

Original Research Article

Aqueous root extract of *Dicoma anomala* Sond ameliorates isoproterenol-induced myocardial infarction in Wistar rats

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Abstract

Purpose: To evaluate the protective potentials of the aqueous root extract of *Dicoma anomala* (AQRED) against isoproterenol (ISP)-induced myocardial damage in Wistar rats.

Methods: Myocardial damage was induced in Wistar rats by isoproterenol (60 mg/kg body weight, b.w.) Various concentrations (125, 250, and 500 mg/kg b.w.) of AQRED and their effects on the rats' feed and water intake, body weight changes, serum enzymes, including aspartate transaminase (AST), alanine transaminase (ALT) creatinine phosphokinase (CPK), as well as tissue antioxidant enzymes, including catalase (CAT), glutathione peroxidase (GP) and lipid peroxidation, during a 30-day experimental period were examined.

Results: ISP-treated rats showed no significant ($p > 0.05$) effect on the feed, water and body weight but increased significantly ($p < 0.05$) AST, ALT, CPK and lipid peroxidation while significantly reducing CAT and GP levels ($p < 0.05$). Treatment with different doses of AQRED significantly ($p < 0.05$) reversed the activity of these enzymes and cardiac lipid peroxidation towards control levels. Histopathological examination of ISP-induced myocardial rats treated with *D. anomala* revealed evidence of oedema and myocardial necrosis at 125 and 250 mg/kg b.w. doses, but these alterations were ameliorated or cleared at 500 mg/kg dose, suggesting attainment of maximum efficacy.

Conclusion: The findings indicate the ameliorative potential of AQRED in myocardial disease, and therefore, could be of therapeutic significance in the management or treatment of cardiac-related diseases.

Keywords: Antioxidant enzymes, *Dicoma anomala*, Lipid peroxidation, Serum enzymes

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INTRODUCTION

Myocardial infarction (MI), the most dreaded among the various forms of ischemic heart disease is the leading cause of morbidity and mortality in developed countries [1]. It is a condition of acute necrosis of the myocardium arising from the imbalance between the coronary blood supply and myocardial demand [2]. This is mostly followed by numerous pathophysiological and biochemical changes such as lipid peroxidation, thrombosis and hyperlipidemia [3],

leading to myocardium alteration. MI preceding the development of heart failure is reported to be due to antioxidant deficit and increased myocardial oxidative stress. Hence, prompt intervention with an antioxidant may be of benefit against cardiac damage [4]. In recent times, due to adverse effects from synthetic drugs, interest in conventional medicines of natural sources have continued to increase particularly in developed and developing countries such as China, United States of America. [1,5]. The use of herbal therapies against numerous diseases

including MI has been encouraged with few people doubting the effectiveness of the treatment modality [6].

Isoproterenol (ISP) is a synthetic catecholamine and a β -adrenergic agonist that causes severe stress in the myocardium, leading to an 'infarct-like' necrosis of the heart muscles [3]. ISP forms free radicals and stimulates lipid peroxidation which perhaps cause irreversible damage to the myocardial membrane [6]. Thus, a high production of reactive oxygen species (ROS) may be a unifying mechanism in ischemic injury progression and antioxidants administration may protect against ISP induced cardiac damage. ISP induced myocardial injury is a well-established model used to study the beneficial effect of arrays of drugs on cardiac dysfunction [7,8]. This is because the pathophysiological signs in animals after ISP administration are similar to myofibrillar degeneration, a typical symptom of MI in humans [9].

South Africa is noted for her rich biodiversity in indigenous plants and she account for 9 % of the higher plants globally [10]. There are has been a continued interest in the development of therapeutic efficacies of medicinal plants in the treatment of numerous ailments. Besides being readily available, affordable and with minimal adverse effect, the presence of flavonoids, vitamins and polyphenols in these plants gave credence to their therapeutic significance and is well buttressed by the WHO [11].

