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Research Article

PHARMACODYNAMIC INTERACTION OF GREEN TEA EXTRACT WITH HYDROCHLOROTHIAZIDE AGAINST ISOPROTERENOL INDUCED MYOCARDIAL DAMAGE IN RATS

Manodeep Chakraborty[1]*, Jagadish V Kamath[2]
[1]Reseach Scholar, Bhagwant University, Ajmer, India
[2]Department of Pharmacology, Shree Devi College of Pharmacy, Mangalore, India
*Corresponding Author Email: manodeep.chakraborty@gmail.com

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ABSTRACT
To evaluate the pharmacodynamic interaction of green tea extract with hydrochlorothiazide against isoproterenol induced myocardial infarction wistar albino rat of either sex were prophylactically treated with hydrochlorothiazide (10 mg/kg for 7 days), low and high dose of green tea extract (100 and 500 mg/kg for 3 weeks) and their combination by oral route. To induce the toxicity apart from normal control all other group subjected to isoproterenol (150 mg/kg, s.c) for two consecutive days. The effect of prophylactic treatment was analyzed by estimation of electrocardiographic changes, serum biomarker levels, antioxidant level in tissue and histopathological report. Green tea in both high and low dose and their combination groups with hydrochlorothiazide significantly reduced the serum biomarker levels, increased antioxidant levels in tissue, restored the electrocardiographic changes compared to isoproterenol only treated group. The combination group showed substantial amount of restoration of all the parameters compared to hydrochlorothiazide alone treated group. To conclude the combination of green tea extract with hydrochlorothiazide reduced the side effect and found to be effective against myocardial stress.
Keywords: Hydrochlorothiazide, Green tea extract, Isoproterenol, Cardio protection

INTRODUCTION

With the strong traditional background, practice of herbalism witnessed a great therapeutic option for the therapist due to its potency and apparent safety profile.[1] Herbs when interact with the modern medicine it can mimic, magnify or oppose the action of one another. Theoretically herb-drug interaction is greater than the drug-drug interaction due to the presence of more than one single entity in the herb.[2,3] Diuretics such as hydrochlorothiazide (HCTZ) are rarely used as a mono therapy for ischemic hypertensive patients. Thiazides by direct action responsible for increase in excretion of sodium and chloride where as by indirect action causes decrease in serum and urinary potassium, plasma rennin activity, aldosterone secretion.[4,5] The patients with cardiac ischemia, heart failure or left ventricular hypertrophy mild to moderate level of hypokalemia leads to development of cardiac arrhythmias.[6-8] Green tea which is processed from *Camellia sinensis* belongs to Theaceae family is an excellent source of poly phenol antioxidants, and mainly contains catechins especially epicatechin, epigallocatechin, epicatechingallate and epigallocatechin gallate.[9,10] Studies have shown that catechins in green tea possess diverse pharmacological properties that include anti-oxidative, anti-inflammatory, anti-carcinogenic and anti-bacterial effects. In the gastrointestinal tract, green tea was found to activate intracellular antioxidants, inhibit procarcinogen formation, and suppress angiogenesis and cancer cell proliferation.[11] It is found that short term consumption of commercial green tea reduces systolic and diastolic BP, fasting total cholesterol, % body fat and body weight[12] and epigallocatechin-3-gallate in green tea preserved cardiac function after ischemia–reperfusion.[13] Till now there is no study which indicates the combine effect of green tea and HCTZ. So the present study has been designed to evaluate the pharmacodynamic interaction of green tea and HCTZ against Isoproterenol induced myocardial infarction.

MATERIALS AND METHODS

Experimental Animals

Healthy adult Wistar albino rats of either sex weighing 175-250 g, were housed in polypropylene cages, maintained under standardized condition (12 h L:D cycles, 25° ± 5°C) with paddy husk bedding at the Central Animal House, Shree Devi College of Pharmacy, Mangalore, India were provided with standard pellet food and had free access to purified drinking water. The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India were followed and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study (SDCP/IAEC-19/2012-13).

