Renal function outcome and antidyslipidaemic effect of polyherbal crude extract in Isoproterenol-Induced Oxidative Damage in Rat Myocardium

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Abstract:

Cardiovascular disease has become a universal cause of morbidity and largely contributes to mortality in developed and developing countries. The present study aimed to evaluate the antidyslipidemic effect and renal outcome of polyherbal extract on isoproterenol induced myocardial infarction in Wistar albino rat model. The rats were divided into eight groups of six animals each. Group, I served as normal control, Group II rats were administered isoproterenol (20mg/g B.wt). Group III and IV were pretreated with PH extract (250 mg/kg B.wt, 500mg/kg B.wt, respectively) for 30 days and received a subcutaneous injection of isoproterenol (20mg/kg, B.wt) at the end of the experimental period for 2 consecutive days. Group V and VI were pretreated with Propranolol (10 mg/ kg B.wt, 20mg/kg B.wt) for 30 days and received a subcutaneous injection of isoproterenol (20mg/kg, B.wt) at the end of the

experimental period for 2 consecutive days. Group VII and VIII received PH extract of 250mg/kg B.wt and 500mg/kg B.wt for 30 days. After the experimental period, blood was collected, serum was separated and used for the estimation of renal and Lipid profiles are studied to access the cardioprotection. The levels of the renal profile(serum urea, uric acid and creatinine), and lipid profiles, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and phospholipids (PL), triglycerides (TG) and Free Fatty acids (FFA) was significantly increased, while the levels of high-density lipoprotein cholesterol (HDL-C) in serum of IPL treated rats. In PH pretreated rats the renal and dyslipidaemic effects of IPL were compensated to near normal levels. The study shows that the extract has a significant antidyslipidaemic effect.

Key words: Cardiovascular disease, antioxidant, oxidative stress, lipid profile and renal profile.

I. INTRODUCTION

Myocardial infarction is a major public health concern and the leading cause of death throughout the world. By 2030, heart disease and stroke will become the leading causes of both death and disability worldwide, with the number of fatalities projected to increase to more than 20 million a year, and to more than 24 million a year by 2030 **(1).** Developing countries like India are struggling to manage the impact of infectious diseases simultaneously with the growing burden on society and the health system caused by non-communicable diseases such as myocardial infarction. Due to changing lifestyles in developing countries, such as India, and particularly in urban areas, myocardial infarction is making an increasingly important contribution to mortality statistics **(2).**

Reactive oxygen species (ROS) play a critical role in the pathogenesis of various diseases such as cardiovascular injury associated with circulatory disturbance **(3).** Isoproterenol-induced myocardial necrosis has the mechanism of generating ROS causing lipid peroxidation damage to the proteins due to the production of carbonyl derivatives. Myocardial infarction is associated with ischemic necrosis of cardiac muscles due to a decrease in the supply of blood to a portion of the myocardium below a critical level necessary for viability and proper physiological function. A disparity between the oxygen requirement of the myocardium and the ability of the coronary artery to meet it results in the ischaemic necrosis of heart muscle **(4).**

Isoproterenol (ISO) is a synthetic catecholamine and [sz]-adrenergic agonist that causes severe stress in the myocardium, resulting in infarct-like necrosis of the heart muscle. The pathophysiological and morphological changes observed in ISO-administered rats are similar to MI in humans **(5).** Further, it undergoes autoxidation generating highly toxic reactive oxygen species (ROS) that stimulate lipid peroxidation, leading to both structural and functional myocardial injury. Therefore, ISO-induced myocardial injury serves as a well-standardized model for human MI to study the beneficial effects of drugs on cardiac function **(6).** Isoproterenol has been found to cause severe stress in the myocardium resulting in infarct-like necrosis of heart muscles. It also increases the level of serum and myocardial lipids **(7)** and also increases the level of cholesterol in the tissues which in turn leads to coronary heart disease **(8).**

The Indian system of medicines, Viz Ayurveda, Siddha, Unani and Homeopathic systems predominantly use plant-based raw materials and most of their preparations and formulations. Herbal medicines are becoming more and more popular nowadays. Among the entire flora, 35,000 to 70,000 species have been used for medicinal purposes **(9).** In India, of the 17,000 species of higher plants, 7500 are known for medicinal uses. This is the highest proportion of medicinal plants known for their medical purposes in any country of the world for the existing flora of that respective country **(10).** Demand for medicinal plants is increasing in both developed and developing countries due to growing recognition of

natural products, being non-narcotic, having no side effects, easily available at a desirable price, and sometimes the only source of health care available to the poor.

