The Role of Sumac in Attenuation of Microcystin-Lr-Induced Renal Oxidative Damage in Balb/C Mice

S. Al-Jassabi and Mohd Sofian Azirun

Institute of Biological Sciences, University of Malaya, 50603, Kuala Lumpur, Malaysia

Abstract: Chemoprotectant studies suggested that membrane-active antioxidants may offer a protection against microcystin toxicity. This type of cyanobacterial potent toxin posing serious water quality problems. The present investigation aimed to evaluate the effect of sumac, an antioxidant, on microcystin (MC-LR)-induced renal oxidative damage in Balb/c mice. 40 male Balb/c mice were assigned randomly to 4 groups, 10 of them were used as a control group (saline treated), the second 10 mice were used as sumac control and the third group was used as toxin control. The fourth group was pre-treated with sumac (300mg /kg mouse b.wt) given orally once a day for 14 days before an interperitoneal injection (i.p) with 75µg/kg b.wt of MC-LR-(according to LD $_{50}$ value). Serum creatinine, blood urea (BUN), urinary protein, glucose, urine gamma glutamyl transferase (GGT) were increased in mice treated with MC-LR, while creatinine clearance decreased compared to controls (P<0.001). Also, protein carbonyl contents increment and DNA-protein cross-links were observed in the kidney homogenates of these mice. Renal biochemical examination revealed tubular damage and sumac significantly normalized the above parameters. MC-LR decreased the activities of antioxidant enzymes, catalase (CAT), glutathione peroxidase (GPHPx), superoxide dismutase (SOD) and the level of glutathione (GSH). Sumac attenuated the MC-LR-induced reduction in the activities of CAT, GPHPx, SOD and level of GSH and the increment in both plasma and kidney homogenate malondialdehyde (MDA) and lipid hydroperxide. In conclusion, these data indicated that the natural antioxidant sumac can be a potent protective agent against renal oxidative damage mediated by world-wide toxin, MC-LR.

Key words: Chemoprotection · Microcystin · Antioxidants · Sumac · Renal · Oxidative damage

INTRODUCTION

The contamination of aquatic ecosystems as a consequence of human activities is a well-established fact. In many cases, the direct effects of pollution processes are cynobacterial blooms, some of which are characterized by the production of toxins [1-3]. This toxin is produced principally by *Microcystis aeruginosa* (*M.aeruginosa*) and is a potent hexapeptide hepatotoxin [4,5] transported specifically into the liver by multispecific bile acid transporte system [6], thereby inducing severe intrahepatic haemorrhaging necrosis and apoptosis [7,8]. MC-LR specifically inhibits serine/ threonine protein phosphatases (PP1 and 2A), resulting in the disruption of many important cellular processes [9,10]. Additionally, this toxin causes oxidative stress and increased reactive oxygen species (ROS) [11,12], as well as lipid peroxidation [13]. The mechanism of MC-LR-induced nephrotoxicity is not completely known. However, studies have implicated ROS particular superoxide anion radical in the pathaophysiology of MC-LR nephropathy [14].

Recently, epidemiological studies have strongly suggested that consumption of antioxidants may reduce the risk of chronic diseases related to oxidative stress on account of their antioxidant activity and promote general health benefits [15]. The southeast asian diet is particularly rich in spices, sumac is one example which is widely used in this area. Sumac is the common name for a genus (*Rhus*) that contains over 250 individual species. Previous phytochemical studies reported that sumac contained flavones, tannins, anthocyanins, phenolic acids, gallic acid and organic acids [16-18] and found that sumac can be used as a natural antioxidant. Sumac was defended as a non-vitamin active antioxidant that has a high oxygen-radical scavenging and quenching capacity. It is very useful in living tissues to reduce the risk of adverse oxidative reactions that produced by hydroxyl radicals and peroxides.

Corresponding Author: S. Al-Jassabi, Institute of Biological Sciences, University of Malaya, 50603, Kuala Lumpur, Malaysia.
E-mail: saaam84@live.com.

The aim of this study was to investigate the role of sumac pre-treatment in protection against MC-LR-induced renal oxidative damage in Balb/c mice.

MATERIALS AND METHODS

Chemicals: All chemicals used in this study was of analytical grade and purchased from Sigma Chemical. Co. (USA). Dried and ground sumac was purchased from local market in Irbid, Jordan.

