

PROTECTIVE EFFECTS OF GARLIC OIL ON ACETAMINOPHEN INDUCED NEPHROTOXICITY IN MALE ALBINO RATS

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ABSTRACT

Introduction: Acetaminophen is a severe hepatotoxic and nephrotoxic drug. This study was undertaken to examine the preventive effects of garlic oil, obtained by steam distillation of crushed garlic, on acetaminophen induced nephrotoxicity in male albino rats. **Materials and Methods:** Twenty four albino rats, 6-8 weeks old, weighing 150-200 gm, were used; these were divided into four groups having six rats in each. Group I served as control and was given normal saline 5ml/kg intraperitoneally on 7th day of the experiment. Group II was treated with a single dose of acetaminophen (1000 mg/kg) dissolved in 5 ml of normal saline, given intraperitoneally on 7th day of the experiment. Group III was given orally garlic oil, 100 mg/kg in 1ml of corn oil, daily for one week before giving an intraperitoneal injection of acetaminophen on 7th day of experiment. Group IV was treated with corn oil orally, 1 ml/kg for one week. At the end of the experiment, the animals were anaesthetized under chloroform and blood from each animal was drawn by cardiac puncture for renal function tests. The animals were then sacrificed under anaesthesia and the kidneys were removed; these were normal in gross appearance with no significant difference between control and experimental groups. Slides were prepared for histological study; these were stained with H & E and PAS, examined under light microscope, evaluated by using the different parameters including measurement of size of glomeruli and nuclei of epithelial cells of proximal and distal convoluted tubules. **Results:** Statistical analysis showed that garlic oil pretreatment significantly reduced acetaminophen induced nephrotoxicity as evidenced by amelioration of histological changes in size of glomerulus from $51.50 \pm 3.60\mu$ in group II to $84.63 \pm 2.89 \mu$ in group III ($p < 0.001$). Garlic oil also reduced deleterious effects of acetaminophen on tubules of kidney as evidenced by absence of vacuolation and granularity of epithelial cells of proximal and distal convoluted tubules and, protein casts in thick ascending limb of loop of Henle in all rats of group III. Value of serum urea was restored from 95.28 ± 2.90 mg/dl in group II to 65.15 ± 2.68 mg/dl in group III ($p < 0.001$) and that of serum creatinine from 2.71 ± 0.68 mg/dl in group II to 1.73 ± 0.04 mg/dl in group III ($p < 0.03$). It was therefore, **concluded** from current results that garlic oil is useful in protecting acetaminophen induced nephrotoxicity.

Key Words: Garlic, Oil, Acetaminophen, Nephrotoxicity.

INTRODUCTION

Acetaminophen (Paracetamol, *N*-acetyl-*p*-aminophenol; APAP) is also known as paracetamol, is widely used as prescription and over the counter analgesic and antipyretic agent.¹ It is a safe drug when given in therapeutic doses but its overdose is fairly common since it has narrow therapeutic index. Its overdose can lead to hepatic and renal damage in both humans and experimental animals.²⁻⁴ Kidney is the second target organ of acetaminophen toxicity and renal dysfunction occurs among patients with marked hepatic injury; however, acetaminophen nephrotoxicity after acute overdose may occur in the absence of hepatotoxicity.⁵

There are three pathways for acetaminophen metabolism; conjugation with sulfate, glucuronide and metabolism by cytochrome p450 oxidase enzyme system.^{6,7} 90% of ingested dose is metabolized through glucuronidation and sulfation pathway and 5% through cytochrome p450 oxidase enzyme system.^{8,9} Metabolism by cytochrome p450 enzyme system produces a metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI) which is toxic to liver and kidney. In therapeutic dose, this is rendered ineffective by reduced glutathione, an antioxidant compound in the liver and NAPQI-reduced glutathione is excreted by kidney.^{10,11} In acetaminophen overdose, sulfation and glucuronidation pathways become saturated. The amount and rate of formation of

NAPQI is greatly increased, depleting body's reduced glutathione stores and outstripping its capability to make new glutathione. NAPQI then binds covalently with cells causing their death, resulting in liver and kidney dysfunction.¹²

Indeed several biological compounds with antioxidant properties proved effective in protecting the kidneys against deleterious effects of acetaminophen overdose.¹³

