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EVALUATION OF AQUEOUS EXTRACT OF *Costus speciosus* (J.König)Sm. LEAF FOR HEPATIC AND RENAL TOXICITIES: BIOCHEMICAL AND HISTOPATHOLOGICAL PERSPECTIVES

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ABSTRACT

Costus speciosus is popular among Sri Lankans as an antidiabetic agent. Various parts of *Costus speciosus* plant are widely used in traditional medicine. Many of its pharmacological properties have scientifically proved. The objective of this study was to investigate the consequences of long-term use of *Costus speciosus* leaf aqueous extract (CSlwex) with respect to hepatic and renal functions of Wistar rats. Wistar rats (170-250 g) were divided into 9 groups (n=5 each) and labelled A to I. Groups A,B and C were kept as normal healthy rats. Insulin resistance was induced in six groups D to I. *Costus speciosus* leaf aqueous extract oral treatment was conducted for 12 weeks as given next. Group A and D- 1 mL of Distilled water, Group B and E- 1500 mg/kg CSlwex, Group F- 2000 mg/kg CSlwex, Group G- 2500 mg/kg CSlwex, Group C and H- 3000 mg/kg CSlwex, Group I- 20 mg/kg pioglitazone. After the therapy, serum ALT, AST and Creatinine were analyzed. Histopathology of liver and kidney were assessed for toxicity. No significant increase in ALT (34.77±6.19 IU/L, p=0.304) or AST (137.55±9.83 IU/L, p=0.928) were found in insulin resistant rats. Also, *Costus speciosus* leaf aqueous extract did not change ALT and AST significantly in healthy rats. Serum creatinine of either insulin resistant or healthy rats treated with CSlwex did not increase significantly compared to the respective controls (insulin resistant- 34.53±1.38 µmol/L; healthy- 42.56±3.27 µmol/L). No features of liver or renal toxicity were observed histopathologically

in CSlwex treated rats or controls. In conclusion, 1500-3000 mg/kg doses of *C. speciosus* leaf aqueous extract did not initiate hepatic or renal toxicity after 12 weeks continuous therapy.

KEYWORDS: *Costus speciosus,* aqueous extract, Wistar rats, renal toxicity, hepatic toxicity.

INTRODUCTION

The use of plants in treatment of illnesses was commenced with the human civilization. Several organized medical systems developed gradually in different parts of the world. Some Asian countries such as India, Sri Lanka and China have well established traditional medicine systems.[1] Their medicinal preparations are mainly derived from plants.[2] *Costus speciosus* (J.König) Sm *(C. speciosus)* is a such medicinal plant widely use in Ayurvedic medicine in Sri Lanka. In addition to the medicinal uses, *C. speciosus* leaves are consumed as a food in day to-day life. *C. speciosus* is frequently found in wet shady lands in Sri Lanka. It is known as 'Thebu' in Sinhala. *C. speciosus* contains many phytochemicals such as flavonoids, alkaloids terpenoids, steroids, saponins and phenolics.[3-5] As stated in Ayurvedic literature, the root of this plant is useful in the treatment of gastritis, leprosy, skin discolouring, asthma, bronchitis, other inflammatory conditions, anemia and diabetes mellitus.[4,6] Many of the pharmacological properties of *C. speciosus* rhizome and leaf have scientifically proved in animal models.

Different extracts of *C. speciosus* rhizome had been investigated in diabetes induced rats, for hypoglycaemic effect by Daisy et al.[7] They have shown that, the hexane crude extract of *C. speciosus* rhizome is effective in lowering blood glucose and HbA_{1c} levels in streptozotocin induced diabetic rats at a dose of 250 mg/kg. [7] Bavarva and Narasimhacharya (2008) revealed a dose dependant hypoglycaemic effect of *C. speciosus* rhizome ethanolic extract in alloxan induced diabetic rats.[8] Methanol and aqueous extracts of *C. speciosus* leaf are effective in reversing insulin resistance in Wistar rats.[9] Further, eremanthin and costunolide are two active principles isolated from hexane crude extract of *C. speciosus* rhizome which showed reductions in plasma glucose in streptozotocin induced diabetic rats.[6,10]

