

## Efficacy of jamun *Syzygium cumini* seed and orange *Citrus sinensis* peel extracts against microcystin LR induced histological damage in the kidney of rat

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**Abstract.** In this study we evaluated the protective effects of jamun *Syzygium cumini* seed and orange *Citrus sinensis* peel extracts on renotoxicity of microcystin LR in male rats. Groups A-F were given daily treatments for 30 days. Group A (Control): No treatment was given; Group B: microcystin (10 µg/kg body wt); Group C: microcystin (10 µg/kg body wt) and jamun seed extract (200 mg/kg body wt); Group D: microcystin (10 µg/kg body wt) and orange peel extract (200 mg/kg body wt); Group E: orange peel extract (200 mg/kg body wt); Group F: jamun seed extract (200 mg/kg body wt). Kidney were fixed at 15th and 30th day after the treatments. In 15 day MCLR (group B) treated rats shrunken glomeruli, hypertrophy of epithelial cells of tubules. vacuolation of cytoplasm and obliterated tubular lumina were noticed. In MCLR+JSE (group C) and MCLR+OPE (group D) treated rats almost similar changes were noticed as seen in MCLR treated rats. In OPE (group E) and JSE (group F) treated rats no visible morphological alterations were noticed. Following 30 day MCLR treatment (group B), increased cellularity of glomeruli, no space between the Bowman's capsule and glomerulus, glomerular degeneration, dilated tubules, separation of tubular epithelial cells from underlying basement membrane, tubular vacuolization and degeneration with necrotic nuclei in lumina and deposition of eosin-positive material in the tubules were observed. In MCLR+JSE (group C) and MCLR+OPE (group D) treated rats the glomeruli were swollen showing increased cellularity. No degeneration was noticed in glomeruli. Tubules were dilated, however, at few places few epithelial cells were degenerating. Necrotic nuclei

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were not seen in tubular lumina. In OPE (group E) and JSE (group F) treated rats the kidney exhibited no histological changes.

**Keywords:** Microcystin; *Syzygium cumini*; *Citrus sinensis*; Kidney; Cyanobacteria.

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## Introduction

In many parts of the world an increasing environmental hazard has been reported due to toxic cyanobacteria which are found in freshwater, eutrophic and municipal water supplies (Filipic et al., 2007). Cyanobacteria produce hepatotoxins, neurotoxins and lipopolysaccharide endotoxins (Srivastava and Srivastav, 2017). Ingestion of cyanobacteria adversely affects domestic and aquatic animals as well as humans (Elleman et al., 1978; Zin and Edwards, 1979; Bell and Codd, 1994; Negri et al., 1995; Benson et al., 2005; Shahi et al., 2012; Srivastava and Srivastav, 2017).

*Microcystis aeruginosa* is commonly observed in cyanobacterial blooms (Shahi et al., 2012) which produce a cyclic hepatotoxin-microcystin LR (Hooser et al., 1990; Carmichael, 1992; Watanabe et al., 1996; Benson et al., 2005; Kim et al., 2006; Srivastava and Srivastav, 2017). Few workers have reported accumulation of microcystin in bivalves (Prepas et al., 1997; Williams et al., 1997; Amorim and Vasconcelos, 1999), snails (Xie et al., 2005; Papadimitriou et al., 2012), shrimp (Xie et al., 2005; Zhang et al., 2009; Papadimitriou et al., 2012), frogs (Papadimitriou et al., 2012) and human liver (Greer et al., 2018). There are reports that microcystin LR produce hepatic damage in rats (Kim et al., 2006; Srivastava and Srivastav, 2017), however, there are evidences which suggest that it also affects kidney (Dias et al., 2009; Shahi et al., 2012; Xiping et al., 2019; Wang et al., 2019), heart (Shahi et al., 2012), germ cell apoptosis (Zhao et al., 2018) and human respiratory system (Brozman et al., 2020).

