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ORIGINAL ARTICLE

Cystone, a well-known herbal formulation improves renal function in rats with acute renal failure (ARF) induced by Glycerol intoxication

MOHAMED RAFIQ*, VISWANATHA GL, MOHAMMED AZEEMUDDIN M, SURYAKANTH DA, UDAY KUMAR VK, and PATKI PS

For author affiliations, see end of text.

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ABSTRACT

The present study was aimed to evaluate the beneficial effect of Cystone syrup in an experimental model of glycerol-induced acute renal failure (ARF) in rats. Biochemical parameters, kidney weight and histopathological evaluation were performed to conclude the beneficial effect of Cystone syrup. Administration of single dose of 50% v/v glycerol (8ml/kg.i.m) caused severe renal dysfunction associated with significant increase in markers of renal function such as serum urea (p<0.01), creatinine (p<0.01), blood urea nitrogen (BUN) (p<0.01), decrease in the Creatinine clearance (Ccr) (p<0.01) and increase in kidney weight to body weight ratio (p<0.01) compared to control group. These changes were in accordance with the histopathological findings showing severe tubular necrosis, degeneration and moderate luminal cast formation. In contrast, pre-treatment with Cystone (5 ml/kg, p.o) for seven days, alleviated the glycerol induced renal dysfunction significantly by maintaining serum urea (p<0.01), creatinine (p<0.05), BUN (p<0.01) and kidney weight to body weight ratio (p<0.05) near to normal range, also improved the creatinine clearance (p<0.05) compared to untreated positive control. In addition, histopathology of Cystone (5 ml/kg, p.o) treated group showed mild to moderate tubular necrosis and degeneration. Thus, the findings of the present study demonstrates the usefulness of Cystone syrup in reversing the biochemical/ structural markers of renal dysfunction observed in experimental model of renal failure in rats.

Keywords: *Acute renal failure, Cystone, Glycerol intoxication, Creatinine clearance*

Acute renal failure (ARF) is frequent in hospitalized critically ill patients and mortality associated with ARF is largely unchanged over many decades. ARF is mainly characterized by acute tubular necrosis. Progress in elucidation of ARF pathophysiology has led to the development and testing of many therapeutic drugs and other interventions in animal and human forms of acute tubular necrosis [1,2]. Renal replacement therapy has promising features in treating of ARF, especially before complications. However, it was reported that the incidence of ARF is been constantly rising over the past two decades [3,4] and mortality rate exceeded 50 % among those who required dialysis support [5,6]. Furthermore, mortality rates have changed little over the past few decades despite significant advances in supportive care. In addition, preventions of the occurrence and progression of ARF has become a very important issue. In recent years, great efforts have been focused on traditional and herbal medicine to provide a safe and therapeutically potential agent for ARF [4,6].

Glycerol is used for the induction of ARF *in-vivo*; intramuscular administration of hypertonic glycerol is the most commonly used animal model of myoglobinuric ARF [7]. It is reported that the acute volume depletion model of glycerol induced ARF is more closely related to the syndrome of ARF in human

beings, than the chronic dehydration model. Glycerol

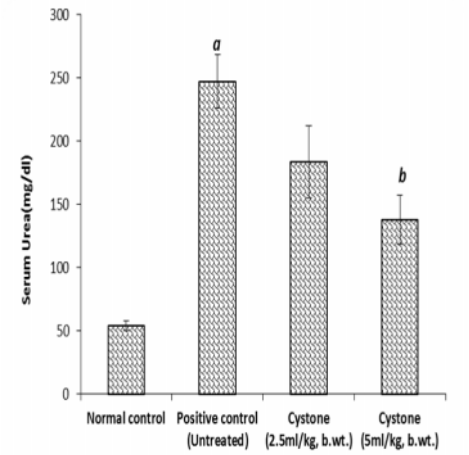

Fig 1. Effect of Cystone on serum urea levels in glycerol-induced ARF in rats. Values are expressed as mean±SEM, and compared by one way ANOVA followed by Tukey's multiple comparison. [a] $p<0.01$ compared to normal control, [b] $p<0.01$ compared to positive control.