C. speciosus rhizome is effective in lowering lipid profile. Hexane extract of *C. speciosus* rhizome had elevated the high density lipoprotein cholesterol (HDL-C) while reducing the low density lipoprotein cholesterol (LDL-C) levels in diabetic rats.[7] Further, 450 mg/kg *C. speciosus* rhizome ethanolic extract being the best dose, showed a reduction in both plasma

and hepatic total lipids, as well as total cholesterol and triglycerides.[8] Also, the two compounds costunolide and eremanthin had individually elevated serum HDL-C and decreased total cholesterol, triglyceride and LDL-C in diabetic rats.[6,10]

Srivastava, et al. had proved the anti-inflammatory and analgesic effects of methanol crude extracts of *C. speciosus* aerial parts.[11] 800 mg/kg dose of this crude extract showed a significant anti-inflammatory action in experimental rats while 400 and 800 mg/kg doses had made potent analgesic effect.[11] There are evidence of diuretic activity of *C. speciosus* rhizome and its aqueous and alcoholic extracts had significantly increased the urine output in Wistar rats.[12] In addition, some research studies reveal *C. speciosus* plant's antioxidant potency.[4,8,13]

In novel research of drug discovery, toxicity studies of therapeutic agents are essential to establish pharmacodynamic parameters such as optimum dose, lethal dose and therapeutic window. Hepatic and renal impairments are potential outcome of medication toxicity. Liver is the main organ responsible for metabolism and physiological homeostasis of an animal. Administration of a drug even at minute doses may sometimes cause acute or chronic toxicity in the liver. Most of these substances in the blood are excreted via the kidneys. Hence, excretion of a drug or any chemical compound for long period of time can damage both structure and function of the kidney. Therefore, hepatic and renal toxicity studies are immensely useful to establish safe doses of therapeutic agents. Although many literature concentrated on therapeutic values of *C. speciosus* plant, very few have focused on toxicity effects of it. This study was planned to investigate the hepatic and renal toxicity effects of *C. speciosus* aqueous leaf extract (CSlwex) in long-term therapy.

MATERIALS AND METHODS

Preparation of aqueous extract of CS leaf

Fresh young leaves of *C. speciosus* were washed with distilled water and shade dried until obtaining a constant weight. Aqueous extract of the leaves was prepared by refluxing this dried material of *C. speciosus* fresh leaves (400 g) in distilled water (3 L) for 6 hours. Excess water was evaporated under reduced pressure. The concentrated crude extract was freeze dried until a constant weight was obtained. Crude aqueous extract was dissolved in distilled water to prepare 1500, 2000, 2500 and 3000 mg/kg concentrations of CSlwex.

Experimental rats

Male Wistar rats (170-250 g) were divided randomly into nine groups (n=5 each). They were acclimatized to animal house conditions for two weeks. Free access to water and pellet diet was allowed. Rat groups were labelled from A to I. Groups A, B and C were kept as normal healthy rats. Insulin resistance (IR) was induced in Groups D to I by feeding a modified high-fat-diet before starting the CSlwex intervention. IR was confirmed by three standard indirect indices; Homeostasis Model Assessment (HOMA) Quantitative Insulin Sensitivity Check Index (QUICKI) and McAuley index (McA). HOMA was calculated by Insulin (µIU/ mL) x Glucose (mmol/L) / 22.5 [14,15]; QUICKI by 1/(log insulin (µIU/mL) + log glucose (mg/dL))[15,16] and McA by exp[2.63-0.28ln(insulin(µIU/mL))-0.31ln(Triglyceride(mmol/L)] [15,17]. HOMA≥2.6, QUICKI≤0.33 and McA ≤5.8 were considered as Insulin resistant.[15,18,19]

Experimental procedure

Ethical approval for this study was obtained from the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka. After confirmation of IR in rats, they were treated with CSlwex as below. Group A and D- 1 mL of Distilled water, Group B and E- 1500 mg/kg CSlwex, Group F- 2000 mg/kg CSlwex, Group G- 2500 mg/kg CSlwex, Group C and H- 3000 mg/kg CSlwex, Group I- 20 mg/kg Pioglitazone. Rats were treated daily for 12 weeks by oral route. After 12 weeks therapy blood was drawn from the lateral tail vein of 12 hour fasting rats. Serum was separated and analysed for Alanine amino transferase (ALT) (BIOLABO, France), Aspartate amino transferase (AST) (BIOLABO, France) and Creatinine (BIOLABO, France) using laboratory test kits. Finally, liver and kidney were obtained from all the rats after euthanizing.