Chemical

All chemicals used were of analytical grade and purchased from standard companies. Pure sample of hydrochlorothiazide was gifted by Bangalore Test House, (Bangalore, India). Biochemical kits were procured from Crest Bio systems (Goa, India)

Preparation of Green Tea Extract (GTE) and dose selection

Green Tea (*Camellia sinensis*) leaves were purchased from the local market of Mangalore, India. The Aqueous extract was prepared by mixing Green tea leaves gently in distilled water maintained at 70°C to 80°C for 30 minutes, filtered and evaporated in same temperature to get a thick gummy mass. The yield was found to be 24.76 %. Extract was freshly dissolved in distilled water before each dose to animals. The dose selection of Green Tea Extract (GTE) was based on acute toxicity studies, carried out according to OPPTS (Office of Prevention, Pesticide and Toxic Substance) guidelines following the limit test procedure.[13,14] The animals were fasted over night prior to the studies. Mice were divided

into two groups of three each. Test dose of 2 g/kg body weight and 5 g/kg body weight were given orally to either group of mice. Mice were observed for 72 hours for mortality. $1/10^{th}$ and $1/50^{th}$ of the maximum safe dose were selected as high and low doses respectively.

Isoproterenol induced myocardial necrosis in rats

At the end of the treatment period, Isoproterenol (ISO) (150 mg /kg, s.c) was administered to all the animals (except the normal control) for two consecutive days. Forty eight hours after the first dose of ISO, the animals were anesthetized with ketamine (70 mg/kg, i.p) and xylazine. (10 mg/kg, i.p) and blood was withdrawn by retro-orbital puncture. Serum was separated by centrifugation for the estimation of biomarkers (LDH, CK-MB, ALT, AST, ALP). E.C.G recordings were made for each animal using lead II method. Thereafter the animals were sacrificed; four hearts were used for the preparation of homogenate to estimate antioxidant (SOD and Catalase) levels. Remaining four hearts were embedded in formal saline (0.7 %) for histological examination[15].

Rats of either sex were divided into 7 treatment groups of eight animals each.

1. Group-I- animals kept as normal control (without pre-treatment).
2. Group-II- animals kept as toxic control (Isoprotrenol only).
3. Group-III- animals treated with Hydrochlorothiazide (HCTZ- 10 mg/kg, p.o) for 7 days and subjected to Isoprotrenol toxicity.
4. Group-IV- animals treated with low dose of Green Tea Extract (GTE-100) for 3 weeks + Isoprotrenol.
5. Group-V- animals treated with high dose of Green Tea Extract (GTE-500) for 3 weeks + Isoprotrenol.
6. Group-VI- HCTZ was during last week of treatment with low dose of Green Tea Extract (GTE-100) + Isoproterenol.
7. Group-VII- HCTZ was during last week of treatment with high dose of Green Tea Extract (GTE-500) for 3 weeks + Isoproterenol

The parameters estimated were:

- LDH, CK-MB, ALT, AST and ALP activity in serum and heart tissue homogenate.
- SOD and Catalase activity in heart tissues homogenate.
- E.C.G recordings: Heart rate, QRS interval, QT segment and RR interval.
- Histipathological Studies: Scoring in myocardial tissue was determined from H-E transverse stain.

Preparation of Heart Tissue Homogenate

The hearts removed after the experiment was made free of the adjacent vessels and fatty tissue mass with the help of scissors. Hearts were then cut open, rinsed with saline (0.9 % NaCl) and dried using filter paper. The weight of the heart was then recorded. Thereafter the heart was homogenized in ice cold 0.25M sucrose solution using a mortar and pestle. The homogenate thus obtained was centrifuged at 5000 rpm for 15 minutes. The supernatant was decanted and used for the estimation of CK-MB, LDH, SOD, Catalase and TBARS[15].

Statistical analysis

Results are expressed as mean ± SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. $P < 0.05$ was considered significant.

RESULTS

Effects on electrocardiographic parameters in Isoprenaline (ISO) induced myocardial infarction (Table 1)

Heart rate of ISO control group showed significant increase compared to normal control. HCTZ treated group reported significant increase in heart rate compared to ISO control. GTE-100 and GTE-500 combined with HCTZ witnessed significant decrease in heart rate compared to HCTZ alone. The experimental results of QRS duration, QT segment RR interval and PR interval demonstrated significant increase for ISO control and HCTZ alone treated group compared to normal group. Prophylactic treated groups GTE-100 and GTE-500 combined with HCTZ reported decrease in electrocardiographic parameters compared to HCTZ alone treated group.

