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POTENTIAL CARDIOPROTECTIVE EFFECTS OF STEVIOSIDE AND VERAPAMIL IN CYCLOPHOSPHAMIDE-INDUCED CARDIOTOXICITY IN RATS

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Abstract
Introduction: Stevioside (STV), obtained from *Stevia rebaudiana* leaves, is a calcium channel blocker and inhibits CYP3A4, the primary enzyme responsible for VPML metabolism, thereby STV may elevate VPML plasma concentrations and potentiate its cardioprotective action when co-administered against oxidative cardiac injury. Cyclophosphamide (CYP), a key alkylating agent in cancer chemotherapy for lymphomas and leukaemias, induces acute cardiotoxicity via metabolites (phosphoramide mustard, acrolein) that trigger oxidative stress, endothelial damage, myocardial haemorrhage, necrosis, and ECG alterations, limiting its therapeutic use. Aim & Objectives: This study aimed to investigate STV's enhancement of VPML's cardioprotective effects against CYP-induced cardiotoxicity in rats, with objectives to evaluate serum biomarker normalization (LDH, CK-MB, CK-NAC, AST, ALT, ALP), ECG restoration (heart rate, PR, QRS, QT, RR intervals), and combinatorial synergy. Methods: Wistar rats of either sex (n=6/group) received CYP (200 mg/kg, i.p.) on day 1. Treatments VPML (9 mg/kg, p.o.), STV (50 mg/kg, p.o.), and STV + VPML were given orally for 10 days post-CYP. Cardiac damage was assessed biochemically and electrocardiographically on day 11. Results: CYP markedly elevated biomarkers levels and disrupted ECG (reduced heart rate, prolonged PR/QRS/QT/RR intervals). VPML, STV and the combination group significantly restored biomarker and ECG changes but the combination group of STV+VPML reported significant protection compared to alone treated groups. Summary & Conclusion: In conclusion, STV with VPML robustly counteract CYP cardiotoxicity, suggesting clinical potential for safer chemotherapy via STV and VPML combination.

1. Introduction

Myocardial infarction (MI) is pathologically characterized by myocardial cell death following a long period of ischemia, which can be diagnosed through electrocardiographic (ECG) findings, increased myocardial necrosis, and release of biomarkers [1]. The advanced age, tobacco smoking, dyslipidemia, diabetes, hypertension, physical inactivity, obesity, chronic kidney disease, and excessive consumption of alcohol are among the key etiological factors. The chemotherapeutic agents associated with cardiotoxicity, such as cyclophosphamide (CYP), doxorubicin, epirubicin, and cisplatin, are of major concern as they contribute significantly in the mortality and morbidity statistics[2]. Cardiotoxicity is life-threatening in the treatment of cancer, and systemic anticancer drugs cause direct toxicity or worsen other agents as they cause adverse effects on other vital organs like the heart, kidneys, and liver. Remarkably, CYP and doxorubicin are involved in serious cardiotoxicity[3]. CYP is an alkylating agent with tumoricidal activity; its metabolites, phosphoramide mustard and acrolein, interact with nucleophilic groups (-COOH, -SH, -NH2, -PO3H2, -OH) to form DNA-protein cross-links; cardiotoxicity is caused mainly by the imbalance of redox in tissues due to oxidative stress[4]. The Synthetic drugs have significant therapeutic advantages, but side effects continue to be an issue. The use of herb-drug interactions has been spreading throughout the world, with herbs potentially imitating, increasing, or decreasing the effects of synthetic agents, and can surpass drug-drug interactions because of the multi-component nature of herbs[5]. The paper examines the synergistic cardioprotective effects of CYP-induced cardiotoxicity and their anti-hypertensive, anti-inflammatory, diuretic and natriuretic actions, and verapamil calcium antagonism using rodent models.

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2. Materials &Method

2.1. Experimental Animal

Experimental rats of either sex weighing 150-250g were housed at 25° ± 5°C, relative humidity 50 ± 5% in a well-ventilated animal house under a 12:12 h light-dark cycle. All the rats were provided with a commercially available standard pellet diet, water ad libitum. The Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of the Committee for Control and Supervision on Experiments on Animals (CPCSEA).