Five millilitres of cystone syrup contains extracts of

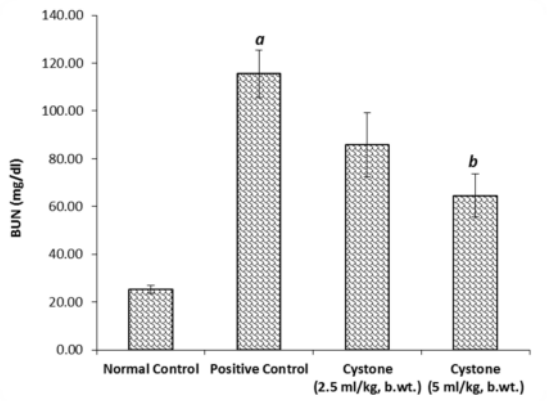

Fig 2. Effect of Cystone on BUN in glycerol-induced ARF in rats. Values are expressed as mean±SEM, and compared by one way ANOVA followed by Tukey's multiple comparison. [a] p<0.01 compared to normal control, [b] p<0.01 compared to positive control.

myoglobin ARF shows many hall marks of the crush syndrome, the archetypical form of human ARF [8,9].

Cystone, a well-known polyherbal formulation is based on ancient Ayurvedic system of medicine, and has been used for many years to treat urinary calculi and UTI. Previously it has been proved that, cystone is very effective in preventing the supersaturation of lithogenic substances and additionally it possesses antioxidant activity [10,11]. In this context, we investigated the beneficial effect of cystone on renal dysfunction in an experimental model of glycerol-induced ARF in rats.

MATERIALS AND METHODS

Animals

Inbred male wistar rats (225-250g) were used in this study. Animals were housed in standard isolation cages under environmental conditions of temperature (22 ± 2°C), relative humidity (60 ± 5%) and light (12 h light/dark cycles). Rats were allowed free access to water and standard laboratory rat chow (Provimi India, Bangalore) *ad libitum*. The protocol was approved by Institutional Animal Ethics Committee (IAEC) of The Himalaya Drug Company, Bangalore, and all the experiments on animals carried out as per the CPCSEA guidelines.

Drugs and Chemicals

Glycerol (Loba Chemie Pvt. Ltd., India), Cystone Syrup (The Himalaya Drug Company, Bangalore), all the biochemical kits were purchased from Erba Diagnostics, Mannheim, Germany. All the other chemicals and reagents were of analytical grade and purchased from HiMedia Laborateries Pvt Limited., India.

the following medicinal plants in definite proportions: Gokshura (*Tribulus terrestris*) 91 mg; punarnava (*Boerhaavia diffusa*) 67 mg; Pashanabheda (*Saxifraga ligulata*) 53 mg; Mustaka (*Cyperus rotundus*) 42 mg; Satavari (*Asparagus racemosus*) 21 mg; Kulattha (*Dolichos biflorus*) 21 mg; Ushira (*Vetiveria zizanioides*) 21 mg and Karchura (*Curcuma zedoaria*) 14 mg.

Experimental protocol

Forty male wistar rats were divided into four groups (G-I to G-IV, n=10). Rats from Group I and II received DM water (10ml/kg.p.o) and served as normal and positive untreated control respectively. Rats from Group III and IV received Cystone syrup at the dose of 2.5 and 5 ml/kg body weight / day, p.o. respectively. After one week of the assigned treatment, all the animals (G-II to G-IV) except G-I received a single intramuscular injection of glycerol (50% v/v) 8 ml/kg body weight, in divided dose to both the hind limbs; animals of G-I were injected with normal saline 8 ml/kg IM. 24-hours after the injection, urine samples were collected and after 48 hrs all the animals were bled to death under deep ether anaesthesia, blood and kidneys were collected for biochemical and histopathological examinations respectively.

Serum creatinine, urea were evaluated by Erba diagnostic kit. BUN and creatinine clearance were calculated using the equation given below.

$$BUN = \frac{serum\ urea\ (mg/dL)}{2.14}$$

$$\text{Creatinine clearance rate} = \frac{\text{Urine Creatinine (mg/l)X Urine output (ml)}}{\text{Serum Creatinine (mg/l)X 1440 (min)}}$$

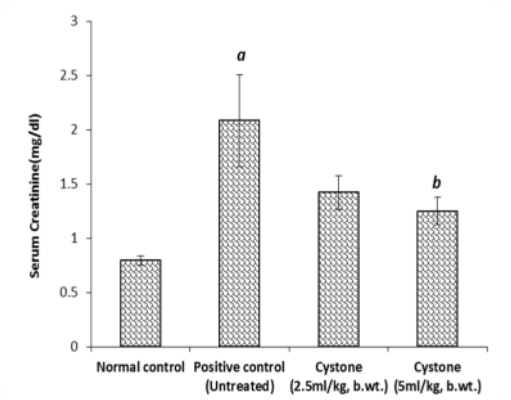

Fig 3. Effect of Cystone on serum creatinine levels in glycerol-induced ARF in rats. Values are expressed as mean±SEM, and compared by one way ANOVA followed by Tukey's multiple comparison.
[a] $p<0.01$ compare to normal control, [b] $p<0.05$ compare to positive control.