Effects on serum CK-MB, CK-NAC, LDH, AST, ALT, ALP activities (Table 2 and 3)

The groups such as ISO control and HCTZ alone treated group reported significant increase in serum biomarker activities where as GTE-100 and GTE-500 groups combined with HCTZ demonstrated significant increase in biomarker level compared to HCTZ alone treated group.

Effect on Tissue SOD, Catalase (Table 4)

ISO control group and HCTZ alone treated group reported significant decrease in SOD and catalase levels in heart tissue homogenate where as groups prophylactically treated with GTE-100 and GTE-500 combined with HCTZ showed significant increase in antioxidant level compared to HCTZ alone treated group.

DISCUSSION

The aim of the present study was to elucidate the pharmacodynamic interaction of green tea extract with HCTZ using isoproterenol induced myocardial dysfunction and metabolic derangement. Observed results suggested that GTE (100 and 500 mg/kg, p.o.) has showed beneficial results dose dependently when combined with HCTZ against ISO induced myocardial injury. Isoproterenol, a β sympathomimetic drug in higher dose can cause myocardial damage. Isoproterenol causes disturbances in coronary microcirculation, development of anoxia, generation of free radicals and increase in calcium uptake and energy consumption which leads to cell death.[16]

Table 1: Effects on electrocardiographic parameters against Isoprenaline induced myocardial infarction

Treatments	Heart rate	QRS duration (ms)	QT segment (ms)	RR interval (ms)	PR interval (ms)
Normal control	193.33 ± 8.81	126.33 ± 2.33	169.33 ± 2.33	214.66 ± 2.60	77.66 ± 2.02
ISO control	380.00 ± 5.07***	247.33 ± 2.18***	261.66 ± 4.41***	294.36 ± 2.18***	146.00 ± 3.21***
HCTZ	310.66 ± 8.81**	220.00 ± 2.30***	231.00 ± 3.05^{***+}	276.66 ± 1.85***	133.00 ± 2.30***
GTE-100	260.00 ± 2.88^{+++}	159.66 ± 1.45^{***+++}	190.00 ± 2.08^{**+++}	240.00 ± 1.73^{*+++}	95.00 ± 1.52^{*+++}
GTE -500	240.00 ± 2.88^{+++}	144.00 ± 2.08^{**+++}	181.00 ± 1.52^{*+++}	222.00 ± 1.52^{+++}	85.00 ± 1.15^{+++}
GTE-100 + HCTZ	280.00 ± 2.88^{+++o}	180.00 ± 2.64$^{***+++oo}$	221.66 ± 2.02$^{***+++ooo}$	256.66 ± 2.90$^{***+++oo}$	121.37 ± 2.02$^{***+++o}$
GTE-500 + HCTZ	270.33 ± 4.41^{+++oo}	166.00 ± 2.64$^{***+++ooo}$	201.33 ± 2.90$^{***+++ooo}$	236.00 ± 2.51$^{***+++ooo}$	111.33 ± 1.20$^{***+++oo}$

All values are mean ± SEM, n = 6, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to normal control; $^{++}P < 0.01$, $^{+++}P < 0.001$ compared to ISO control and $^{oo}P < 0.01$, $^{ooo}P < 0.001$ compared to Hydrochlorothiazide. GTE-100 (Green Tea Extract-100 mg/kg), GTE-500 (Green Tea Extract-500 mg/kg) and HCTZ (Hydrochlorothiazide -10 mg/kg)

Table 2: Effects on serum CK-MB, CK-NAC, LDH against Isoprenaline induced myocardial infarction