Histopathological observations for toxicity

Liver and kidney tissues of all the rats were fixed with 10% formalin solution. Sections of the tissues were processed and stained with haematoxylin and eosin. They were observed microscopically for toxic changes occurred due to CSlwex.

Statistical analysis

ALT, AST and creatinine of IR groups treated with CSlwex were compared with IR distilled water treated group using One-way ANOVA. Further, ALT, AST and creatinine of healthy groups treated with CSlwex were compared with healthy distilled water treated group using One-way ANOVA. In addition, ALT, AST and creatinine of healthy and IR rats treated with distilled water were compared using independent t-test.

RESULTS

Biochemical analysis

Table 1 shows the serum ALT, AST and creatinine of healthy rats and table 2 indicates those of IR rats after 12 weeks *C. speciosus* aqueous extract therapy.

Table 1 Serum ALT, AST and Creatinine of healthy rats

Rat group	Drug	ALT	AST	Creatinine
A	Healthy- Distilled water (control)	21.55 ± 3.52	92.43 ± 14.74	42.56 ± 3.27
B	Healthy- 1500 CSlwex	35.89 ± 3.98	121.78 ± 14.43	39.29 ± 4.72
C	Healthy- 3000 CSlwex	23.86 ± 0.01	119.60 ± 14.84	43.96 ± 5.15

This table illustrates ALT, AST and Creatinine levels in healthy rats treated with distilled water and different doses of CSlwex. These parameters were measured after 12 weeks of continuous therapy. ALT and AST are given in IU/l while creatinine is given in µmol/l. Data are presented as Mean± SE. Data were analysed by One-way ANOVA and $P<0.05$ was considered as statistically significant.

Table 2 Serum ALT, AST and Creatinine of IR rats

Rat group	Drug	ALT	AST	Creatinine
D	IR-Distilled water (control)	34.77 ± 6.19	137.55 ± 9.83	34.53 ± 1.38
E	IR-1500 CSlwex	40.16 ± 4.50	141.72 ± 17.36	36.03 ± 6.34
F	IR-2000 CSlwex	35.21 ± 4.54	142.08 ± 19.19	43.82 ± 4.14
G	IR- 2500 CSlwex	30.61 ± 2.16	141.64 ± 20.67	42.28 ± 4.44
H	IR- 3000 CSlwex	26.97 ± 2.61	138.78 ± 8.62	33.31 ± 6.40
I	IR- pioglitazone	30.19 ± 1.65	162.38 ± 14.85	42.28 ± 2.72

This table illustrates ALT, AST and Creatinine levels in insulin resistant rat groups treated with distilled water, different doses of CSlwex and pioglitazone. These parameters were measured after 12 weeks of continuous therapy. ALT and AST are given in IU/l while creatinine is given in µmol/l. Data are presented as Mean± SE. Data were analysed by One-way ANOVA and $P<0.05$ was considered as statistically significant.

ALT and AST

Mean ALT of both distilled water treated healthy and IR rats were not significantly different. Serum ALT levels of 1500 and 3000 mg/kg CSlwex treated healthy rats were not statistically different than distilled water treated healthy rats ($p=0.063$, table 1) after 12 weeks therapy. There were no significant differences in ALT of IR rat groups treated with CSlwex compared to IR controls ($p=0.304$, table 2). Mean AST of healthy and IR rats treated with distilled water were 92.43 ± 14.74 IU/l and 137.55 ± 9.83 IU/l respectively, after 12 weeks ($p=0.064$,

table 1and 2). There was no significant difference in serum AST of CSlwex treated IR rat groups compared to IR controls (p=0.928, table 1). Serum AST levels of CSlwex treated healthy rat groups were not statistically significant compared to distilled water treated healthy rats (p=0.374). This results emphasis that, consumption of *C. speciosus* leaf water extract continuously for 12 weeks did not create toxic changes in liver tissues.