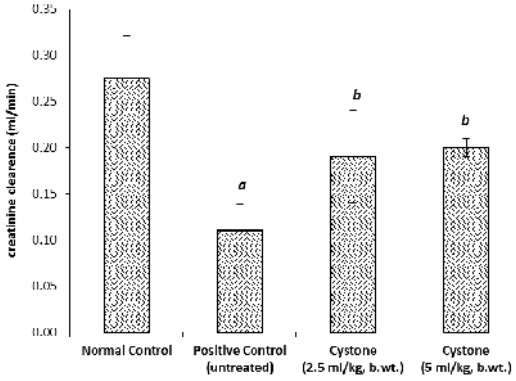

Fig 4. Effect of Cystone on creatinine clearance in glycerol-induced ARF in rats. Values are expressed as mean±SEM, and compared by one way ANOVA followed by Tukey's multiple comparison. [a]$p<0.01$ compared to normal control, [b]$p<0.05$ compared to positive control.

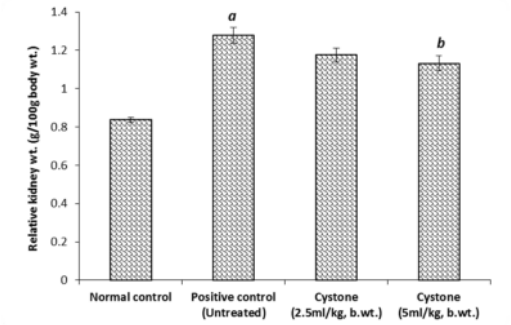

Fig 5. Effect of Cystone on kidney weight in glycerol-induced ARF in rats. Values are expressed as mean±SEM, and compared by one way ANOVA followed by Tukey's multiple comparison. [a] $p<0.01$ compare to normal control [b] $p<0.05$ compare to positive control.

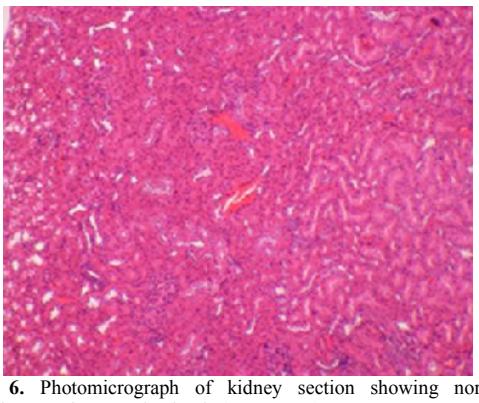

Fig 6. Photomicrograph of kidney section showing normal architecture in control animals (H&E x100)

Renal histopathology

The kidneys were isolated immediately after sacrificing the animal, washed with ice cold saline and fixed in 10% neutral buffered formalin solution and processed for histopathological evaluation.

Statistical analysis

The results were expressed as mean ± SEM and analyzed statistically by One Way ANOVA followed by Tukey's multiple comparison test using Graph pad Prism software package (Version 4.0). The minimum level of significance was fixed at $p<0.05$.

RESULTS

Effect of Cystone on glycerol-induced renal dysfunction

Intramuscular injection of 8 ml/kg of hypertonic glycerol produced a marked derangement in the renal function and lead to a significant increase in the level of serum urea, creatinine, BUN and a severe fall in the clearance values of creatinine. Also, there was significant increase in kidney to body weight ratio. Pre-treatment with Cystone (5 ml/kg, p.o.) produced significant improvement in the renal functions by maintaining the all biochemical parameters and kidney to body weight ratio near to control group (Fig. 1-5).