Many herbal secondary metabolites, chemical compounds, and herbal formulations have been studied for their biological actions related to preventing human diseases by using models such as IPL-induced myocardial infarctions **(11).** Since IPL-induced myocardial infarction serves as a well-standardized model to study the beneficial effects of many drugs. IPL, a non-selective β-adrenergic agonist, has been reported to cause oxidative stress in the myocardium resulting in infarct-like necrosis of the heart muscle and an increase in the levels of lipids in the myocardium **(12).** Free radical generation and lipid peroxidation could be involved in IPL-induced cardiac damage **(13).** The pathophysiological changes during IPL induction are comparable to those taking place in human myocardial infarction **(14),** due to altering lipid metabolism. In many countries, herbal therapies are among the most popular of all "alternative treatments" **(15, 16).** Hence, In this line, a polyherbal formulation of crude extract consisting of 9 medicinal plants in all with specific morphological parts of the plants used and each ingredient being of a desired equal amount.

The nine medicinal plants includes *Punica granatm rind, Catharanthus roseus L, Gymnea sylvestre ,Cissus quadrangularis, Terminalia Arjuna, Tinosporia Cordifolia, Urginea Indica, Garcinia Gummi- gutta, Ficus racemosa.* These plants individually reported by various authors for their medicinal uses by *invitro* and *invivo* studies. In traditional literature these plants are used for various pharmacological activities such as *Punica granatm rind*- antidiabetic **(17),** antimalarial **(18)**, *Catharanthus roseus L* – antidiabetic **(19)**,wound healing **(20)** *Gymnea sylvestre*- antidiabetic **(21),** anti-inflammatory **(22)** ,*Cissus quadrangularis* – CNS **(23,24)**, *Terminalia Arjuna* – Cardiotonic **(25,26,27,28)** hypercholesterolaemia **(29)**, antidiabetic **(30)** *Tinosporia Cordifolia* – Cardioprotective **(31),** antidiabetic **(32),** *Urginea Indica* – phytochemical constituents **(33)**, *Garcinia Gummi- gutta* -Anti LPO **(34),** *Ficus racemosa*- CNS **(35)**, Cardiac glycosides **(36).**

II. MATERIALS AND METHODS

PLANT COLLECTION AND AUTHENFICATION

The plants and fruits are collected in and around Coimbatore. They are authenticated by Botanical Survey of India (BSI) in "Tamil Nadu Agriculture University" Coimbatore. A voucher specimen (No: BSI/SRC/5/23/2012-13/Tech 44) has been deposited at the Herbarium of the Botany Department. Various parts of the plant such as fruit rind, flowers, leaves and bark are used for the current study.

PREPARATION OF POLYHERBAL EXTRACT

One gram of polyherbal formulation consisted of equal amount of Punica granatum (rind), Catharanthus roseus (leaves), Gymnema sylvestre (leaves), Cissus quadrangularis (leaves and stem), Garcinia cambogia (fruit), Tinospora cordifolia (dimber), Terminalia arjuna (bark), Urginea indica (bulb) and Ficus racemosa (fruit and leaves). Ten grams of dried powder of each plant was added to cold macerated ethanolic solvent with occasional stirring for 3 days. After 3 days, the suspension was filtered through a fine muslin cloth and the filtrate was evaporated to dryness at low temperature

Selection of animals: Male albino rats of Wistar strain weighing about 130-150 g, obtained from the Animal Breeding Station, Thrissur, Kerala, India were used for the study. The animals were few standard pellets under standard laboratory conditions: humidity (40-70%), temperature (25±20ºC) and light (12 hrs light/ dark). The protocol of the study was approved (KMCERT/PhD/ 15/2015-2016) by the Institutional Animal Ethic Committee (IAEC) of Kovai Medical Centre Research and Education Trust.