Treatment	Blood serum level U/L		
	CK-MB	CK-NAC	LDH
NC	73.71 ± 8.66	91.63 ± 7.35	322.82 ± 3.59
ISO	668.43 ± 10.34***	1215.77 ± 45.68***	955.51 ± 5.60***
HCTZ	631.24 ± 10.07***	1100.24 ± 8.48***	890.02 ± 7.20***
GTE-100	505.02 ± 7.97^{***+++}	528.11 ± 22.70^{***+++}	656.61 ± 7.90^{***+++}
GTE-500	414.71 ± 10.11^{***+++}	447.70 ± 14.70^{***+++}	573.63 ± 3.62^{***+++}
GTE-100 + HCTZ	555.61 ± 10.40$^{***+++ooo}$	851.16 ± 23.18$^{***+++ ooo}$	793.89 ± 8.48$^{***+++ooo}$
GTE-500 + HCTZ	511.41 ± 8.59$^{***+++ooo}$	760.23 ± 9.999$^{***+++ooo}$	674.00 ± 9.54$^{***+++ooo}$

All values are mean ± SEM, n = 6, $^{***}P < 0.001$ when compared to normal control; $^{+++}P < 0.001$ compared to ISO control and $^{ooo}P < 0.001$ compared to Hydrochlorothiazide. GTE-100 (Green Tea Extract-100 mg/kg), GTE-500 (Green Tea Extract-500 mg/kg) and HCTZ (Hydrochlorothiazide -10 mg/kg)

Table 3: Effects on serum AST, ALT, ALP against Isoprenaline induced myocardial infarction

Treatment	Blood serum level (U/L)		
	AST	ALT	ALP
NC	139.92 ± 4.13	66.55 ± 4.77	155.22 ± 7.42
ISO	669.68 ± 35.26***	329.96 ± 21.86***	403.45 ± 9.35***
HCTZ	605.45 ± 8.16***	304.98 ± 11.52***	399.18 ± 24.34***
GTE-100	277.26 ± 34.03$^{***###}$	237.46 ± 15.43$^{***#}$	241.46 ± 5.65$^{***###}$
GTE-500	173.33 ± 14.53$^{***###}$	185.06 ± 3.14$^{***###}$	201.66 ± 6.00$^{***###}$
GTE-100 + HCTZ	485.06 ± 3.14***	285.06 ± 1.04***	365.06 ± 3.44***
GTE-500 + HCTZ	365.06 ± 2.44$^{***# ooo}$	215.06 ± 3.74$^{***# ooo}$	286.06 ± 1.66$^{***# ooo}$

All values are mean ± SEM, n = 6, $^{***}P < 0.001$ when compared to normal control; $^{+++}P < 0.001$, $^{+}P < 0.05$ compared to ISO control and $^{ooo}P < 0.001$ compared to Hydrochlorothiazide. GTE-100 (Green Tea Extract-100 mg/kg), GTE-500 (Green Tea Extract-500 mg/kg) and HCTZ (Hydrochlorothiazide -10 mg/kg)

Table 4: Effect on Tissue SOD, Catalase and Histopathological scoring against Isoprenaline induced myocardial infarction

Treatment	Heart Tissue Homogenate U/Mg		Histopathological Score
	SOD	CATALASE	
NC	15.25 ± 0.19	6.46 ± 0.43	0.00 ± 0.00
ISO	4.80 ± 0.15***	0.32 ± 0.06***	2.50 ± 0.22***
HCTZ	5.59 ± 0.26^{***+++}	0.54 ± 0.18^{***+++}	2.41 ± 0.21***
GTE-100	7.38 ± 0.29^{***++}	3.09 ± 0.43^{***+++}	1.83 ± 0.26***
GTE-500	9.13 ± 0.27^{***+++}	3.79 ± 0.03^{***+++}	1.15 ± 0.17^{**+}
GTE-100 + HCTZ	6.67 ± 0.73$^{**+++ooo}$	1.48 ± 0.24^{**+++}	2.10 ± 0.17^{***o}
GTE-500 + HCTZ	7.46 ± 0.33^{+++ooo}	2.68 ± 0.17^{+++ooo}	2.06 ± 0.21^{***o}

All values are mean ± SEM, n = 6, $^{***}P < 0.001$, $^{**}P < 0.001$ when compared to normal control; $^{+++}P < 0.001$, $^{+}P < 0.05$ compared to ISO control and $^{ooo}P < 0.001$, $^{o}P < 0.05$ compared to Hydrochlorothiazide. GTE-100 (Green Tea Extract-100 mg/kg), GTE-500 (Green Tea Extract-500 mg/kg) and HCTZ (Hydrochlorothiazide -10 mg/kg)