Effect of Cystone on glycerol-induced changes on renal morphology

The renal morphology of control group animals was found to be normal (Fig. 6). In contrast, the kidneys of rats treated with glycerol showed marked histological changes in the cortex and outer medulla. The renal sections showed severe tubular necrosis, degeneration and moderate luminal cast formation (Fig.7). Treatment with Cystone (2.5 ml/kg, p.o) did not show any significant morphological protection. However, Cystone

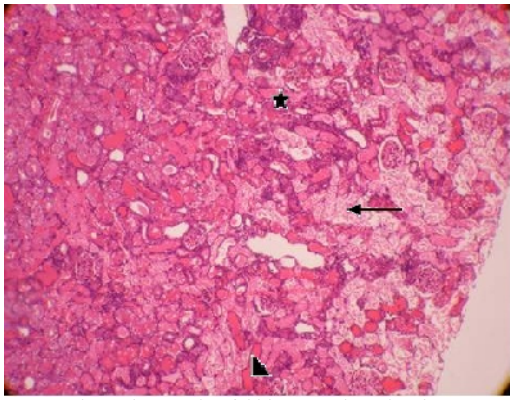

Fig 7. Photomicrograph of kidney section showing severe tubular necrosis (arrow) and degeneration (asterisk) and moderate luminal cast formation (arrow head) in positive control animals (H&E ×100)

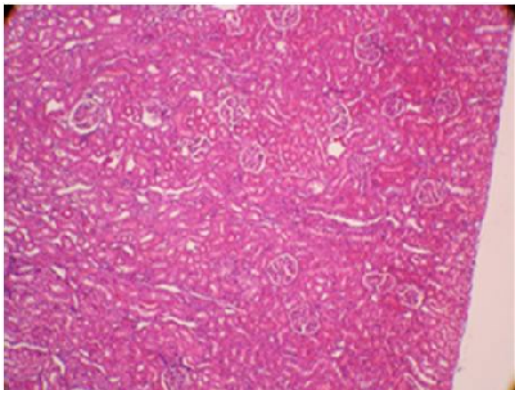

Fig 8. Photomicrograph of kidney section showing mild to moderate tubular necrosis and degeneration in animals treated with Cystone (H&E ×100).

(5 ml/kg, p.o) treated group showed very mild tubular necrosis and degeneration (Fig.8) and hence found to promising in preventing the glycerol-induced renal damage.

DISCUSSION

ARF is characterized by rapid decline in glomerular filtration rate and retention of nitrogenous waste products. Skeletal muscle accounts to about 40% of the body weight, and when massive necrosis occurs following injury it is termed as rhabdomyolysis [12,13]. Number of studies have shown that rhabdomyolysis - induced myoglobinuric ARF accounts for about 10-40 % of all cases of ARF [14].

The intramuscular administration of hypertonic glycerol induces myolysis and hemolysis and affords a faithful and widely utilized model of heme protein-induced renal injury [15,16].

Myoglobinuric ARF has three pathogenic mechanisms: tubular obstruction, renal vasoconstriction and oxidative stress [17]. The latter is generated through the iron released from the heme group of the myoglobin. Iron induces the formation of high-activity oxygen free radicals that increase oxidative stress and provoke lipid peroxidation and cellular death [18,19].

In the present study, 48 h after glycerol administration the levels of serum urea, creatinine and BUN were significantly increased. Our findings are in consistent with previous reports [14]. These results indicated that renal function was severely impaired in ARF rats. However, pre-treatment with Cystone prevented the elevation of serum urea, creatinine and BUN. Furthermore, pre-treatment with Cystone prevented the renal morphological changes caused due to administration of glycerol. These results suggested that Cystone plays an important role as a renoprotective agent against ARF. It was reported that Cystone could alleviate oxidative stress by scavenging free radicals and reducing lipid peroxidation by enhancing antioxidant defence mechanisms [11,20].

In conclusion, Cystone, a polyherbal preparation used clinically for many years for urinary complications, has been shown to provide partial but significant protection against renal damage - induced by the glycerol intoxication. The anti-oxidant property of cystone could be one of the mechanisms behind the beneficial effect observed in the present study.

CONFLICTS OF INTEREST

Author declares that there are no conflicts of interest.