Creatinine

Serum creatinine of distilled water treated healthy rats were not significantly different from that of distilled water treated IR rats after 12 weeks of therapy (p=0.083, table 2). In addition, There were no significant differences in serum creatinine of CSlwex treated IR rat groups compared to IR controls (p=0.419, table 1). Serum creatinine levels of CSlwex treated healthy rat groups were not significantly different compared to distilled water treated healthy rats (p=0.755, table 1). It reveals administration of *C. speciosus* leaf water extract continuously for 12 weeks did not initiate renal toxicity in rats.

Histopathological observations

Liver

Hepatic tissue of all nine rat groups were observed microscopically for toxicity after 12 weeks therapy. There were mild portal and lobular inflammation found in all groups, but they were within normal limits. Microvesicular or macrovesicular fatty changes were not seen in hepatic tissue of any group. No ballooning degeneration or feathery degeneration was observed. No hepatocyte necrosis was noted. There was no evidence of fibrosis nor features of cirrhosis in any rat group (Fig. 1). Therefore, hepatic tissues of all nine rat groups were normal and no toxicity features were observed microscopically.

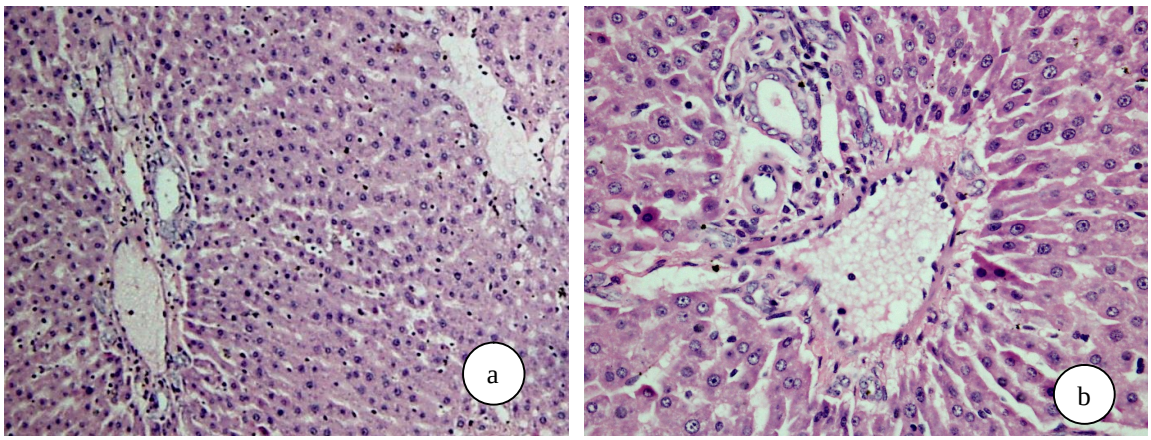

Fig. 1: Photomicrograph of a cross section of the liver of a normal healthy rat. a-magnification X10, b-magnification X40

Kidney

Renal tissues of all healthy and IR rat groups were observed microscopically for toxicity after 12 weeks therapy. In the longitudinal section of normal rat kidney tissue, renal cortex and medulla were clearly identified. Scattered glomeruli and renal tubules were noticed. The glomerulus consisted of a tuft of capillaries surrounded by glomerular basement membrane. The tubules consisted of proximal and distal convoluted tubules. Interstitium was not expanded by inflammation or fibrosis. Blood vessels were scattered within the interstitium. There was no evidence of glomerulonephritis, acute tubular necrosis nor interstitial inflammation. Similarly, there was no evidence of drug hypersensitivity nor vascular involvement (fig. 2). Therefore, renal tissues of all nine rat groups were normal and no toxicity features were observed microscopically.