2.2. Chemicals & Drugs

Cyclophosphamide (CYP)was purchased (Endoxan, Zydus Oncosciences, Ahmedabad). Verapamil (VPML) and stevioside (STV) were obtained (Calaptin, Hoechst). The dissociative Anaesthesia ketamine HCl was procured from Neon Pharmaceuticals, and the muscle relaxant xylazine was procured from Indian Immunologicals. Cardiac biomarkers were assayed using commercial kits using the Robonik semi-autoanalyzer. The AST, ALT, and ALP were obtained from Robonik India Pvt Ltd, Mumbai, CK-MB and CK-NAC from Lab-Care Diagnostics Pvt Ltd, Mumbai, and LDH from Accurex Biomedical Pvt Ltd, India.

2.3. Experimental Model

The in vivo experimental grouping was as follows

Group I (Normal control): Rats received saline orally for 10 days.

Group-II (CYP control): Rats received 200mg/kg of Cyclophosphamide (CYP)intraperitoneally on the first day of the experimental period[6].

Group-III(VPML 2mg/kg, p.o): Rats received 2mg/kg Verapamil (VPML) orally during 10 days and, in addition, received 200 mg/kg Cyclophosphamide (CYP) intraperitoneally on the first day of the experimental period[6,7].

Group-IV(STV 50mg/kg,p.o)- Rats received 50mg/kg Stevioside (STV) orally and, in addition, received 200 mg/kg Cyclophosphamide (CYP) intraperitoneally on the first day of the experimental period[6–8].

Group-V (STV +VPML): Rats received 50mg/kg Stevioside (STV) orally, and 2 mg/kg Verapamil (VPML) orally during 10 days, and, in addition, received 200 mg/kg Cyclophosphamide (CYP) intraperitoneally on the first day of the experimental period.

2.4. Electrocardiography (ECG) Recording

Rats were anaesthetised with Ketamine (70 mg/kg i.p) and Xylazine (10 mg/kg i.p). A digital physiograph was used to record bipolar limb leads (I, II, III) and a near-left-ventricle chest lead at 0.5 mV sensitivity and 100 mm/s paper speed[9]. Rats were placed on their backs with the front legs slightly bent and hind legs straight[10]. Electrodes were placed on limbs using subcutaneous needles. The measured parameters included heart rate, PR, RR, QRS, and QT[11].

2.5. Biochemical Estimation

Blood samples were collected through the retro-orbital plexus puncture method from the anaesthetised rats. The obtained blood was quickly put in centrifuge tubes, and serum was centrifuged at 3000 rpm for 15 minutes[12]. The clear serum supernatant was carefully aliquoted and analyzed for cardiac injury markers using a semi-automated clinical chemistry analyzer with commercially available diagnostic kits following the manufacturers' standardized protocols: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) kits, creatine kinase-MB (CK-MB), creatine kinase N-acetyl cysteine (CK-NAC) and lactate dehydrogenase (LDH)[6,13]. Each enzyme activity was measured and recorded as international units per litre (IU/L) of serum using predetermined calibration curves included with each kit[14].

2.6. Statistical Analysis

Results are expressed as mean ± SE. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. $P<0.05$ was considered significant[15].

3. Results

3.1. Effect on electrocardiographic parameters:

Effect on heart rate

The CYP control group demonstrated an extremely significant ($p\leq0.001$) decrease in heart rate compared to the normal control group.

All the treatment groups, such as Verapamil 9mg/kg, p.o. (VPML), Stevioside 50 mg/kg, p.o. (STV), STV + VPML, showed extremely significant ($p\leq0.001$) increase in heart rate compared to the CYP control group.

STV + VPML showed an extremely significant ($p\leq0.001$) increase in heart rate compared to the VPML alone-treated group. **(Figure 1) (Table 1)**

Effect on QRS duration

The CYP control group demonstrated an extremely significant ($p\leq0.001$) increase in QRS duration compared to the normal control group.

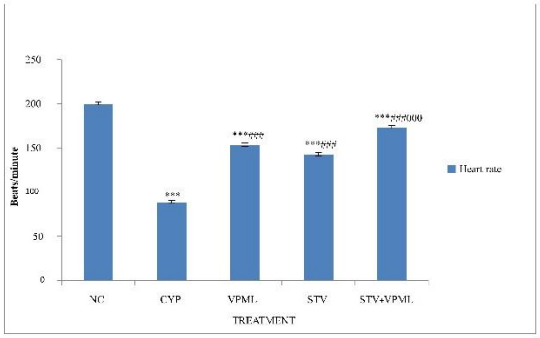
All the treatment groups, such as Verapamil 9mg/kg p.o (VPML), Stevioside 50mg/kg p.o (STV), STV+VPML showed a highly significant (p≤0.001) decrease in QRS duration compared with the CYP control group.