Black plum *Syzygium cumini* is found in Asian subcontinent, Eastern Africa, South America, Madagascar, and some parts of United States of America (Swami et al., 2012; Srivastava and Srivastav, 2017). It is also known as java plum, Indian blackberry, jamun, jambolao, jambul etc. (Srivastava and Srivastav, 2017; Chagas et al., 2018). Various workers have attributed some biological activities to *Syzygium cumini*, namely cardioprotective, anti-inflammatory, hepatoprotective, antineoplastic, hypoglycemic, hypolipidemic, antibacterial and antiallergic (Giri et al., 1985; Raza et al., 2017; Srivastava and Srivastav, 2017; Chagas et al., 2018).

Orange *Citrus sinensis* peel is a rich source of flavonoids, carotenoids, limnoids, acridone alkaloids, vitamin C and B complex, essential oils and minerals (Calabro et al., 2004; Hegazy and Ibrahim, 2012; Madhuri et al., 2014; Muhtadi et al., 2015; Ashraf et al., 2017; Srivastava and Srivastav, 2017). Orange peels have been reported to possess antioxidant, antibacterial, larvicidal, antifungal activity (Gorinstein et al., 2001; Ghasemi et al., 2009; Ramful et al., 2010; Hegazy and Ibrahim, 2012; Parashar et al., 2014; Madhuri et al., 2014; Bashandy et al., 2019). It has been reported that flavonone and hesperidin present in *Citrus* are anti-inflammatory and antihypertensive (Gil-Izquierdo et al., 2001; Bashandy et al., 2019). Muhtadi et al. (2015) have reported that *Citrus sinensis* peel extract possess antidiabetic and antihypercholesterolemic potential in rats.

The present study was aimed to evaluate the protective effects of extracts of jamun (*Syzygium cumini*) seeds and orange (*Citrus sinensis*) peels against microcystin LR (MCLR) induced histological changes in the kidney of male rats. Prior to this study no report exists regarding protective effects of extracts of seed of *Syzygium cumini* (JSE) and peels of *Citrus sinensis* (OPE) on renotoxicity induced by microcystin LR.

## Materials and methods

Male Wistar rats (70-90 g) were housed in polypropylene cages and acclimatized for 2 weeks under laboratory conditions prior to different treatments. During entire experimental period rats were maintained on the standard laboratory feed and water *ad libitum*. Animal handling and sacrifice were carried out following the guidelines provided by Ethics Committee of the University.

After acclimation rats were divided into six groups - A, B, C, D, E, and F, each consisting of 20 animals. Following treatments were orally given daily to these groups at 08:00 each day throughout the experiment:

Group A (Control): No treatment was given.

Group B (Microcystin-treated rats; MCLR): Received daily only microcystin (10 µg/kg body wt).

Group C (Microcystin+jamun seed extract; MCLR+JSE): These rats were given daily microcystin (10 µg/kg body wt) and jamun seed extract (200 mg/kg body wt) simultaneously.

Group D (Microcystin+orange peel extract; MCLR+OPE): These rats were given daily microcystin (10 µg/kg body wt) and orange peel extract (200 mg/kg body wt) simultaneously.

Group E (Orange peel extract; OPE): Rats received daily orange peel extract (200 mg/kg body wt).

Group F (Jamun seed extract; JSE): Rats received daily jamun seed extract (200 mg/kg body wt).

Rats from all the groups (10 from each group) were sacrificed 24 h after last dose on 15th and 30th day after initiation of the experiment under light ether anesthesia. Animals were fasted overnight before sacrifice.

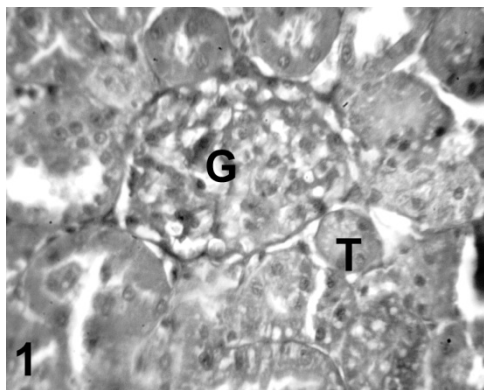
For use in experiment purified Microcystin LR (Enzo Life Sciences, Inc.) was dissolved in 0.9% NaCl. The preparation of jamun seed and orange peel extracts have been described in detail by Srivastava et al. (2018).