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REFERENCES

1. Kellum JA. What can be done about acute renal failure? Minerva Anestesiol 2004; 70: 181-188.
2. Lameire NH, De Vriese AS, Vanholder R. Prevention and non-dialytic treatment of acute renal failure. *Curr Opin Crit Care* 2003; 9:481-90.
3. Hou SH, Bushinsky DA, Wish JB, Cohen JJ, Harrinigton JT. Hospital-acquired renal insufficiency: a prospective study. *Am J Med* 1983; 74:243-8.
4. Nash K, Hafeez A, Hou S. Hospital-acquired renal insufficiency. *Am J Kidney Dis* 2002; 39:930-6.
5. Metnitz PG, Krenn CG, Steltzer H, Lang T, Ploder J, Lenz K, Le Gall JR, Druml W. Effect of acute renal failure requiring renal replacement therapy on outcome in critically ill patients. *Crit Care Med* 2002; 30:2051-8.
6. Mehta RL, Pascual MT, Soroko S, Savage BR, Himmelfarb J, Ikizler TA, Paganini EP, Chertow GM. Spectrum of acute renal failure in the intensive care unit: the PICARD experience. *Kidney Int* 2004; 66:1613-21.
7. Zurovsky Y. Models of glycerol-induced acute renal failure in rats. *J Basic Clin Physiol Pharmacol* 1993; 4:213-28.

8. Allen L, Donald E. Glomerular hemodynamics in established glycerol-induced acute renal failure in the rat. *J Clin Invest* 1989; 84:1967-73.
9. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. *Method Enzymol* 1990; 186:1-85.
10. Rao M, Praveen Rao PN, Kamath R. Reduction of cisplatin-induced nephrotoxicity by cystone, a polyherbal ayurvedic preparation, in C57BL/6J mice bearing B16F1 melanoma without reducing its antitumor activity. *J Ethanopharmacol* 1999; 681: 77–81.
11. Rao M, Rao MN. Protective effects of cystone, a polyherbal preparation on cisplatin induced renal toxicity in rats. *J Ethanopharmacol* 1998; 62:1-6.
12. Zhang HA, Wang M, Zhou J, Yao QY, Ma JM, Jiang CL. Protective Effect of Ginsenoside against Acute Renal Failure and Expression of Tyrosine Hydroxylase in the Locus Coeruleus. *Physiol Res* 2010; 59: 61-70.
13. Devinder S, Vikas C, Kanwaljit C. Protective effect of naringin, a bioflavonoid on glycerol-induced acute renal failure in rat kidney. *Toxicol* 2004; 201:143–51.
14. Chander V, Chopra K. Molsidomine, a nitric oxide donor and L-arginine protects against rhabdomyolysis induced myoglobinuric acute renal failure. *Biochim Biophys Acta* 2005; 1723: 208-14.
15. Zager RA. Rhabdomyolysis and myohemoglobinuric acute renal failure. *Kidney Int* 1996; 49:314–26.
16. Dubrow A, Flamenbaum W. Acute Renal Failure. In: Brenner, B.M., Lazarus, J.M. (Eds.), Churchill Livingstone, New York, US. 1988.
17. Polo RFJ, Fernández FA, Broseta VL, Atienza MP, Sánchez GF. Effect of N-acetylcysteine on antioxidant status in glycerol-induced acute renal failure in rats. *Renal Fail* 2004; 26:613-8.
18. Vlahović P, Cvetković T, Savić V, Stefanović V. Dietary curcumin does not protect kidney in glycerol-induced acute renal failure. *Food Chem Toxicol* 2007; 45:1777-82.
19. Guidet B, Shah SV. Enhanced *in vivo* H_2O_2 generation by rat kidney in glycerol-induced renal failure. *Am J Physiol* 1989; 257: F440-F445.
20. Satyakumar V, Puttanarasaiah M, Pralhad SP. Cystone – An ayurvedic polyherbal formulation inhibits adherence of uropathogenic *E. coli* and modulates H_2O_2-induced toxicity in NRK-52E cells. *J Exp Pharmacol* 2010; 2v:19–27.

CURRENT AUTHOR ADDRESSES

Mohamed Rafiq*, Department of Pharmacology, R&D Center, The Himalaya Drug Company, Makali, Bangalore-562123, Karnataka, India. Email: dr.rafiq@himalayahealthcare.com

Viswanatha GL, Department of Pharmacology, R&D Center, The Himalaya Drug Company, Makali, Bangalore-562123, Karnataka, India.

Mohammed Azeemuddin M, Department of Pharmacology, R&D Center, The Himalaya Drug Company, Makali, Bangalore-562123, Karnataka, India.

Suryakanth DA, Department of Pharmacology, R&D Center, The Himalaya Drug Company, Makali, Bangalore-562123, Karnataka, India.

Uday Kumar VK, Department of Pharmacology, R&D Center, The Himalaya Drug Company, Makali, Bangalore-562123, Karnataka, India.

Patki PS, Department of Pharmacology, R&D Center, The Himalaya Drug Company, Makali, Bangalore-562123, Karnataka, India.