Garlic (*Allium Sativum*), belonging to Lilaceae family of herbs, is widely used as an ingredient in many food items; besides it has a great medicinal value.^{14,15} Commercial and non commercial preparations of this plant are increasingly used as health supplements; these include garlic powder, oil, water and ethanolic extract of raw garlic or aged garlic.^{16,17} It contains more than 30 components such as Allin, Allicin, Ajuen, lectins, prostaglandins, pectin, adenosine, vitamin B₁, B₂, B₆, C, biotin, nicotinic acid, fatty acids, glycolipid, phospholipids and essential amino acids. They are all present in oily extract of garlic.^{18,19} Garlic had proven successful in protecting different organs against oxidative and inflammatory injuries in many experimental models.^{20,21}

The prophylactic action of garlic oil was observed in rats with acetaminophen induced hepatotoxicity.²² However, the effect of garlic oil on acetaminophen induced nephrotoxicity had not yet been studied. The present study was, therefore, designed to investigate protective effects of garlic oil against acetaminophen induced nephrotoxicity.

For this purpose, garlic oil was given prophylactically to rats which were then treated with acetaminophen. Histological preparations of the kidney were examined with the light microscope in addition to measuring serum urea and creatinine levels.

MATERIAL AND METHODS

Acetaminophen powder was purchased from Merck pharmaceuticals and was dissolved in normal Saline. Garlic oil was prepared by subjecting the crushed garlic to direct steam distillation method. It was then dissolved in commercially available corn oil. One gram of oil was obtained from 1300gm of fresh garlic cloves.

Twenty four male albino rats, 6-8 weeks old and weighing 150-200 gm, were procured from the National Institute of Health, Islamabad. The animals were weighed before the experiment and the difference in weight was not statistically significant. They were housed in experimental research laboratory of the University of Health Sciences, Lahore, under controlled temperature (23-25°C), humidity (60 ± 10%) and light and dark cycles each of 12 hours. They were fed on standard rat diet, water ad libitum and allowed to acclimatize for one week be-

fore starting the experiment. The animals were randomly divided into four groups having 6 rats in each. Group I served as a control, whereas groups II, III and IV were used as experimental.

Group I was given single dose of normal saline

intraperitoneally, 5 ml/kg on 7th day of the experiment; group II was given intraperitoneally single dose of acetaminophen, 1000 mg/kg in 5 ml of normal saline on 7th day of the experiment; group III animals were given garlic oil orally, 100 mg/kg in 1 ml of corn oil daily for one week prior to acetaminophen given on 7th day and group IV was given only corn oil orally 1 ml/kg for one week.

The experiment continued for a period of 8 days when the animals were anaesthetized with chloroform. 6ml of blood was drawn in 10ml disposable syringe by cardiac puncture. Blood samples were collected in a test tube and centrifuged at a speed of 3000r/pm for 10 minutes. The clear serum was collected in sterilized disposable plastic tubes and stored in a freezer set at -20°C for subsequent measurement of serum urea and creatinine using commercially available kits prepared by Human Company.

The animal was killed under anaesthesia. Its extremities were fixed to the dissection board with drawing pins. Vertical midline incision was given from xiphoid process to pubic symphysis and skin and abdominal muscles were retracted laterally before fixing them. Kidneys were freed from connective tissue coverings and gently removed, weighed and examined macroscopically.

3-5mm² thick tissue pieces were excised from the organ and were fixed in 10% formaline solution, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. 5µm thick sections were obtained and subsequently stained with eosin and haematoxylin and PAS and, examined under light microscope (Leica DM 5000B).

STATISTICS

The data was entered and analyzed using SPSS (statistical package for social Sciences, version 16.0). Mean ± SEM is given for quantitative variables. Frequencies and percentages are given for qualitative variables. One way ANOVA was applied to observe mean differences between groups and Post hoc Tukey's test was applied to observe which group mean differs. Fisher's exact test was applied to observe associations between qualitative variables. A p-value of <0.05 was considered statistically significant.

RESULTS

Body weight of rats:

Rats were weighed again towards the end of experiment. Mean weight of rats was 134.87 ± 11.53 , 120.52 ± 4.64 , 142.12 ± 11.86 and 147.74 ± 4.72 gm in groups I, II, III and IV respectively (Table 1). The difference in weight of rats was not statistically significant when compared between groups I, II, III

and IV respectively ($p < 0.20$).