A total of forty eight animals were used and divided into eight treatments containing six animals each. Treatment I: served as control; Treatment II : rats were administered Isoproterenol (20 -1 mg g b.wt.) subcutaneously twice at an interval of 24 hrs dissolved in normal saline; Treatment III and Treatment IV: rats -1 were pretreated with polyherbal extract (250 and 500 mg g b.wt.) -1 for a period of 30 days and isoproterenol (20 mg g b.wt.) subcutaneously twice at an interval of 24 hrs at the end of treatment period on 29th and 30th day; Treatment V and Treatment VI: rats were pretreated with Propranolol (10 and 20 -1 -1 mg g b.wt.) for a period of 30 days and isoproterenol (20 mg g b.wt.) subcutaneously twice at an interval of 24 hrs at the end of treatment period on 29th and 30th day; Treatment VII and Treatment VIII: rats were treated with polyherbal extract (250 and -1 500 mg g b.wt.) for a period of 30 days.

After the last treatment, all the rats were sacrificed by cervical dislocation. The collected blood was centrifuged at 2500rpm for 10 min and collects the serum. The serum was used for various biochemical experiments.

BIOCHEMICAL ANALYSIS

LIPID PROFILE IN SERUM

The following Lipid profile and renal profile were estimated by the standard biochemical techniques **(39-45).**

Statistical analysis: Results were expressed as mean ± SD. The data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT), using a statistical package program (SPSS 10.0 for windows) taking $P < 0.05$ as significant (46).

III. RESULT AND DISCUSSION

Table: 1, depict the levels of total cholesterol, phospholipids, triglycerides and free fatty acids in the serum of control and experimental rats.

Table. 1 EFFECT OF POLYHERBAL CRUDE EXTRACT ON SERUM LIPID PROFILE IN CONTROL AND EXPERIMENTAL ANIMALS

GROUPS	Total Cholesterol (mg/dl)	Phospholipids (mg/dl)	Triglyceride (mg/dl)	FFA (mg/dl)
GROUP I	60.45 ± 0.12[a]	81.68 ± 0.53[a]	86.21 ± 0.01[a]	25.46 ± 0.01[a]
GROUP II	115.25 ± 0.32[b]	90.87 ± 0.77[b]	158.76 ± 0.47[b]	32.63 ± 1.41[b]
GROUP III	86.97 ± 1.58[c]	59.20 ± 8.83[c]	115.52 ± 0.02[c]	27.68 ± 0.01[c]
GROUP IV	54.12 ± 0.04[d]	43.24 ± 1.16[d]	95.53 ± 0.01[d]	26.98 ± 0.01[d]
GROUP V	86.12 ± 0.04[c]	50.26 ± 0.06[c]	99.50 ± 0.09[c]	25.27 ± 0.18[c]
GROUP VI	56.00 ± 2.88[d]	44.22 ± 0.08[d]	92.17 ± 0.10[d]	23.10 ± 0.00[d]
GROUP VII	60.32 ± 0.46[a]	81.12 ± 0.04[a]	86.18 ± 0.04[a]	25.53 ± 0.05[a]
GROUP VIII	60.78 ± 0.15[a]	81.31 ± 8.17[a]	86.08 ± 0.04[a]	25.73 ± 0.05[a]

Values are mean ± SD of six samples in each group

[a-f] Mean values with in a column no common superscript differ significantly at (<0.05) by DMRT

[a-f] Mean values with in a column of common superscript means Non significant.

A significant ($p<0.05$) elevation in serum total cholesterol, phospholipids, triglycerides, and free fatty acids was observed in Isoproterenol induced myocardial rats (Group II), when compared with normal control rats (Group I). Pretreatment with the 50% hydroethanolic polyherbal crude extract (250

and 500 mg/kg Body weight) for 30 days significantly ($p<0.05$) reduced the values as compared to Group II of myocardial rats. No significant ($p<0.05$) variation was observed in the Propranolol treated group of rats (Group V and VI) when compared with Group III and IV respectively. The 50% hydroethanolic polyherbal crude extract treated rats (Group VII and VIII) did not show any significant ($p<0.05$) changes in the serum lipids when compared to control rats (Group I). The higher dose of 500 mg/kg Body weight showed an efficient result when compared to 250 mg/kg Body weight, likewise, the Standard drug Propranolol at the concentration of 20 mg/kg shows a competent result when compared to 10 mg/kg Body weight.