Extraction of Phenolic Components of Sumac: Phenolic components of sumac was extracted according to the method described by Kosar *et al.* [18].

Cyanobacteria and Cyanotoxin: *Microsistis aeruginosa* was collected from selected sites of King Talal Reservoir, identified and isolated as previously reported [5,7]. Toxin, microcystin was extracted [6,19].

Animals and Treatment: Male Balb/c mice 6-7 weeks old, (average body wt.30g) were used in this study. Mice were kept on standard laboratory diet and tap water *ad libitum* through out the experiments. Five animals were housed stainless metal cages under a 12-12h ligh-dark cycle and room temperatures of 23°-26° C.

LD_{50} Determination: LD_{50} value of the toxin was determined according to Fawell's up-down method [19] to reduce the number of mice used and quantity of toxin required.

Toxicological Studies: Forty mice were divided randomly into four groups (10 mice each). Group1, was the control group with mice that did not receive toxins or sumac supplementations (saline treated group); group 2, received sumac supplementation (300 mg/kg/ day) orally [20] for 14 days, prior to sacrification. Group 3 of mice were received a single i.p. dose of toxin (75μg/kg) and sacrificed after 24h. Group 4, received the same dose of sumac for 14 days then received a single i.p.; dose of toxin (75μg/kg) only and sacrificed after 24h after toxin treatment. Twenty four hours following the last treatment, 24h urine free of food and faeces was collected into ice-cold graduated cylinders for the determination of proteins, creatinine, glucose and the activity of gamma glutamyl transferase.

Mice were sacrificed by cervical dislocation and the kidneys were excised, washed in ice-cold saline. The kidneys were homogenized in ice-cold 0.1M Tris-HCl buffer (pH 7.4) using Ultra homogenizer. The homogenates were first centrifuged at 10,000xg for 15 min and the supernatants were then centrifuged at 100,000xg for 1h. Supernatant (cytosolic fraction) was recovered and the protein concentration was determined [7], aliquoted were used for the determination of enzymatic activities and lipid peroxidation as malondialdehyde (MDA) production from the thiobarbituric acid reaction in kidney homogenates and serum [20]. Blood was obtained by heart puncture technique into centrifuge tubes. Serum was prepared by centrifugation for 10 min at 3000xg. Heparinized blood samples were centrifuged at 1500xg for 10 min and plasma was removed.

Blood urea nitrogen (BUN) [21], creatine [22] and creatinine clearance [23] were determined. Urinary glucose was measured using commercial kit. Gamma-glutamyl transferase [24], Catalase (CAT) activity [25] Superoxide dismutase (SOD) activity [25] were determined. GSH peroxidase (GSH-Px) activity was determined in kidney homogenates [26]. GSH was determined in the 10,000xg supernatant fraction of the kidney homogenate [27]. Lipid peroxidation was determined as malondialdehyde (MDA) and lipid hydroperoxide (LOOH) in both plasma and kidney cytosol, respectively [28]. Protein carbonyl contents was determined as described previously [29,30]. Protein content of all samples was determined by the method of Biuret using bovine serum albumin as standard.

The extent of DNA-protein cross-links were assayed by the method of Carmichael [31].

Statistical Analysis: All results were expressed as the mean ± S.E.M from ten mice per group. One way analysis of variance (ANOVA) followed by a Tukey test was used to determine the significance of the differences between the groups. Statistical significance was declared when P value was equal to or less than 0.05. The statistical analysis was performed using the Sigma State Statistical Software version 3.5.

RESULTS

In this study, MC-LR treated mice show increased liver body mass index ratio due to massive intrahepatic hemorrhage and pooling of blood in the liver, as shown in table (1). The liver of mice which received sumac only was within the normal value, but this value increased slightly after the exposure to the toxin.

The effect of sumac on BUN, creatinine and creatinine clearance in Bulb/c mice treated with MC-LR (75μg/kg) are shown in Table 1.