Gross examination of kidney:

Mean length of kidney on right side was 1.35 ± 0.09 , 1.29 ± 0.11 , 1.39 ± 0.15 , and 1.60 ± 0.15 cm and on left side it was 1.49 ± 0.02 , 1.3 ± 0.13 , 1.50 ± 0.19 , and 1.49 ± 0.17 cm for groups I, II, III and IV respectively. The difference was not statistically significant in length of both right ($p < 0.41$) and left ($p < 0.73$) kidneys respectively

when compared between groups I, II, III and IV (Table 2).

Mean weight of kidneys was 0.95 ± 0.05 , 0.88 ± 0.04 , 0.88 ± 0.57 , and 0.82 ± 0.07 percent of body weight in groups I, II, III and IV respectively. The difference in weight of kidneys was not statistically significant when compared between groups I, II, III and IV (Table 1, $p < 0.67$).

Gross examination of kidney specimen showed that it was brown in colour with firm consistency, reddish brown cortex and light red colored medulla in all rats of groups I, II, III and IV.

Histopathological assessment:

Examination of cortex of kidney of group I showed renal corpuscles which appeared as dense rounded structures comprising of glomeruli, surrounded by Bowman's capsule lined by squamous epithelial cells (Fig. 1a). The cortical tubules made the bulk of parenchyma and mainly consisted of proximal and distal convoluted tubules in addition to collecting tubules. Proximal convoluted tubules were lined by simple cuboidal epithelium with prominent brush border and distal convoluted tubules were identified on account of simple cuboidal epithelium, more cle-

arly defined lumen and closely packed nuclei per section (Fig. 1a). In sections of renal medulla of group I, thin descending limb of loop of Henle was lined by simple squamous epithelium but differentiated from vasa recta by absence of erythrocytes and by their regular and rounded shape; thick ascending limb of loop of Henle and collecting tubules were lined by simple cuboidal epithelium but were wider and less regular in shape. Collecting ducts were recognized by pale low columnar epithelium.

Examination of kidney of acetaminophen treated group showed small sized glomeruli, loss of brush border of proximal convoluted tubules and presence of protein casts in distal convoluted tubules. Vacuoles and desquamating cells were seen in both proximal and distal convoluted tubules (Fig. 1b). Protein casts were also observed in lumen of thick ascending limb of loop of Henle.

Garlic oil pretreatment prevented acetaminophen induced changes in glomerular size which almost appeared normal (Fig. 1c). Proximal and distal convoluted tubules did not show any difference when compared to control group (Figs. 1a, 1c)

Table 1: Comparison of mean body weight of animals of groups I, II, III and IV.

Weight of rats	Group I	Group II	Group III	Group IV	P-value
	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM	
	134.87 ± 11.53	120.52 ± 4.64	142.12 ± 11.86	147.74 ± 4.72	0.20
Weight of kidney	0.95 ± 0.05	0.88 ± 0.04	0.88 ± 0.57	0.82 ± 0.07	0.67

* $p < 0.05$ is considered statistically significant

Table 2: Comparison of mean length of right and left kidneys between groups I, II, III and IV.

Length of kidney	Group I	Group II	Group III	Group IV	P-value
	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM	
Right side	1.35 ± 0.09	1.29 ± 0.11	1.39 ± 0.15	1.60 ± 0.15	0.41
Left side	1.49 ± 0.02	1.30 ± 0.13	1.50 ± 0.19	1.49 ± 0.17	0.73

* $p < 0.05$ is considered statistically significant

Examination of medulla of the histological preparations of kidney specimens from group I and III did not revealed any significant difference.

Effects of garlic oil on serum urea and creatinine levels:

Table 4 shows that value of serum urea was 49.76 ± 2.90 mg/dl in group I which was raised to 95.28 ± 2.90 mg/dl in group II whereas in garlic oil pretreated group, its level was reduced to 65.15 ± 2.68 mg/dl. Value of serum urea was 41.55 ± 1.56 mg/dl in group IV. Garlic oil lowered serum urea significantly in group III when compared to that in group II ($p < 0.001$) but was still statistically significantly raised when compared with group I ($p < 0.001$).