Elevation in cholesterol levels could be due to an increase in biosynthesis and a decrease in its utilization. Isoproterenol induces free radical formation, which may cause cellular cholesterol accumulation by increasing cholesterol biosynthesis, decreasing cholesterol ester hydrolysis, and reducing cholesterol efflux **(47, 22, 23).**

The rise in the serum phospholipid and free fatty acid content in myocardial rats is due to the liberation of free fatty acids from the adipose tissue also entering into the myocardium, and the process is proportional to the free fatty acid concentration in the coronary sinus **(48,32).** Though the heart can utilize free fatty acid for its energy requirement, the excess free fatty acid is used for the synthesis of triglycerides, resulting in hypertriglyceridemia.

An increase in the triglyceride level is due to the elevated flux of fatty acids and impaired removal of VLDL from serum **(49, 29).**

In 250 and 500 mg/kg B wt of the 50% hydroethanolic polyherbal crude extract treated animals, the lipid profile was reduced to a normal level. The lipid-lowering effect is due to the inhibition of the hepatic cholesterol biosynthesis which increased fecal bile acid secretion and stimulation receptor-mediated catabolism of LDL cholesterol or increases the uptake of LDL from blood by the liver **(50, 24, 58).**

This may also be due to the cardioprotective activity of *TerminaliaArjuna, Gymnema Sylvestre, and Cissusquandragularis*, which are present in the 50% hydroethanolic polyherbal crude extract, which contains an enormous amount of the active constituents like saponins, phenols, and gallic acids that are responsible to inhibit the increased accumulation of lipid by its hypolipidaemic and hypocholesterolemic activity, which might prove valuable for the management of CVD **(51, 49).**

The standard drug Propranolol treated groups (Group V and VI) also showed non significantly ($p<0.05$) decrease in the serum total cholesterol, phospholipids, triglycerides, and free fatty acids similar to Group III and IV of the 50% hydroethanolic polyherbal crude extract. Propranolol is a selective β-blocker. The antioxidant property of the Propranolol and increase in NO by reducing its antioxidant

inactivation is responsible beneficial in maintaining the lipid and carbohydrate metabolic profile **(52, 44, 47).**

LEVEL OF HDL, LDL, VLDL IN THE SERUM

The activity of HDL, LDL, VLDL in the serum of control and experimental rats is given in the **Table: 2**

TABLE. 2 EFFECT OF POLYHERBAL CRUDE EXTRACT ON SERUM LIPOPROTEINS IN CONTROL AND EXPERIMENTAL ANIMALS

GROUPS	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
GROUP I	36.78 ± 0.01[a]	57.39 ± 2.56[a]	99.17 ± 2.61 [a]
GROUP II	26.86 ± 0.65[b]	84.27 ± 1.06[b]	111.13 ± 2.87[b]
GROUP III	32.15 ± 0.01[c]	70.81 ± 1.51[c]	102.96 ± 3.02 [c]
GROUP IV	36.18 ± 0.02[d]	54.80 ± 1.95[d]	90.98 ± 2.11 [d]
GROUP V	34.07 ± 0.01 [c]	77.11 ± 1.57 [c]	111.18 ± 2.14 [c]
GROUP VI	31.40 ± 0.26 [d]	50.13 ± 1.24 [d]	91.53 ± 1.98 [d]
GROUP VII	36.46 ± 0.01 [a]	57.29 ± 1.95 [a]	99.75 ± 2.38 [a]
GROUP VIII	36.32 ± 0.00 [a]	57.98 ± 1.03 [a]	99.3 ± 2.26 [a]

Values are mean ± SD of six samples in each group

[a-f] Mean values with in a column no common superscript differ significantly at (<0.05) by DMRT

[a-f] Mean values with in a column of common superscript means Non significant

The activity of the serum lipoproteins (LDL and VLDL) was significantly ($p<0.05$) elevated and a significant ($p<0.05$) reduction in the HDL cholesterol was observed in the myocardial rats of Group II. Pretreatment of the doses 250 and 500 mg/kg Body weight of the 50% hydroethanolic polyherbal crude extract (Group III and IV) showed a significant ($p<0.05$) reduction in LDL, VLDL and a significant ($p<0.05$) increase in HDL cholesterol when compared with Isoproterenol induced Group II rats. Oral doses of Propranolol for 30 days to myocardial rats (Group V and VI) also showed significantly ($p<0.05$) decreased LDL, VLDL, and increased HDL. No significant ($p<0.05$) variation was shown when the

