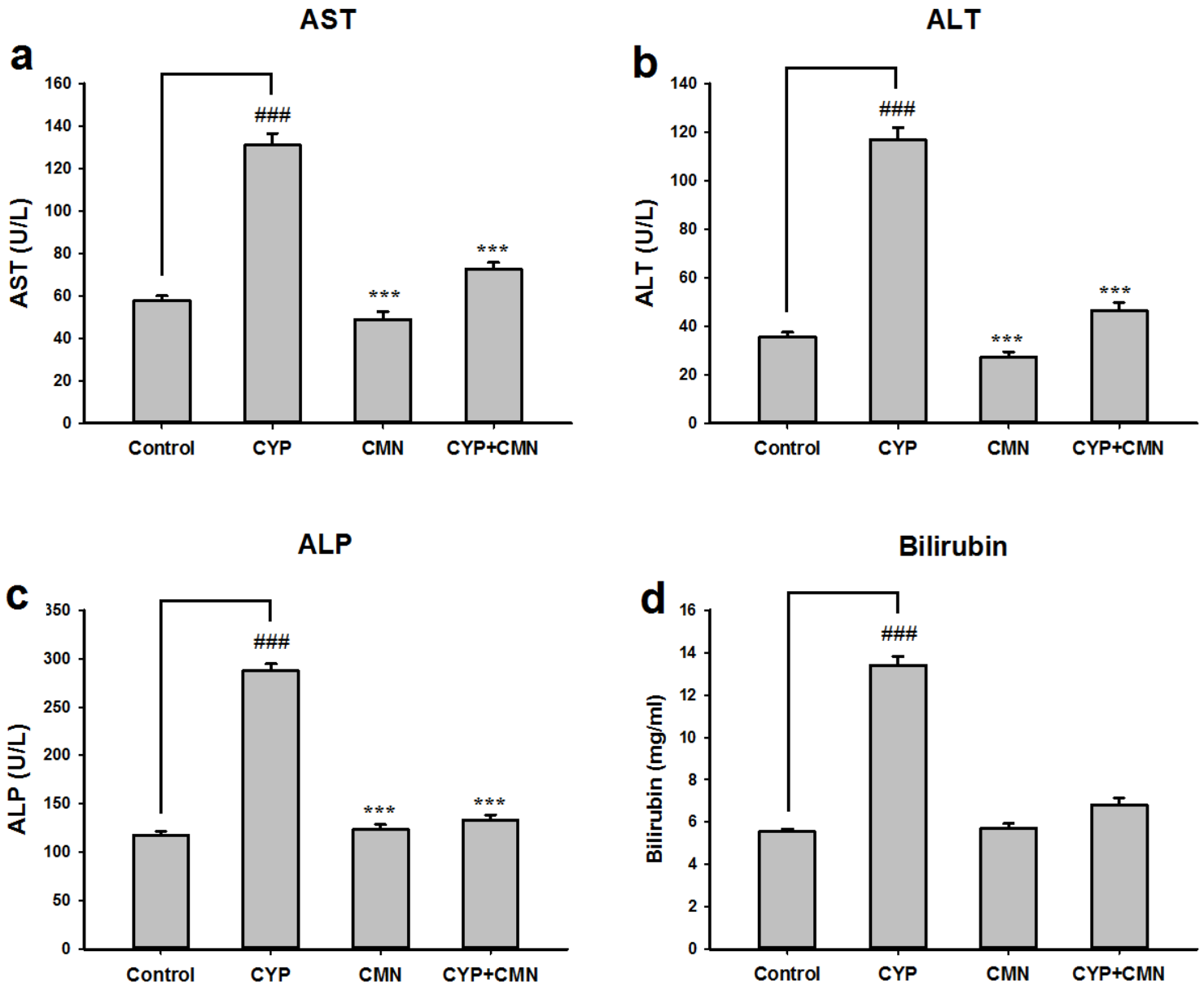
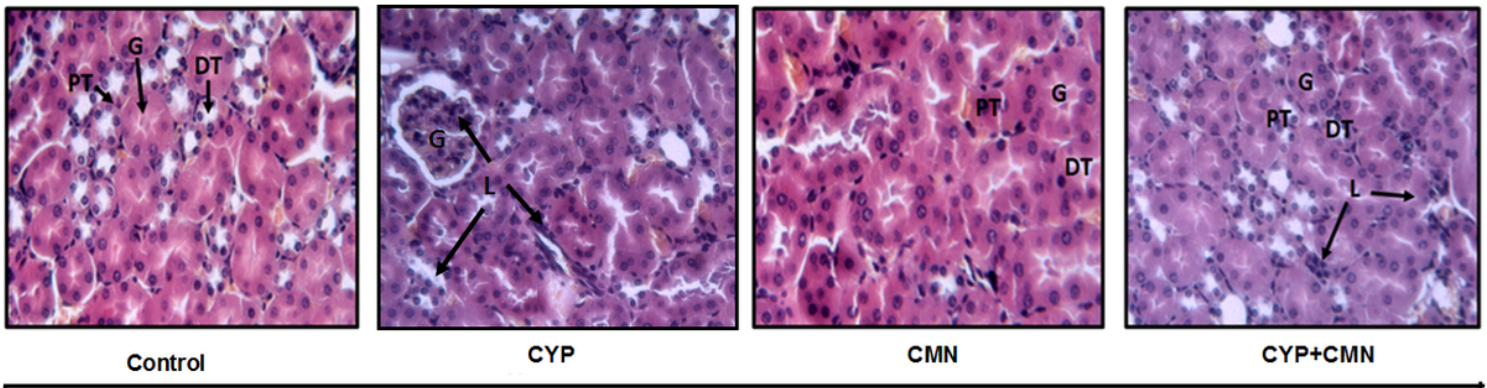