Serum creatinine concentration was 1.16 ± 0.13 mg/dl in group I which was raised to 2.71 ± 0.68 mg/dl in group II. Garlic oil pretreatment reduced its level to 1.73 ± 0.04 mg/dl in group III. Value of serum creatinine was 1.65 ± 0.86 in group IV. Statistically significant difference in values of serum creatinine was observed when group I was

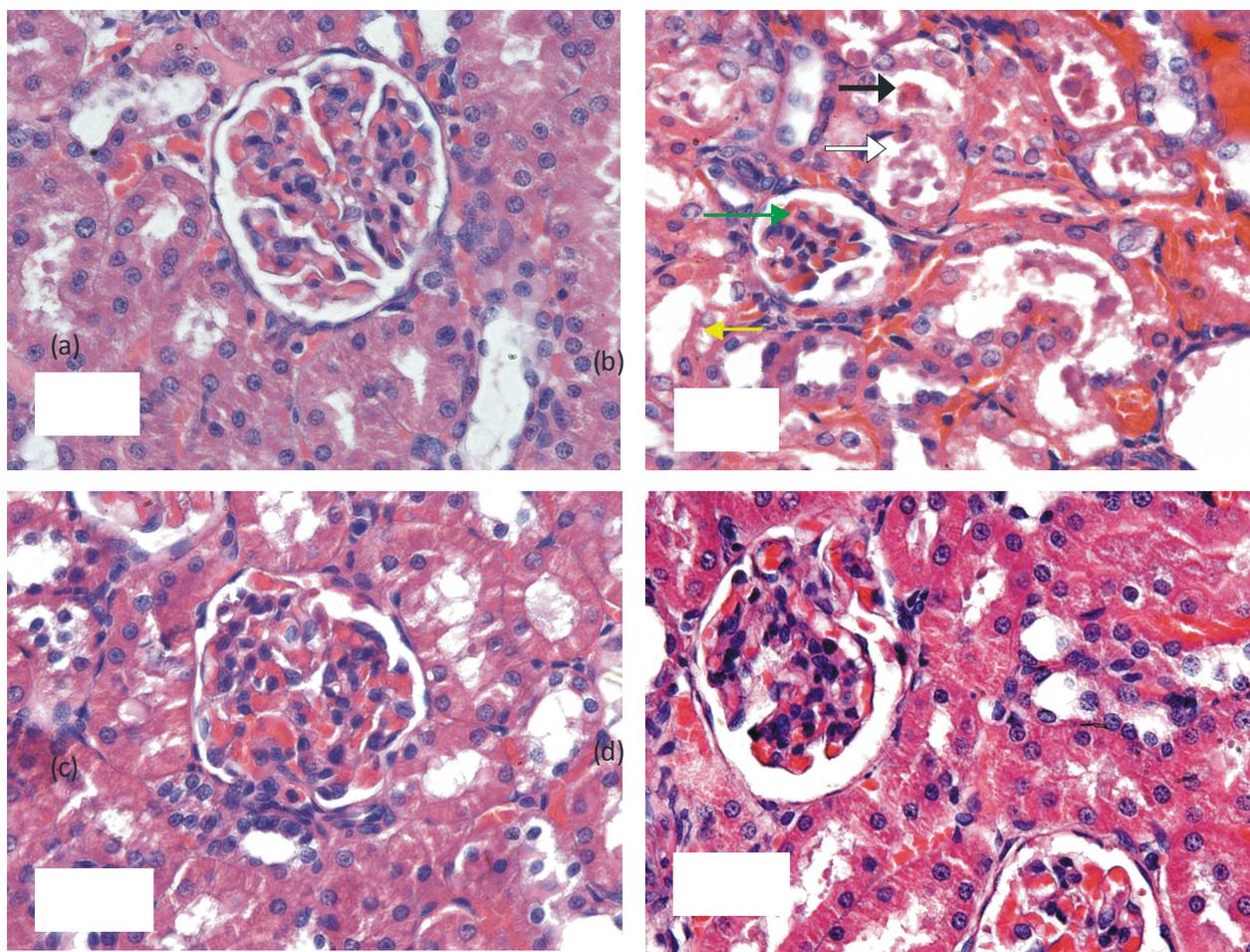


Fig. 1: Photomicrograph of rat kidney from (a) control group showing normal renal architecture; (b) acetaminophen treated group showing small sized glomerulus (green arrow), severe tubular necrosis,

vacuolar degeneration of tubules (yellow arrow), epithelial desquamation (white arrow), and intra-luminal casts (black arrow); (c) garlic oil plus acetaminophen treated group showing marked improvement with no sign of vacuolation and epithelial desquamation; (d) vehicle treated group showing comparable picture as that in control group (eosin and hematoxylin 400 X).