Group III and IV animals are compared with Group V and VI respectively. Comparison of Group I with Group VII and VIII also showed no significant ($p<0.05$) changes. When compared with Group III rats, Group IV rats showed an effective activity. The 500 mg/kg Body weight showed an efficient result when compared to 250 mg/kg body weight, likewise, the Standard drug Propranolol at the concentration of 20 mg/kg shows a competent result when compared to 10 mg/kg Body weight.

In the present investigation increased LDL and VLDL in the Isoproterenol induction rats were observed. This may be due to the fat cells, which are mediated by the cAMP cascade, in which lipolytic hormones activate adenylate cyclase, thereby increasing cAMP formation, Subsequently cAMP promotes lipolytic activity by activating cAMP-dependent protein kinase which phosphorylates hormone-sensitive lipase (Morumoto*et al*., 2010). LDL formation occurs primarily by the catabolism of VLDL, whereas HDL inhibits the uptake of LDL from the arterial wall and facilitates the transport of cholesterol from tissues to the liver where it is catabolized and excreted from the body **(53, 47).** The present results are in agreement with the study reported **(54, 26, 10).**

The 50% hydroethanolic polyherbal crude extract treatment showed a significant ($p<0.05$) high in the HDL level and a drop in the LDL and VLDL levels. The reason may be due to the chemical constituents like flavonoids, saponins, pectins, and phenols present in the polyherbal crude extract, which are required for maintaining the lipoproteins level **(55, 39).**

Flavonoids rich in polyherbal crude extract reduced the LDL and VLDL levels thereby maintaining the fluidity and function of the myocardial membrane. Polyphenols particularly gallic acid and catechin hydrolyses the cholesterol esters and liberate free cholesterol in the reduction of the small intestine. Thus polyphenols present in the polyherbal crude extract binds with the bile acids, to increase fecal secretion, which has been hypothesized as a possible mechanism for lowering LDL and VLDL level **(56, 22, 23).**

Standard drug Propranolol treated Group V and VI shows no significant ($p<0.05$) changes. This is because; Propranolol blocks the alterations of myocardial lipids by inhibiting the cAMP and there maintain the normal fluidity and fewer alterations in the property of lipids and functions of the myocardial membrane **(57).**

SERUM UREA, URIC ACID AND CREATININE

The results obtained on serum urea, uric acid and creatinine in control and experimental rats are presented in **Table: 3**

Table. 3 EFFECT OF 50% HYDROETHANOLIC POLYHERBAL CRUDE EXTRACT ON SERUM RENAL PROFILE IN CONTROL AND EXPERIMENTAL ANIMALS

GROUPS	UREA (mg/dl)	URIC ACID (mg/dl)	CREATININE (mg/dl)
GROUP I	26.9 ±0.19[a]	5.1 ± 0.18[a]	25.03 ± 0.56[a]
GROUP II	59.7 ± 0.27[b]	10.6 ± 0.30[b]	48.54 ± 0.21[b]
GROUP III	38.2 ± 0.22[c]	7.1 ± 0.19[c]	36.37 ± 0.51[c]
GROUP IV	37.10 ± 0.20[d]	6.2 ± 0.17[d]	23.11 ± 0.55[d]
GROUP V	38.6 ± 0.21 [c]	7.1 ±0.36 [c]	36.39 ± 0.27 [c]
GROUP VI	37.30 ± 0.24 [d]	6.1±0.40 [d]	23.111 ± 0.45 [d]
GROUP VII	26.8 ± 0.20[a]	5.3 ± 0.55 [a]	25.01 ± 0.08 [a]
GROUP VIII	26.4 ± 0.18 [a]	5.1 ± 0.34 [a]	25.32 ± 0.15 [a]

Values are mean ± SD of six samples in each group

[a-f] Mean values with in a column no common superscript differ significantly at (<0.05) by DMRT

[a-f] Mean values with in a column of common superscript means Non significant.