Figure 2

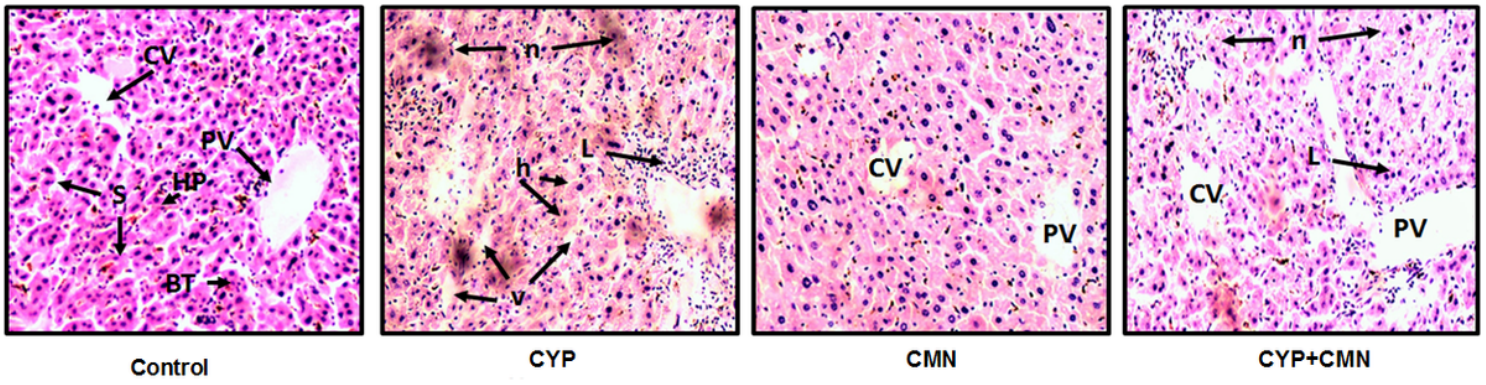
Effect of on CMN on liver function. CMN reduced CYP-induced elevation in (a) AST (b) ALT (c) ALP, and (d) bilirubin level. Analysis were performed at the end of experiment in blood plasma of rabbits from each group. Data collected was presented as mean ± SD. Groups were compared for statistical significances by one way ANOVA followed by pairwise comparison. *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$ represents a statistically significant difference from CYP-induced group. (###) indicates comparison to normal control group.



Groups	Glomerulus (G)	Proximal Tubule (PT)	Distal Tubule (DT)	Leukocytes infiltration (L)
Control	Normal	Normal	Normal	No
CYP	Degenerated	Degenerated	Degenerated	High
CMN	Normal	Normal	Normal	No
CYP+CMN	Normal	Normal	Normal	No

Figure 3

Histopathological analysis of rabbit's kidney. Kidneys tissue from control, CYP-, CMN-, and CYP+CMN-treated animals were stained with H&E and observed with microscope. Administration of CYP resulted in degeneration of glomerulus, renal tubules as well as infiltration of leukocytes.



Groups	Hepatic cytoplasm inflammation Leukocytes infiltration (L)	Centrilobular necrosis (n)	Cellular hypertrophy (h)	Vacuolization (v)	Steatosis (st)
Control	-	-	-	-	-
CYP	+	+	+	+	-
CMN	-	-	-	-	-
CYP+CMN	-	-	-	-	-

CV: Central Vein, **PV:** Portal vein, **HP:** Hepatocytes, **BT:** Bile Duct, **S:** Sinusoid

Figure 4

Histopathological analysis of rabbit’s liver. Livers tissue from control, CYP-, CMN-, and CYP+CMN-treated animals were stained with H&E and observed microscope. The histological changes have indicated with various symbols such as (-) showing no changes and (+) showing changes.