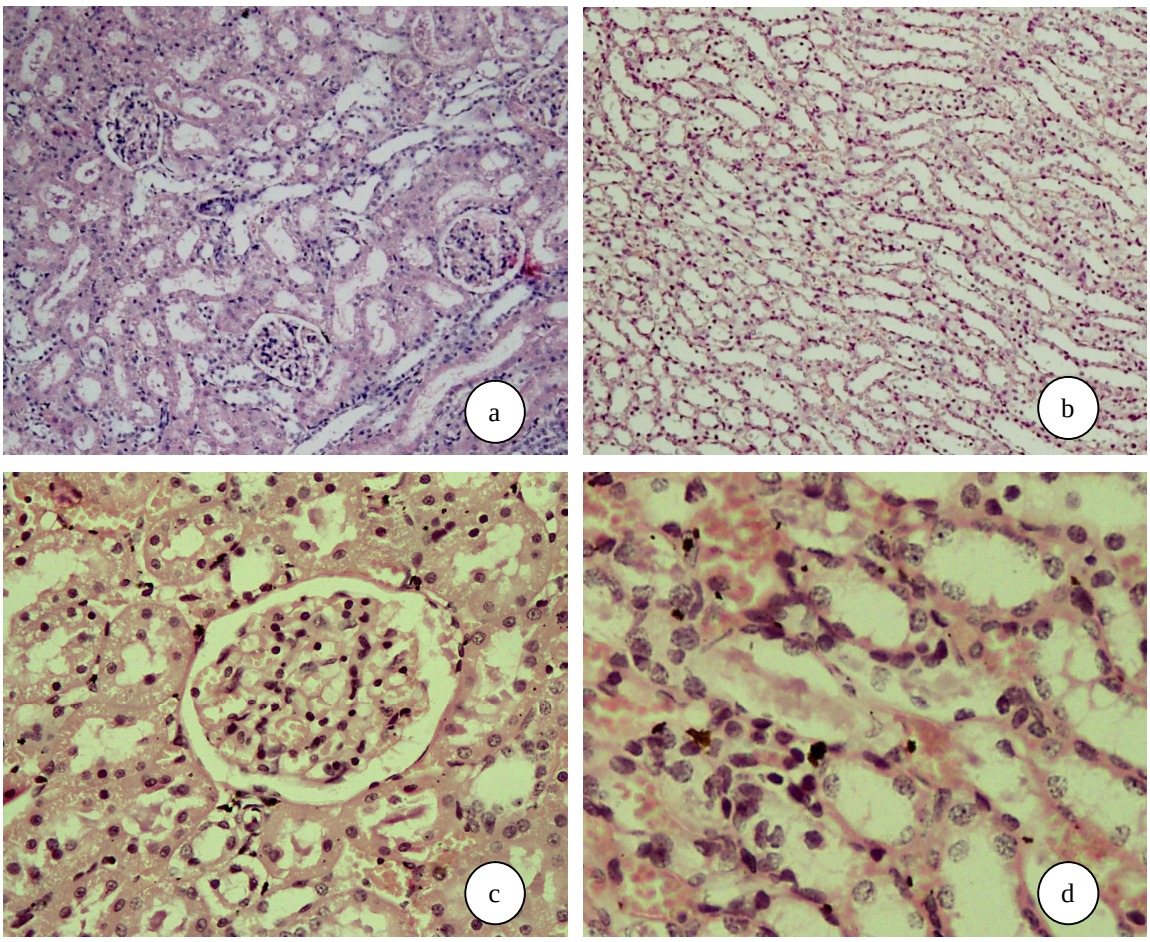

Fig. 2 Photomicrograph of a longitudinal section of the kidney of a normal healthy rat. a - renal cortex X10, b - renal medulla X10, c - glomeruli X40, d - renal tubules X40