Kidney of control and treated rats were fixed in Bouin's fluid. These fixed tissues (kidney) were dehydrated in an ethanol gradient, treated with a clearing agent, infiltrated and embedded in paraffin, sectioned at 6 µm, floated on a heated water bath and mounted to glass slides. After drying overnight, paraffin was removed with a clearing agent, tissue was rehydrated in an ethanol gradient and then stained with hematoxylin and eosin (HE) for light microscopic examination.

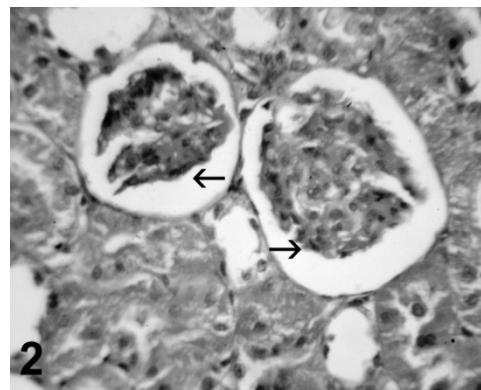
For electron microscopic studies, small kidney pieces were fixed in paraformaldehyde and glutaraldehyde mixture for 4 h at 4 °C, washed with phosphate buffer and stored at 4 °C. These tissues were processed at Sophisticated Analytical Instrument Facility, All India Institute of Medical Sciences, New Delhi, India.

## Results

Kidney of control rats contain numerous nephrons, each nephron consist of a dilated portion, the renal corpuscle; the proximal tubule; loop of Henle and the distal tubule. The renal corpuscles contain a tuft of capillaries, the glomerulus which is surrounded by the Bowman's capsule (Figure 1). There is present the urinary space between the glomerulus and Bowman's capsule. The proximal tubule is lined by simple cuboidal (Figure 2) or columnar epithelium whereas the distal tubule is lined by simple cuboidal epithelium (Figure 1).

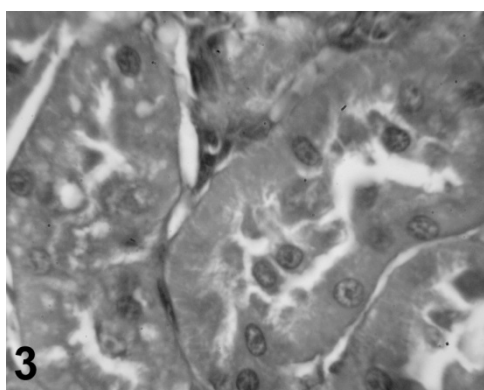


**Figure 1.** Kidney of control rat showing glomerulus (G) and tubule (T). Hematoxylin-eosin X 200.

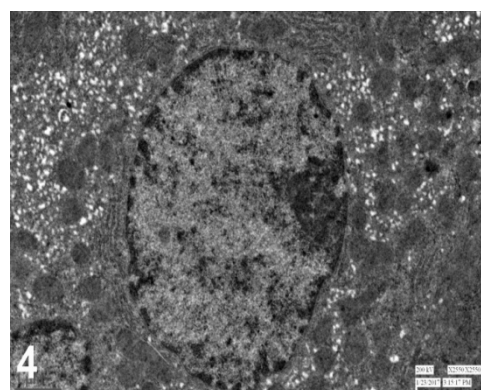


**Figure 2.** Shrunken glomerulus (arrows) in the kidney of 15 day microcystin treated rat. Hematoxylin-eosin X 200.

In 15 day MCLR treated rats (group B) the glomeruli at few places are noticed to be shrunken thus more space is visible between the Bowman's capsule and glomerulus (Figure 2). The proximal and distal tubules show hypertrophy of epithelial cells, vacuolation of cytoplasm and exhibit obliterated lumina (Figures 3 and 4). In MCLR+JSE (group C) and MCLR+OPE (group D) treated rats almost similar changes are noticed as seen in MCLR treated rats. In OPE (group D) and JSE (group F) treated rats no visible morphological alterations are seen.