compared with groups II, III and IV ($p < 0.001$). Garlic oil lowered serum creatinine and the difference was statistically significant when group III was compared with group II ($P < 0.03$). Statistically significant difference was not observed when the values of the serum creatinine were compared between groups I and III

Table 3: Comparison of mean value of serum urea and creatinine of groups I, II, III and IV.

Groups	Group I Mean \pm SEM	Group II Mean \pm SEM	Group III Mean \pm SEM	Group IV Mean \pm SEM	p-value
Serum urea	49.76 \pm 2.90	95.28 \pm 2.90	65.15 \pm 2.68	41.55 \pm 1.56	0.001*
Serum creatinine	1.16 \pm 0.13	2.71 \pm 0.68	1.73 \pm 0.04	1.65 \pm 0.86	0.001*

* $P < 0.05$ is considered statistically significant

($p < 0.35$).

DISCUSSION

Microscopic examination of histological preparations of the kidney showed that glomerular size was significantly reduced in rats treated with acetaminophen ($p < 0.001$). Vacuoles and fine granules were scattered in the cytoplasm of the proximal

and distal convoluted tubules of group II. Loss of brush border and desquamating cells were seen in proximal convoluted tubules and protein casts were observed in the lumen of distal convoluted tubules of the rats treated with acetaminophen. These changes were indicative of toxic effects of acetaminophen on kidney (Fig. 1b).

Fouad et al. (2009) reported severe tubular vacuolar necrosis with dilatation, degeneration, epithelial desquamation; they reported intraluminal cast formation mainly in distal convoluted tubules together with apoptotic bodies after a single dose of 2.5 g/kg of acetaminophen through a stomach tube.²³

Our results are in agreement with Khorsandi and Orazizadeh (2008) who reported glomerular damage evident by glomerular bleeding and partial epithelial rupture in Bowman's capsule.²⁴ They also reported proximal tubular dilatation with loss of cell boundary, intraluminal cell debris, karyorrhexis, glassy pink cytoplasm, loss of brush border of proximal tubule. They also observed debris and granules from epithelial cells which leaked into tubular lumen after administration of a single dose of 500mg/kg of acetaminophen suspension which was prepared by gum tragacant (0.5%) in normal saline.²⁴

Our results agree with those reported earlier by Abdel-zaher (2007) who observed moderate cloudy swelling of proximal convoluted tubules and severe vacuolar degeneration of distal convoluted tubules after single oral dose of 2.5 g/kg of acetaminophen (20% suspension in saline stabilized by 0.2% gum).¹³ In our investigation swelling of proximal convoluted tubules was not observed. The reason for this difference could be explained on basis of difference in dosage and route of administration of acetaminophen.

Li Chengxiu (2003) reported cortical interstitial congestion, degeneration and necrosis 16 hours after a single dose of 600 mg/kg of acetaminophen dissolved in 25 ml/kg saline given intraperitoneally.²⁵

In our investigation, examination of renal medulla of acetaminophen treated group showed protein casts in the lumen of thick ascending limb of loop of Henle. Trumper (1998) reported many granular casts and epithelial cells in urinary sediments after single intraperitoneal dose of 1000 mg/kg acetaminophen dissolved in 5ml/kg of propylene glycol.²⁶

In the present study, value of serum urea was significantly raised from 49.76 ± 2.90 mg/dl in group I to 95.28 ± 2.90 mg/dl ($p < 0.001$) and that in group II of serum creatinine from 1.16 ± 0.13 mg/dl in group I to 2.71 ± 0.68 mg/dl in acetaminophen treated group respectively, p-value being 0.001 (Table 3); these findings were in accord with findings

of Adenye (2008), who reported significant rise in blood urea and serum creatinine after intraperitoneal administration of 200 mg/kg/day acetaminophen for 14 days ($p < 0.05$).²⁷ Comparable results were also reported earlier by Sener (2003), who observed increased levels of serum creatinine and blood urea nitrogen 4, and 24 hours after administration of single intraperitoneal dose of 900mg/kg of acetaminophen dissolved in water.²⁸