Dicoma anomala (Asteraceae) commonly called fever or stomach bush (English) or hloenya (South Sotho) is a prostrate, decumbent or erect perennial herb with underground tuber. *D. anomala* is widely distributed in sub-Sahara Africa including South Africa within North West, Gauteng, Mpumalanga, Free State, Northern Cape and KwaZulu- Natal Provinces [12]. Traditionally, the plant is indicated in the treatment of various diseases such as coughs, cold, fever, ulcers, dermatosis and venereal ailments linked to numerous pharmacological activities including antiplasmodial, antibacterial, anthelmintic, antiviral and anti-inflammatory [13]. Previous studies on the plant revealed the antibacterial, anti-inflammatory and antioxidant activity of the root extracts [13] but little or no information has been reported on its cardioprotective or ameliorative efficacies. In this paper, we present the report on the ameliorative potential of aqueous root extract of *D. anomala* in ISP -induced myocardial infarcted rats.

EXPERIMENTAL

Chemicals

Isoproterenol hydrochloride (ISP), assay kits such as alanine transaminase (ALT), aspartate transaminase (AST), creatine phosphokinase (CPK) were purchased from Sigma-Aldrich (South Africa). While water used was glass-distilled, other chemicals and reagents used were of analytical grades.

Plant collection and extraction

Fresh rootstocks of *D. anomala* were collected from the wild within Phuthaditjhaba area of QwaQwa, Maluti- A - Phofung municipality of the Free State Province, South Africa in April 2014. The identity of this taxon was confirmed by Dr. AOT Ashafa of the Department of Plant Sciences, UFS, QwaQwa campus and the voucher specimen was deposited in the herbarium of University of Free State, South Africa. A total of 5.2 kg of fresh rootstock was washed, oven dried (40 °C) and ground with a hammer mill to yield 3.063 kg fine powdered plant material. Of this, 200 g was extracted with 2 litres distilled water, filtered and concentrated on a water bath to yield 48.87 g of brown gummy (24.435 % w/w of dry plant material) crude extract.

Experimental animals

Male and female Wistar rats (weighing 150 - 200 g) were used in this study. They were procured from the animal facility of the University of the Free State, Bloemfontein. The animals were acclimatized for one week and maintained at (18 \pm 2 °C under 12 h dark/light cycle). They were fed with standard rat feed (Epol mice cubes, Westville, South Africa) and water *ad libitum*. The husks in the cages were renewed thrice weekly to ensure proper hygiene and maximum comfort for animals. Ethical clearance for the animal studies was obtained from Institutional Interfaculty Animal Ethics Committee of the University of the Free State (no NR 02/13), prior to the commencement of the study in line with the internationally accepted guidelines of National Research Council for animal care and use [14].

Experimental design

The animals were randomly divided into six groups of 6 rats each. Group A animals served as normal control and were orally administered 1 mL normal saline for 30 days. The rats in Groups B, C, D, E and F were respectively treated with 1

mL normal saline, simvastatin (30 mg/kg b.w.) and 125, 250 and 500 mg/kg b.w. aqueous extract of *D. anomala* for 30 days via oral intubation. The rats in all the groups except group A were given ISP (60 mg/kg b.w.) [15], intraperitoneally on the first day of the experiment prior to administration of test drugs and the extracts.

At the end of the experimental period, all the rats were anaesthetized with halothane and blood was collected from the retro-orbital plexus. About 5 mL of the blood collected into a non-heparinized bottle was centrifuged at 1285 g for 10 min and the resulting serum was used for marker enzymes determination. The heart tissue was excised immediately and divided into two portions; a portion was immediately fixed in 10 % formalin for histopathological studies while the other portion was homogenized in ice-cold 0.1 mol/L Tris-HCl buffer (pH 7.2). The supernatant obtained was used for the antioxidant assays as well as tissue activities of catalase (CAT), glutathione peroxidase (GP) and the level of lipid peroxidation in terms of thiobarbituric acid reactive species (TBARS) was estimated. The daily measurement of food intake and water consumption was recorded.

Assessment of biochemical parameters

The activities of ALT, AST, and CPK in the serum were determined following the procedures described in the Sigma-Aldrich assay kits.

Determination of antioxidant parameters

Glutathione peroxidase activity in tissues was assayed according to the method of Nicholas [16] while catalase activity determined based on the adapted method of Aebi [17]. Lipid peroxidation assayed by the formation of thiobarbituric acid reactive substances according to the method of Niehaus and Samuelson [18].