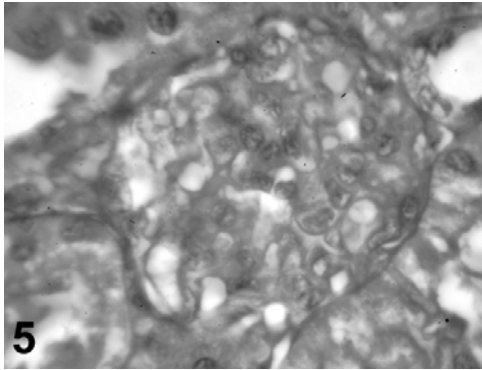
**Figure 3.** Kidney of 15 day microcystin treated rat showing hypertrophy of tubular epithelium and obliterated lumen. Hematoxylin-eosin X 500.



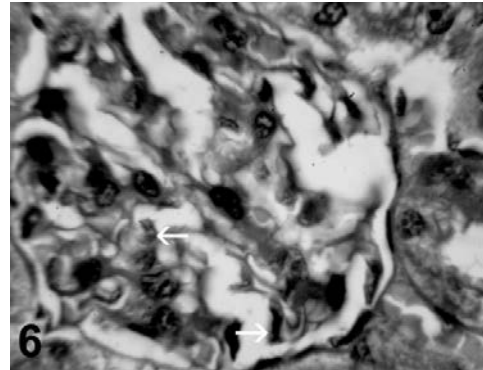
**Figure 4.** Electron micrograph showing vacuolization in the kidney tubule of 15 day microcystin treated rat.

Following 30 day MCLR treatment (group B), the cellularity of glomeruli is increased at several places thus there is no space between the Bowman's capsule and glomerulus (Figure 5). However, at some places glomeruli are seen degenerating (Figure 6). The proximal and distal tubules are dilated (Figure 7). Tubules also display separation of epithelial cells from underlying basement membrane, vacuolization and degeneration having necrotic nuclei in lumina (Figures 7, 8 and 9). Large deposits of eosin-positive material appear in the tubules (Figures 8 and 10). In MCLR+JSE (group C) and MCLR+OPE (group D) treated rats the glomeruli are swollen showing increased cellularity. No degeneration is noticed in glomeruli. Tubules are dilated, however, at few places few

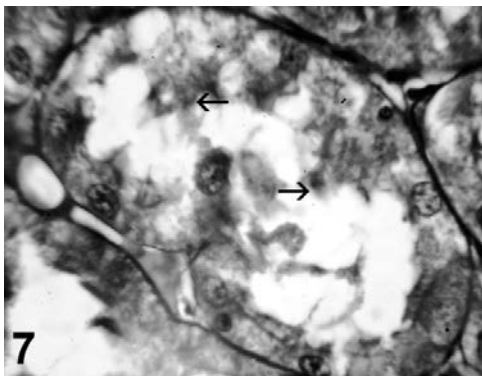
epithelial cells are degenerating. Necrotic nuclei are not seen in tubular lumina. In OPE (group E) and JSE (group F) treated rats the kidney architecture is almost similar to control rats.