Table 1: The effect of sumac supplementation (300mg/kg bwt) on the liver mass and clinical parameters in Balb/c mice received single dose of MC-LR (75 ug/kg)

Parameters	CON	MC-LR	SUM	MC-LR+ SUM
Liver weight (g)	1.52±0.03	2.88±0.06	1.49±0.02	1.71±0.03
Urinary protein mg/24h	18.6±0.9	31.2±1.61*	16.6±2.1	22.4±2.1**
Glucose mg/24h	8.4±1.1	48.6±2.1*	6.3±1.1	10.6±1.8**
Creatinine clearance ml/min/100g	0.52 ± 0.003	0.08± 0.001*	0.51± 0.004	0.46± 0.003**
Creatinine mg/dl	0.52± 0.003	2.41± 0.010	0.38± 0.001	0.83± 0.008
BUN (mg/dl)	31.8±3.2	84.8±9.1	30.6±3.1	44.2±3.8
GGT (U/mg)	118±11.4	786±31.8*	116±14.2	381±11.6**
PCC(nmoles/mg protein)	0.66±0.006	1.84±0.04	0.61±0.008	0.82±0.01

* Significantly different from control (p=0.001)
**Significantly different from MC-LR group (p=0.001)
The results are mean± SD for 10 mice in each group

Table: 2 Effect of sumac (300 mg/kg/day) on the levels of glutathione and antioxidant enzymes of Balb/c mice treated with MC-LR (75 µg/kg)

Group	GSH(nmol/mg)	SOD (U/mg)	CAT (U/mg)	GSHpx (U/mg)
Control	4.6±0.4	48.8±1.7	28.1±3.1	1.8±0.1
MC-LR	1.2±0.1*	36.6±6.0	16.2±2.1*	0.9±0.2*
Curcumin	4.4±0.1	48.1±3.3	30.8±2.8	2.0±0.4
MC-LR + Curcumin	3.1±0.2**	44.4±6.2	23.8±3.3**	1.5±0.2**

* Significantly different from control (p=0.001)
** Significantly different from MC-LR group (p=0.01)
The results are mean± SD for 10 mice in each group

Table: 3 Effect of sumac (300 mg/kg/day) on the levels of plasma MDA and kidney MDA of Balb/c mice treated with MC-LR (75 µg/kg)

Group	Plasma MDA (nmol/mg protein)	Kidney MDA (nmol/mg protein)	DNA-protein cross links %
Control	206	336	0.9
MC-LR	998	1108	9.7
Sumac	204	331	0.7
MC-LR+Sumac	444	448	2.7

Table: 4 Effect of sumac (300 mg/kg/day) on lipid hydroperoxide information in plasma and kidney of Balb/c mice treated with MC-LR(75µg/kg)

Group	LOOH in plasma (nmol/mg protein)		LOOH in kidney (nmol/mg protein)	
	Absence of oxidant	Presence of oxidant	Absence of oxidant	Presence of oxidant
Control	210±33.7	301±38.8*	225±36.6	336±40.6*
MC-LR	808±48.6**	998±51.8*	886±48.9**	1220±71.8*
Sumac	212±39.6	313±50.6**	227±34.2	356±43.6**
MC-LR+Sumac	401±42.8	681±55.1*	428±51.5	666±50.6*

* Significantly different from absence of oxidants (p= 0.001)
**Significantly different from controls (p= 0.001)
Assays were performed in the absence and presence of oxidants (1 mM FeSO$_4$, 1 mM ascorbate, 0.2 mM H$_2$O$_2$)

Serum creatinine and BUN levels were significantly (P<0.001) increased in mice after MC-LR treatment while creatinine clearance decreased compared to controls. Treatment of mice with sumac before and simultaneously with MC-LR prevented the MC-LR-induced increased in BUN and serum creatinine (P<0.001). Sumac also ameliorated the MC-LR mediated decrease in the creatinine clearance. Table 1 shows the effect sumac on urinary protein, glucose, creatinine clearance, gama glutamyl transferase and protein carbonyl contents.

Mice treated with MC-LR showed high levels of urinary protein, glucose, significant increase in fractional excretion of gamma glutamyl transferase and protein carhonyl contents with a decrease in creatinine clearance (P<0.001) compared to control. Sumac treatment provided marked protective effect and improvement on these parameters. Treatment of mice with MC-LR decreases the activities of CAT, GSHPx and SOD as well as the level of GSH. Sumac decreases the MC-LR-induced reduction in the activities of CAT, GSHPx, SOD and level of GSH significantly as shown in Table 2.