Histopathological studies

A portion of the heart tissue from each group was fixed immediately in 10 % neutral formalin for a period of at least 24 h, dehydrated in graded (50 – 100 %) alcohol, embedded in liquid paraffin, cut into 4-5 μ m thick sections and stained with hematoxylin-eosin. The sections were evaluated and photographed for the pathological/rejuvenative changes in the myocardial tissue under a canon power shot S72 digital camera (x 200) attached to a light microscope (Amscope, model B409A).

Statistical analysis

Data analysis were done by one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test and results are expressed as mean \pm SEM using Graph Pad Prism version 3.0 for Windows software, Graph Pad software, San Diego, California, USA. Statistical significance was considered at ($p < 0.05$).

RESULTS

Effect of AQRED on feed and water intake

The effects of oral administration of AQRED on feed and water intake is presented in Table 1. ISP had no effect on feed and water intake of the experimental rats when compared with control. A significant ($p < 0.05$) increase in average feed and water intake of the cardiotoxic group with a similar increase in food and water consumption in all the treatment groups on days 10, 20 and 30 was observed.

Effect of AQRED on body weight changes

Table 2 presents the effect of AQRED on average body weight changes of the experimental rats. As observed from the results, administration of ISP had no significant ($p > 0.05$) effect on the weight of the animals treated with different doses of AQRED and simvastatin when compared with control group.

Effect of AQRED on serum enzymes

The effect of AQRED on serum enzymes of ISP-induced cardiac damage is presented in Table 3. ISP showed no effect on the activity of ALT when compared with control. However, the ISP - mediated increased serum activities of AST, ALT, and CPK were significantly ($p < 0.05$) and dose-dependently attenuated following treatments with simvastatin and the extracts although, simvastatin tends to restore the activities of these enzymes better towards normal than the extracts except in AST

Effect of AQRED on tissue antioxidant status

Table 4 shows the effects of AQRED on tissue marker enzymes. ISP significantly ($p < 0.05$) decreased the activities of the antioxidant marker enzymes (catalase and glutathione peroxidase) while It increased the level of TBARS when compared with control. Simvastatin and the three concentrations of the plant extract significantly ($p < 0.05$) increased the activities of the enzymes