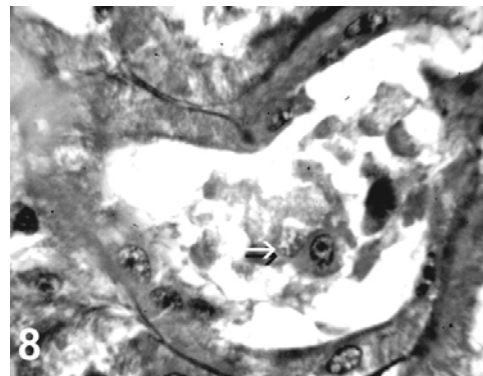
**Figure 5.** Glomerulus exhibiting hypercellularity after 30 day microcystin treatment. Note the absence of space between the glomerulus and Bowman's capsule. Hematoxylin-eosin X 500.



**Figure 6.** Degeneration of glomerulus (arrows) in 30 day microcystin treated rat. Hematoxylin-eosin X 500.



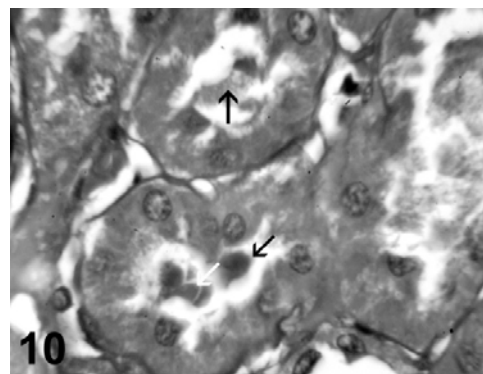
**Figure 7.** Degeneration (arrows) and dilation of kidney tubules of 30 day microcystin treated rat. Hematoxylin-eosin X 500.



**Figure 8.** Degenerated tubules displaying necrotic nuclei (arrow) in the lumen of 30 day microcystin exposed rat. Hematoxylin-eosin X 500.



**Figure 9.** Electron micrograph of kidney of 30 day microcystin exposed rat displaying degeneration of tubular nucleus.



**Figure 10.** Deposition of eosin-positive substances (arrows) and degenerating nuclei (white arrow) in the tubular lumina of 30 day microcystin treated rat. Hematoxylin-eosin X 500.

## Discussion

In the present study histological alterations in the kidney has been noticed in MCLR exposed rats. The present study demonstrated glomerular swelling after 30 day in MCLR treated rats. This derives support from studies of other workers who have reported glomerular swelling in kidney of toxicant exposed rat (cadmium - Aughey et al., 1984; Brzoska et al., 2003; Abdel-Moneim and Said, 2007; Tripathi and Srivastav, 2011), rabbit (fluoride - Shashi et al., 2002), quail (lead - Al-Mansour et al., 2009) and mice (MCLR - Xiping et al., 2019). Rats treated with MCLR exhibited degeneration of glomeruli and deposition of eosin-positive substances. In past, degeneration of glomeruli has been reported after treatment with microcystin (Atencio et al., 2008; Suput, 2011). Several investigators have noticed toxicant induced glomerular degeneration in vertebrates such as fish - after treatment with chlorpyrifos (Srivastava et al., 1990), deltamethrin (Cengiz, 2006), cadmium (Wangsongsak et al., 2007) and cypermethrin (Korkmaz et al., 2009); chick - after treatment with cypermethrin (Aslam et al., 2009) and rat - after treatment with paraquat (Lamfon and Al-Rawi, 2007), chlorpyrifos (Tripathi and Srivastav, 2010) and cadmium (Tripathi and Srivastav, 2011). Amorphous cellular debris in glomerulus has been noticed in rat exposed to paraquat (Lamfon and Al-Rawi, 2007).

In the foregoing study shrinkage of glomerulus has been observed in MCLR treated rats. Shrinkage of glomerulus concordant with this study has also been reported in rats after exposure to paraquat (Abdel-Mageid, 1994), chlorpyrifos (Tripathi and Srivastav, 2010) and cadmium (Tripathi and Srivastav, 2011). The degeneration of glomerulus in MCLR treated rats may cause decreased GFR as Barrouillet et al. (1999) have noticed decrease in GFR in their *in vitro* studies of cadmium exposure.