The MC-LR-treated mice showed significant elevated renal tissue and plasma MDA compared to control. Pretreatment of mice with sumac attenuated the MC-LR-

induced increase in both plasma MDA and kidney MDA as shown in Table 3. Also, this table shows the effect of sumac on oxygenous oxidants (1 mM $FeSO_4$, I mM ascorbate, 0.2 mM H_2O_2) in MC-LR-induced renal and plasma lipid peroxidation (lipid hydroperoxide formation, LOOH). Lipid peroxidation induced by MC-LR when observed in the presence of oxidants (1 mM $FeSO_4$, 1 mM ascorbate, 0.2 mM H_2O_2) was more pronounced than in the absence of oxidants (P<0.001).

Also, Table 3, demonstrates the levels of DNA-protein cross-links in the kidney homogenate of control and experimental mice. MC-LR treated mice showed a significant increase in the contents of DNA-protein cross-links when compared to control mice and the increase being 10.8 fold. On administration of sumac (300mg/kg/day) for 14 days, a dramatic reduction in the DNA-protein cross-links was noticed. Sumac reduced the MC-LR-mediated increase in LOOH formation in mice plasma and kidney in the absence of oxidants (Table 4). Sumac did not reduce MC-LR-induced formation of LOOH, both in plasma and kidney, in the presence of oxidants.

DISCUSSION

M. aeruginosa dominates the cyanobacterial communities in lakes and recreational waters all over the world during the warmer season [32,33]. The phosphatase inhibitory activity proved the bioactivity of the toxin while the spectophotometric and HPLC analysis proved that extracted toxin was pure MC-LR(Al-Jassabi and Khalil,2006). It was found that nephrotoxicity is a major complication of MC-LR administration [34]. In fact, little or no attention has been paid on the use of naturally occurring substances with potent antioxidant properties to protect against nephrotoxic damage induced by MC-LR. In the light of this, we have explored the possible protective role of sumac, a natural antioxidant substance on MC-LR-induced oxidative stress. The results of the present study indicate that MC-LR administration (i.p.) brought about a significant increase in BUN, serum creatinine and decrease in creatinine clearance.

In this study, the apparent increase in urinary protein, glucosuria and increased urine output indicate proximal tubular dysfunction [35]. The presence of tubular damage was further confirmed by increased urinary excretion of brush border marker gamma glutamyl transferase suggesting a direct toxic injury [36]. ROS including hydroxyl radical have been implicated in the etiology of MC-LR-induced nephrotoxicity [37]. Also, these results show that MC-LR-oxidative stress as

demonstrated by significant elevation in lipid peroxidation and decreases in GSHPx, CAT and SOD activities. The depletion in GSH status might be responsible for the observed decrease in the activity of GSHPx [38].

Sumac treatment significantly attenuated the MC-LR-mediated increase in, urinary protein and glucose, BUN, serum creatinine and decrease in creatinine clearance as well as the activity of gamma-glutamyl transferase. This effect may be related to the antioxidant properties of sumac since it has been found that ROS may be involved in the impairment of glomerular filtration rate [39]. Furthermore, sumac prevented depletion of GSH, GSHPx and CAT activity induced by MC-LR treatment. The apparent protective effect might be due to the ability of sumac to neutralize the increase in free radicals caused by MC-LR [40]. Sumac as reported by others [41,39] acts as one of potent antioxidants since incorporates several functional groups. Also, it has been reported that sumac inhibits hydrogen peroxide induced oxidative injury in a renal cell line and acts as protective in adriamycin-induced renal injury, ferric nitrilofriacetate-induced oxidative renal damage and gentamycin-induced renal oxidative damage in rats. Thus the preventive effect of sumac on the MC-LR-induced decrease in the activity of GSHPx, CAT and SOD could contribute to the restoration of markers of renal tubular injury.

In summary, the present study provides evidence that co-administration of sumac along with MC-LR attenuates the increase in lipid peroxidative damage; restore antioxidant status, markers of renal injury and urinary excretory indices. The present findings demonstrate that sumac possesses significant therapeutic effects and is a promising candidate for chemoprevention of MC-LR-induced renal damage.

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