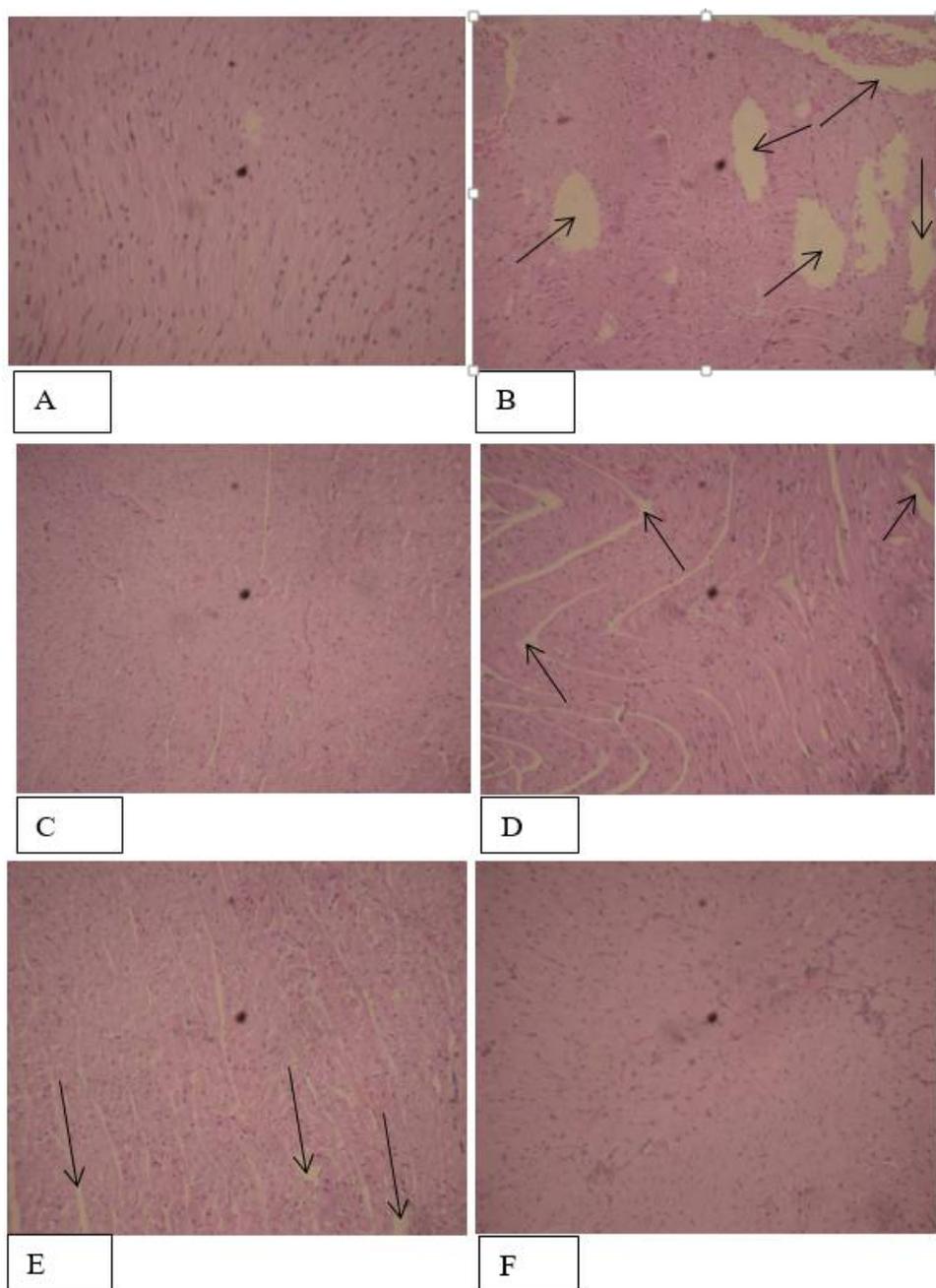


Figure 1: Photomicrograph showing: (A) normal heart architecture in control group (H&E 150x); (B) extensive tissue granulation, myocardial necrosis and coronary congestion (H&E 150x); (C) normal cardiomyocytes with mild oedema (H&E 150x); (D) myocardial necrosis and severe oedema (H&E 150x); (E) myofibres disintegration and oedema (H&E 150 x); (F) normal cardiomyocytes (H&E 150x). Figures are representative of four independent experiments

Table 1: Effect of AQRED on feed and water intake of ISP -induced myocardial infarcted rats

Parameters	Control	Isoproterenol	Simvastatin	125 mg/kg	250 mg/kg	500 mg/kg
AFI (g) Day 1- 10	148.90 ± 3.36 ^a	150.90 ± 9.61 ^a	137.94 ± 8.48 ^a	113.70 ± 7.07 ^a	122.34 ± 8.92 ^a	116.78 ± 7.95 ^a
AFI (g) Day 11-20	148.50 ± 1.62 ^a	167.02 ± 8.54 ^b	178.06 ± 5.71 ^b	137.72 ± 5.60 ^b	157.32 ± 5.15 ^b	147.06 ± 3.99 ^b
AFI (g) Day 21- 30	148.14 ± 1.98 ^a	181.84 ± 5.13 ^c	169.40 ± 4.93 ^c	154.56 ± 1.91 ^c	160.50 ± 3.53 ^b	159.92 ± 1.56 ^c
AWI (ml) Day 1 -10	166.26 ± 4.45 ^a	140.76 ± 5.89 ^a	155.00 ± 5.55 ^a	138.50 ± 11.54 ^a	148.50 ± 9.01 ^a	146.50 ± 8.25 ^a
AWI (ml) Day 11-20	171.20 ± 4.97 ^b	146.00 ± 6.45 ^b	164.80 ± 3.73 ^b	162.20 ± 11.32 ^b	169.00 ± 7.03 ^b	168.00 ± 7.36 ^b
AWI (ml) Day 21- 30	179.20 ± 5.97 ^c	167.80 ± 4.54 ^c	171.80 ± 1.60 ^c	173.60 ± 5.45 ^c	189.40 ± 4.32 ^c	194.80 ± 8.43 ^c