The kidney is involved in the excretion of many toxins, and metabolites waste products, particularly nitrogenous compounds; it would therefore be worthwhile to examine the effects of Isoproterenol induced rats. Plasma levels of urea, uric acid, and creatinine are the biomarkers of nephrotoxicity **(58, 59).** Uric acid is considered to be the risk factor in the development of cardiotoxicity **(60).**

The serum urea, uric acid, and creatinine levels were significantly ($p<0.05$) raised in Group II of Isoproterenol-induced myocardial infarction in rats. The levels were reduced ($p<0.05$) in 50 % hydroethanolic polyherbal crude extract (250 and 500 mg/kg Body weight) treated Group III and IV as well as standard drug Propranolol(Group V and VI). Whereas in the 50% hydroethanolic polyherbal crude extract alone treated rats (Group VII and VIII) the levels of serum urea, uric acid, and creatinine were similar to the control group. There was no significant difference in the levels of Group III and IV when compared with Group V and VI respectively. Group IV animals showed more recovery of urea, uric acid, and creatinine levels than the Group III animals. The higher dose of 500 mg/kg Body weight showed an efficient result when compared to 250 mg/kg Body weight, likewise standard drug Propranolol at the

concentration of 20 mg/kg Body weight shows a competent result when compared to 10 mg/kg Body weight.

The elevated level of uric acid is due to the increased free radical production by the Isoproterenol **(61, 44, 46)**. In hypoxic tissue, ATP depletion occurs, causing accumulation of hypoxanthine. When tissues are disturbed, the enzyme xanthine dehydrogenase is converted to xanthine oxidase by the oxidation of the essential –SH group. Xanthine oxidase catalyzes the conversion of hypoxanthine to xanthine, and uric acid to superoxide **(62, 47, 15).**

The increased creatinine indicates renal injury in myocardial infarction **(63,57)**. The amount of creatinine excreted depends upon the glomerular filtration rate. When this excretion fails to balance the creatinine production, the level of serum creatinine rises **(64).** Blood urea is one of the most used and traditional markers for detecting renal function. Heart failure or systolic dysfunction assessed with urea and creatinine. The increased creatinine indicates renal injury in myocardial infarction **(65).** Most experimental studies show the relationship between venous pressure and deterioration in kidney function **(66, 57).**

In 50% hydroethanolic polyherbal crude extract 250 and 500 mg/kg Body weight treated rats, the elevated levels of serum urea, uric acid, and creatinine were almost brought back to near normal levels which is due to the presence of flavonoids, tannins, sterols, glycosides present in polyherbalcrude extract which prevented the increase in renal profile, thereby decreasing the generation of O2- and H2O2 in Isoproterenol induced rats by their free radical scavenging activity **(67, 36)** and these played a vital role on bringing back the elevated level of serum urea, uric acid, and creatinine to near normal level **(65).**

The result obtained in the present study are in concordance with **(67)** reported in "Biological Evaluation of polyherbal Ayurvedic cardiotonic preparation *"Mahamrutyunjaya rasa"*

Propranolol is a β-blocker and β-blockers prevent Isoproterenol–mediated overstimulation of the sympathetic system **.**Propranolol is a nonselective vasodilating β-adrenergic antagonist with α1-blocking activity. It has antioxidant activity and the renal effects of Propranolol include essential hypertension, renal hypertension, hypertension with evidence of CKD, hemodialysis, renal transplantation, congestive HF, and hypertension with diabetes **(62, 55)**

IV CONCLUSION

Thus the present finding exemplifies that flavonoids, phenols, tannins, sterols, and other chemical constituents were present in an enormous amount in the polyherbal crude extract, thus proving its hypolipidaemic role in lipid metabolism by lowering the lipogenesis process in the affected myocardium against the Isoproterenol induced myocardial infarction. These constituents

also bring back the elevated levels of serum urea, uric acid, and creatinine to near normal levels by protecting against Isoproterenol induced myocardial infarction in rats which proves the additional significant action of the polyherbal crude extract against glomerular filtration rate or kidney failure in case of myocardial Infarction by protecting against Isoproterenol induced myocardial infarction in rats.

CONFLICT OF INTEREST

All the authors have equally contributed to carry out the research work. Conflict of interest declared none.

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