DISCUSSION

Due to the range of known therapeutic values assembled with *C. speciosus* plant, many people are used to consume its leaves and rhizomes in their day-today lives with or without a specific disease condition. *C. speciosus* plant contains many phytochemicals such as flavonoids, alkaloids terpenoids, steroids, saponins and phenolics which are tested for pharmacological properties.[3-5] The overuse of plant materials or their extractions for a lengthy period can create harmful effects in the body. Liver is the main body organ responsible for metabolism. The chemical compounds we consume in the form of medicines, as well as their metabolites can damage the liver during the process of metabolism. Also, these compounds can potentially damage kidney tissues while excretion. Therefore, toxicity studies of a therapeutic compound are mandatory to establish the pharmacological properties of a drug whilst it is important to determine the therapeutic window. Although there are many studies available on medicinal values of *C. speciosus* plant, only few have focused towards its hepatic and renal influences. Hence, this study was designed to investigate the toxicity effects of CSlwex mainly on liver and kidney of normal healthy rats and insulin resistant rats. Serum levels of two liver enzymes ALT and AST were tested as the biochemical markers of liver function and serum creatinine was measured as the indicator of renal activity. In addition, histopathology of liver and kidney were observed microscopically for any toxic changes due to CSlwex therapy. Increased serum levels of hepatic transaminases such as ALT and AST reflect the liver dysfunction or liver damage.[20,21] Oral administration of 1500 to 3000 mg/kg CSlwex for twelve weeks did not show significant change in ALT or AST in IR rats. In addition, these doses did not alter ALT or AST level in healthy rats. It indicates the aqueous extract of *C. speciosus* leaf did not damage the hepatocytes enabling the secretion of ALT and AST to blood stream. In parallel to biochemical observations, no toxic changes in liver tissue were observed microscopically by any dose of CSlwex either in healthy or IR rats. There were no significant hepatic inflammation, fatty changes or hepatocyte necrosis observed. Hence it is understood that, even the higher doses of *C. speciosus* leaf aqueous extract (3000mg/kg) did not make hepatic toxicity at long-term consumption. Supporting these observations, some researchers have revealed about hepatoprotective effects of *C. speciosus* plant. The ethanolic extract of *C. speciosus* rhizome had been effective in preventing the carbon tetrachloride induced liver toxicity in Wistar rat model.[3] This extensive effect is due to the availability of certain steroidal saponins and glycosides that associates a hepatoprotective action.[3] These chemical compounds are more commonly found in aqueous extract of *C. speciosus* leaves too.[3,4] The two active chemical compounds;

costunolide and eremanthin isolated from *C. speciosus* rhizome are effective in reducing the elevated ALT and AST, in diabetic rats.[6,10] Moreover, the ethanolic extract of *C. speciosus* rhizome possesses a potent antioxidant property which would help to protect the liver from reactive oxygen species and reduce the oxidative stress in the hepatocytes.[4,8,13] There are no reported acute or chronic toxic effects of *C. speciosus* rhizome yet. Thus, *C. speciosus* can be identified as a source of hepatoprotective compounds. These evidence further justifies the use of *C. speciosus* against jaundice in traditional medicine systems.[3]

Creatinine is a metabolic product found in blood which also excretes in urine via kidneys. Assessing the serum creatinine level is considered as a screening method of renal function because it is simple and economical.[22-24] A reduction in glomerular filtration or tubular secretion could increase serum creatinine level. Ethanol and hexane extractions of *C. speciosus* rhizome reduced serum creatinine in diabetic rats and it may be due to improved renal function by the extracts.[7,8] Oral administration of 1500 - 3000 mg/kg CSlwex doses did not make significant changes in serum creatinine level in healthy or IR Wistar rats even after twelve weeks of therapy. It reflects that, aqueous extract of *C. speciosus* leaf did not change the physiological state of kidney, specially glomerular filtration and tubular secretion. Besides, microscopical view of renal tissue was not different than normal either in healthy or IR CSlwex treated rats. There were no signs of glomerulonephritis, acute tubular necrosis, interstitial inflammation or drug hypersensitivity. Therefore, aqueous extract of *C. speciosus* leaf, even at much higher doses such as 3000 mg/kg did not damage the renal tissues.

CONCLUSION

The present study reveals that, aqueous extract of *C. speciosus* leaf does not create toxic effects in liver or kidney of Wistar rats at the doses of 1500 to 3000 mg/kg. Further, the extract can be recommended for daily use upto 12 weeks in both normal and IR-induced rats. However, more extensive studies should be conducted to confirm these effects in humans.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article is reported.

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