Values with different superscript along the same column for each parameter are significantly ($p < 0.05$) to each other **Key:** AFI: Average feed intake; AWI: Average water intake

Table 2: Effect of AQRED on body weight of ISP -induced myocardial infarcted rats

Parameters	Control	Isoproterenol	Simvastatin	125 mg/kg	250 mg/kg	500 mg/kg
Initial weight (g)	141.80 ± 10.55 ^a	141.90 ± 5.94 ^a	142.60 ± 11.52 ^a	140.80 ± 9.52 ^a	144.10 ± 9.53 ^a	139.70 ± 6.64 ^a
ABW (g) Day 1– 10	172.80 ± 12.84 ^a	168.70 ± 8.51 ^a	169.20 ± 14.92 ^a	164.00 ± 12.63 ^a	162.30 ± 8.37 ^a	165.90 ± 10.82 ^a
ABW (g) Day 11 – 20	185.70 ± 14.64 ^a	179.20 ± 12.57 ^a	180.10 ± 16.10 ^a	170.30 ± 8.60 ^b	174.30 ± 10.34 ^a	175.40 ± 12.89 ^b
ABW (g) Day 21 – 30	200.80 ± 16.50 ^a	194.10 ± 15.90 ^a	194.10 ± 18.67 ^a	187.80 ± 13.59 ^b	192.70 ± 12.76 ^a	192.40 ± 15.69 ^a

Values with different superscript along the same row for each parameter are significantly different ($p < 0.05$) to each other; **Key:** ABW: Average body weight

Table 3: Effect of AQRED on cardiac marker enzymes of ISP -induced myocardial infarcted rats

Parameters	Control	Isoproterenol	Simvastatin	125 mg/kg	250 mg/kg	500 mg/kg
ALT IU/L	12.96 ± 2.40 ^a	13.49 ± 0.37 ^a	7.15 ± 3.30 ^b	9.91 ± 0.67 ^b	8.87 ± 0.90 ^b	7.22 ± 2.24 ^b
AST IU/L	211 ± 1.16 ^a	290 ± 1.15 ^b	207 ± 0.58 ^a	193 ± 1.16 ^c	185 ± 0.57 ^c	178 ± 0.58 ^d
CPK IU/L	76.07 ± 0.88 ^a	224.60 ± 12.77 ^b	83.57 ± 21.48 ^a	181.00 ± 0.60 ^c	178.20 ± 9.30 ^c	140.20 ± 8.56 ^d

Values are presented as mean ± SEM (n = 6). *Values with different superscript along the same row for each parameter are significantly different ($p < 0.05$); ALT: Alanine transaminase; AST: Aspartate transaminase; CPK: Creatine phosphokinase

Table 4: Effect of AQRED on tissue marker enzymes of ISP-induced myocardial infarcted rats

Parameters	Control	Isoproterenol	Simvastatin	125 mg/kg	250 mg/kg	500 mg/kg
Catalase (U/mg protein)	66.20 ± 0.27 ^a	7.20 ± 0.56 ^b	57.28 ± 2.44 ^a	20.97 ± 0.56 ^c	21.75 ± 4.08 ^c	38.97 ± 2.71 ^d
Peroxidase (U/mg protein)	71.01 ± 0.10 ^a	27.25 ± 0.13 ^b	54.83 ± 0.26 ^c	35.08 ± 0.13 ^b	36.39 ± 0.11 ^b	58.51 ± 1.25 ^c
TBARS (mM/ 100 mg tissue)	90.34 ± 1.93 ^a	201.40 ± 0.84 ^b	113.50 ± 1.74 ^c	104.80 ± 0.97 ^c	110.10 ± 2.21 ^c	116.90 ± 0.97 ^d

Values are presented as mean ± SEM (n = 6). *Values with different superscript along the same row for each parameter are significantly different ($p < 0.05$). TBARS: Thiobarbituric acid reactive substances

while they reduced TBARS level in a dose-related manner by bringing towards normal the cardiac damage done to the rats.