In our investigation, garlic oil pre-treatment reduced changes in glomeruli which almost looked normal; glomerular size was restored to $84.63 \pm 2.89\mu$ in group III from $51.50 \pm 3.60\mu$ in group II ($p < 0.001$). Statistically significant difference was not observed between glomerular size of group I ($84.01 \pm 1.85\mu$) and III ($84.63 \pm 2.89\mu$) ($p < 0.99$). Protein casts were not seen in rats treated with garlic oil prior to administration of acetaminophen (Fig. 1c). Garlic oil lowered serum urea significantly in group III and the difference was statistically significant when it was compared to group II ($p < 0.001$, Table 3); but the difference was statistically significant when it was compared to groups I and IV ($p < 0.001$). Serum creatinine concentration in group III was lowered (Table 3); the difference was statistically significant when compared to group II ($p < 0.03$). The difference in serum creatinine concentration was statistically insignificant when group III was compared with that of groups I and IV ($p < 0.35$ and $p < 0.99$ respectively).

The protective effects of garlic oil upon acetaminophen induced nephrotoxicity remain almost unexplained; Kalantari (2001), however, investigated the effects of garlic oil in preventing acetaminophen induced hepatotoxicity. He reported increased levels of ALT, AST and extensive necrosis of liver. Garlic oil given intraperitoneally immediately after acetaminophen administration in a dose of 200 mg/kg reduced the ALT, AST levels and area of liver damage. Garlic and garlic extracts are reported to provide protection against free radical damage in body through antioxidant activities.²²

It had also been reported earlier that seasoned (aged) garlic extract ameliorated deranged renal functional and histological alterations like vacuolation, hydropic degenerative changes or necrosis produced by cyclosporine A administration.²⁹ Pretreatment with garlic preparations restored glutathione levels and offered significant protection to myocardium in isoproterenol induced myocardial infarction in rats.³⁰

Garlic oil had also been shown to reduce oxidative stress in streptozotocin induced diabetes.³¹ Fresh garlic and garlic powder through their combined antioxidant and antimicrobial effects are potentially useful in preserving meat products.³² Similarly

diallyl disulfide, a constituent of garlic, is effective in exerting antioxidant action by inhibiting testosterone induced oxidative stress.³³

Trumper in 1998 reported that intraperitoneal administration of single dose of 200 mg/kg of Probenecid in 10ml of isotonic saline, 30 min before giving a single intraperitoneal dose of 1000 mg/kg of acetaminophen in 5ml of propylene glycol, improved urine flow and glomerular filtration rate, restored urea and creatinine plasma levels changed by acetaminophen.²⁶ Abraham (2005) reported that oral administration of 100, 200 and 400 mg/kg of Propylthiouracil one hour before single intraperitoneal dose of 1000 mg/kg of acetaminophen in saline significantly reduced its nephrotoxicity as evidenced by reduction in plasma creatinine and amelioration of interstitial congestion, tubular degeneration and necrosis in kidney.³⁴

Pre-treatment of rats with alpha-lipoic acid given orally in a dose of 100mg/kg one hour before acute toxic dose of acetaminophen (2.5 mg/kg) administered orally protected acetaminophen induced nephrotoxicity and hepatotoxicity. Which was assessed by reduction in levels of SGOT, SGPT, serum urea, serum creatinine, swelling of proximal convoluted tubule and vacuolar degeneration of distal convoluted tubules.¹³

Treatment of rats with an oral dose of 100-400 mg/kg/day of aqueous leaf and seed extract of *Phyllanthus amarus* one hour before intraperitoneal injection of 200mg/kg of acetaminophen for 14 days reduced its nephrotoxicity as evidenced by reduction in serum creatinine and blood urea levels; it is also supported by reduction in focal tubulonephritis and lymphocytic infiltration in a dose related manner.¹¹

Our study showed that garlic oil was effective in protecting acetaminophen induced renal injury; the exact mechanism is not properly understood. Further investigations are required to elaborate and understanding the way garlic oil operates to prevent nephrotoxic effect of acetaminophen.

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