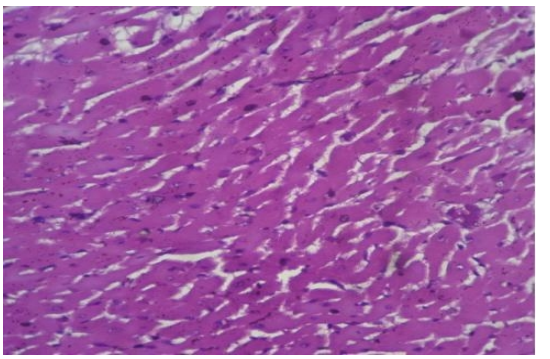

Figure 1A: Heart tissue of normal: Normal texture of cell

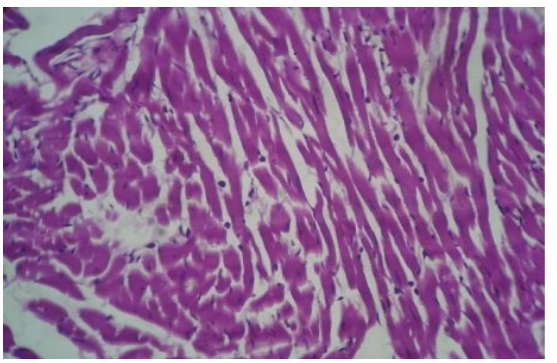

Figure 1B: Isoproterenolol treated, Group: damage in myocardial fibres

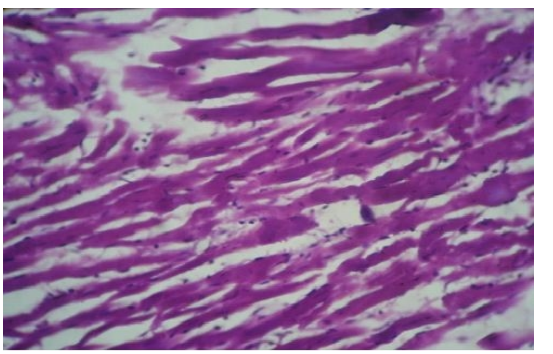

Figure 1C: HCTZ treated group: Marked diffuse inflammation and interstitial space

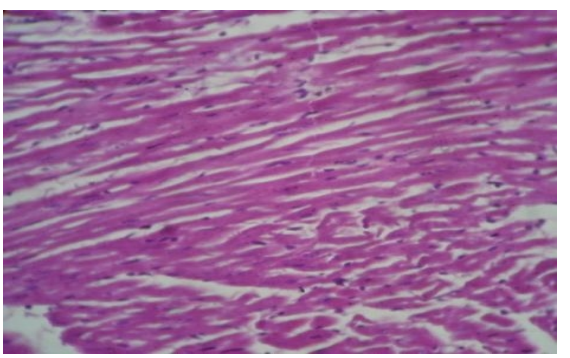

Figure 1D: GTE-100 treated group: show early coagulated necrosis, few fibres show loss of nucleus

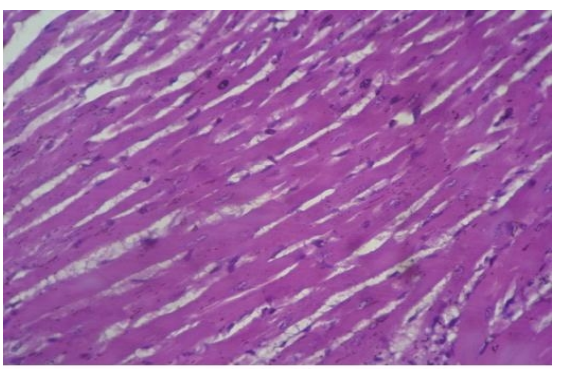

Figure 1E: GTE-500 treated group: Few inflammatory cells seen in the interstitial tissue