Dilatation of renal tubules have been observed in MCLR exposed rats. This is in agreement with the similar reports by Hooser et al. (1989), Suput (2011) and Xiping et al., (2019), after MCLR treatment. The present study regarding dilatation of kidney tubules derives support from observations on rats (lead - Nunes et al., 2001; cadmium - Kukner et al., 2007, Tripathi and Srivastav, 2011; fenthion - Kerem et al., 2007; fenitrothion - Afshar et al., 2008; endosulfan - Khan and Kumari, 2012; chlorpyrifos - Tripathi and Srivastav, 2010), wood mouse (landfill leachates containing toxic metals - Sanchez-Chardi et al., 2009), rabbit (fluoride - Shashi et al., 2002), quails (lead - Al-Mansour et al., 2009) and fish (chlorpyrifos - Srivastava et al., 1990). Degeneration and vacuolization in the tubular cells have been noticed in MCLR treated rats. This is in concordant with the reports of other investigators who have also noticed similar changes in tubules of microcystin exposed organisms (Hooser et al., 1989; Rabergh et al., 1991; Kotak et al., 1996; Atencio et al., 2008; Suput, 2011; Rangel et al., 2014; Al-Sultan et al., 2015; Al-Majeed et al., 2016). Tubular degeneration has also been reported by other investigators after exposure to various toxicants to vertebrates - fish (chlorpyrifos--Srivastava et al., 1990; cadmium - Wangsongsak et al., 2007; cypermethrin - Ayoola and Ajani, 2008); chick (cypermethrin - Aslam et al., 2009); quail (lead - Al-Mansour et al., 2009); wood mouse and white-toothed shrew (landfill leachates containing toxic metals - Sanchez-Chardi et al., 2009); monkey (lead - Colle et al., 1980); mice (chlorpyrifos - Thijssen et al., 2007); rabbit (fluoride - Shashi et al., 2002); rat (chlorpyrifos - Aughey et al., 1984; Karmakar et al., 1986; Gatta et al., 1989; Brzoska et al., 2003; Abdel-Moneim and Said, 2007; Kukner et al., 2007; Jihen et al., 2008; Tripathi and Srivastav, 2010; metal mixture - Jadhav et al., 2007; paraquat - Damain et al., 1992; Abdel-Mageid, 1994; Lamfon and Al-Rawi, 2007; cypermethrin - Muthuviveganandavel et al., 2008; fenthion - Kerem et al., 2007; cigarette smoke - Kuru et al., 2009; cadmium - Tripathi and Srivastav, 2011). However, Velisek et al. (2009) have not recorded any change in the kidney of fish treated with bifenthrin.

In MCLR treated rats degenerated cells have been encountered in the tubular lumina. Hosser et al. (1989) and have reported eosinophilic to basophilic material within

the lumina of renal cortical tubules after MCLR treatment to rats. After microcystin treatment Suput (2011 - in rats) and Xiping et al. (2019 - in mice) have noticed dilated tubules filled with eosinophilic material. Brzoska et al. (2003) and Shashi et al. (2002) have also reported occurrence of degenerated cells in tubular lumina of rats (exposed to cadmium) and rabbit (exposed to fluoride), respectively.

Kidney alterations such as tubular vacuolization, dilatation and degeneration caused by toxicants might be due to hydrolic changes in the renal tissue. These changes clearly indicates that toxicants provoke renal failure in pump transport of tubular cells and also cause functional disturbances in kidney. In past, it has been suggested that dilatation may be a compensatory mechanism after the loss of renal excretory function (Sanchez-Chardi et al., 2009).

## Conclusion

We can conclude that exposure to microcystin adversely affected the kidney structure of the rats. The structural damage caused by microcystin to this vital organ could be protected by supplementation of extracts of jamun seed and orange peel. It is suggested that the microcystin exposed organisms should be given dietary supplement of these botanical extracts which would ease the toxic symptoms.

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## Conflict of interest

The authors declare that they do not have any conflict of interest.

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