Effect of AQRED on histopathological alterations

The result of histopathological examination of the heart sections from control and cardiotoxic rats is shown in Figure 1. Control rats showed normal cardiomyocytes with no visible lesions (Figure 1A). The ISP-treated rats revealed necrotic cardiomyocytes, marked granulation of tissue characterized by collagenous tissue deposition and presence of coronary congestion (Figure 1B). Simvastatin-treated rats exhibited normal cardiomyocytes with mild oedema (Fig 1C). Treatment with 125 mg/kg AQRED shows multiple areas of myofibres disintegration, presence of necrotic myocytes and severe oedema (Figure 1D). 250 mg/kg AQRED revealed myofibres disintegration and severe oedema (Figure 1E). However, at 500 mg/kg

dose, the heart appeared to be normal (Figure 1F).

DISCUSSION

Destruction of myocardial cells arising from myocardial ischemia results in ruptured or damaged cardiac membrane thus, leading to leakage of heart enzymes [19]. MI occurs when there is an insufficient oxygen supply or glucose to the heart resulting in necrosis of the myocardium. CPK and LDH are marker enzymes diagnostically used to measure the level of cardiac damage and the release of these enzymes from the myocardial tissues of the heart into the bloodstream is an indication of myocardial damage induced by ISP. This does not only increase their concentration in the blood [20] but causes increase in heart rate, abnormal electrocardiogram (ECG) pattern [21,22] due to the formation of reactive oxygen species which ultimately leads to tissue degenerative changes resulting in the necrosis of the myocardium. The pathogenesis of MI has not been fully elucidated but insight into studies involving ISP-induced

cardiotoxicity suggests oxidative stress involvement. Moreover, increase in the serum activities of AST and ALT might also be attributed to myocardial injury [19].

The results of our study revealed increased activities of AST, ALT, and CPK following intraperitoneal administration of ISP, this affirms the preliminary prevalence of myocardial necrosis [23] and the leakage of marker enzymes from the heart to the blood. The treatment with different doses of AQRED reduced the level of these enzymes compared to control; this suggests the protective action of the plant in ameliorating cardiac damage arising from ISP. This result corroborates the previous report of Suchalatha and Shyamala-Devi [3] for *Arogh* as well as Sunmonu and Afolayan [24] for *A. afro*.

Free radical formation and accumulation of lipid peroxides have been established as one of the probable biochemical mechanisms leading to myocardial damage from catecholamine such as ISP [25]. Catecholamine undergoes rapid oxidation and the oxidation products cause the necrosis of the cell and contractile failure in the rat heart [7]. CAT, superoxide dismutase, and GP are free radical scavenging enzymes concerned foremost with cellular defence against oxidative damage, O₂ and H₂O₂ decomposition prior their interaction to form more reactive hydroxyl radical. It is, therefore, essential that equilibrium is maintained among these enzymes for effective removal of oxidative stress within intracellular organelles. It has been reported that glutathione protect the myocardium from free radical-induced injury and reduction in cellular glutathione content could hinder the recovery of ischemia after a short period [19]. The results from the present study indicate that treatment with doses of AQRED increased the level of catalase and glutathione peroxidase suggesting an improved antioxidant status in the system. The result of our study was in line with the submission of Raju *et al* [26] who reported a reduction in the activity of tissue antioxidant enzymes in rats with myocardial damage.

Tissue damages are caused by free radicals; these free radicals attack the cellular membrane of the poly-unsaturated fatty acids (PUFAs) thereby causing lipid peroxidation [27]. Myocardial damage induced by ISP in rats causes lipid peroxidation and this signifies an elevated free radical generation [28]. The results of the present study reveal that rats treated with ISP show a significant increase in TBARS when compared with control group. Treatment with different doses of aqueous root extract of *D. anomala* reduced the elevated TBARS level.

Histopathological analysis of the heart of ISP-induced myocardial disease in rats treated with AQRED revealed the normal structure of the heart with some traces of oedema and necrotic myocytes when compared with the heart of ISP-treated rats. This shows effective protection of AQRED most especially at the highest dose of 500 mg/kg on the heart against ISP-induced cardiotoxicity.

CONCLUSION

The findings of this study indicate the ameliorative activity of AQRED in rats with myocardial disease. Thus, *D. anomala* might be a potential alternative source of medicine for the management and prevention of cardiovascular disease. Further studies are, however, required to identify the active components of the plant as well as elucidate their mechanisms of action.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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