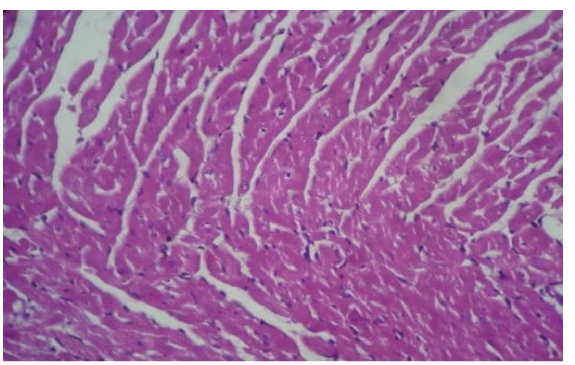

Figure 1F: GTE-100 + HCTZ treated group: Early coagulated necrosis in few areas, Loss of nuclei

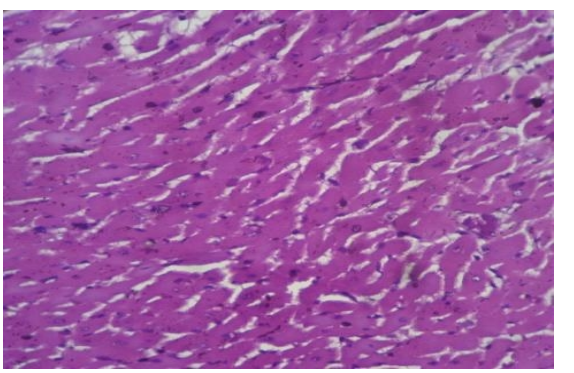

Figure 1G: GTE-500 + HCTZ treated group: Recovery from necrosis, less inflammation process, less interstitial space

Hydrochlorothiazide (HCTZ) alters the renal tubular mechanisms of electrolyte re absorption. The direct action of HCTZ responsible for increase in the excretion of sodium and chloride and by the indirect action causes reduction in plasma volume, serum and urinary potassium loss, plasma renin activity and increase in aldosterone secretion. It has been observed that patients with cardiac ischemia, heart failure or left ventricular hypertrophy, mild-to-moderate hypokalemia increase the likelihood of cardiac arrhythmias. HCTZ causes vasodilatation by activating calcium-activated potassium channels (large conductance) in vascular smooth muscles and inhibiting various carbonic anhydrases in vascular tissue. It is possible that by this vasodilatation, HCTZ may have promoted the healing and prevented the ischemic damage to myocardium. In this study prophylactic treatment with HCTZ alone showed protection against myocardial damage but it is not substantial to prove its protective behavior.[17,18] Green Tea belongs to family *Camellia sinensis*, having rich source of poly phenols and reported its potency against cardiovascular disease risk factors. Green Tea is responsible to reduce in body weight by interfering within the sympathoadrenal system and fatty acid synthesis, decrease cholesterol absorption and plasma levels, have strong free radical-scaven ging activity inhibiting LDL oxidation, reduce the adhesion molecule expression and have antithrombotic activities by inhibiting platelet aggregation and decrease systolic and diastolic blood pressures.[19]

It is evidenced that isoproterenol is responsible for sympathetic over activity which leads to vagal hypoactivity and produces severe myocardial damage. The extent damage can be reflected from the disturbances of electrocardiographic parameters. Prolongation of QT interval is the prime indication for the development of cardiac arrhythmia.[16] The groups combined with GTE (100 and 500 mg/kg p.o.) and HCTZ is responsible for substantial decline in HCTZ induced ST segment elevation, prolongation of QRS complex. The presence of different biomarkers such as LDH, CKMB, ALT, AST and ALP indicates the myocardial cell integrity. Administration of high dose of isoproterenol is responsible for severe cellular damage and loss of membrane integrity, due to that biomarkers leaked out from the tissue and indicates higher levels in serum.[16] Prophylactic group treated with combination of GTE (100 and 500 mg/kg p.o.) and HCTZ showed better protection by reducing serum biomarker levels compared to HCTZ alone treated group. Isoproterenol is also responsible for generation of free radicals and further decrease in antioxidant levels in tissue.[16] Both high and low dose of GTE combined with HCTZ causes remarkable increase in antioxidant profile compared to HCTZ alone treated group.

CONCLUSION

From this present study it may be concluded that green tea extract when combined with HCTZ showed better result to reduce the myocardial stress compared to HCTZ alone treated group. This finding can be beneficial for the ischemic hypertensive patients but further study is required to establish this fact clinically. Apart from that, this study establishes the potential of herb drug interaction and provokes further research in this area.

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