Table 1: Effect of drugs on heart rate and electrocardiographic parameters in cyclophosphamide-induced cardiotoxicity.

Treatment	Heart rate (Beats/minute)	QRS duration (ms)	QT segment (ms)	RR interval (ms)	PR interval (ms)
Normal control	199.52±4.27	120.28±3.52	111.90±5.95	192.80±3.88	79.38±3.40
CYP control	88.12±4.02***	267.78±4.64***	244.45±5.91***	303.86±3.36***	178.51±5.50***
VPML	152.81±1.8***###	198.50±3.21***###	179.61±2.97***###	252.80±4.84***###	109.36±3.82***###
STV	141.78±1.71***###	223.40±3.16***###	193.45±2.27***###	269.19±1.77***###	141.03±2.87***###
STV+VPML	172.87±2.61***###000	175.82±3.34***###00	143.08±2.27***###000	232.20±5.55***###000	98.52± 3.91***###00

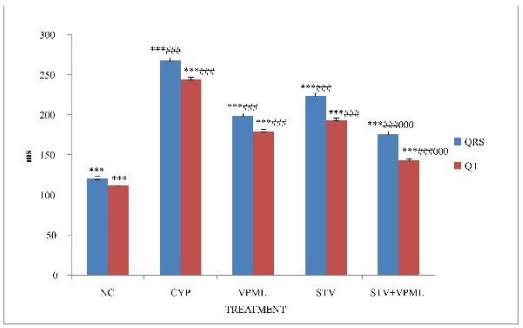
All values are mean ± SEM, n=6, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to Normal control, ###P< 0.001 compared to Cyclophosphamide control and 000P<0.001 when compared to VPML (9mg/kg).

Figure 1: Effect on heart rate in cyclophosphamide-induced cardiotoxicity



All values are mean ± SEM, n=6, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to Normal control, ###P< 0.001 compared to Cyclophosphamide control and 000P<0.001 when compared to VPML (9mg/kg).

Figure 2: Effect on QRS & QT intervals in cyclophosphamide-induced cardiotoxicity.



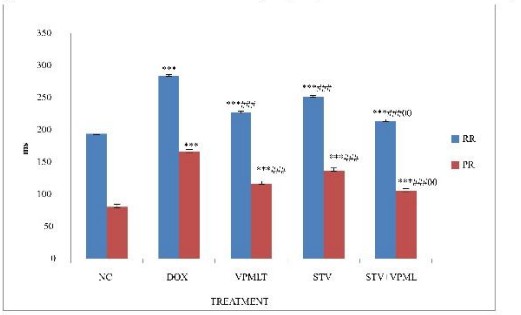
All values are mean ± SEM, n=6, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to Normal control, ###P< 0.001 compared to Cyclophosphamide control and 000P<0.001 when compared to VPML (9mg/kg).

STV+VPML shows a moderately significant (p≤0.01) decrease in QRS duration compared to the VPML-treated group. **(Figure 2) (Table 1)**

Effect on the QT segment

The CYP control group demonstrated an extremely significant (p≤0.001) increase in QT duration compared to the normal control group.

Figure 3: Effect on RR & PR intervals in cyclophosphamide-induced cardiotoxicity



All values are mean ± SEM, n=6, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to Normal control, ###P< 0.001 compared to Cyclophosphamide control and 000P<0.001 when compared to VPML (9mg/kg).

Figure 4: Effect of drugs on electrocardiographic parameters in Cyclophosphamide-induced cardiac stress.

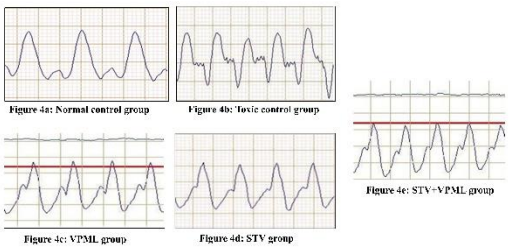
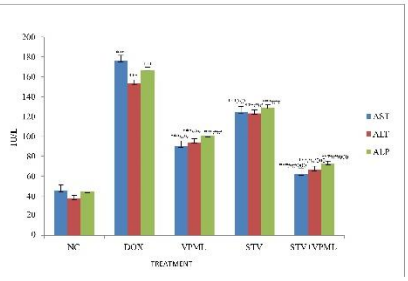


Figure 4a: Normal control group **Figure 4b: Toxic control group** **Figure 4c: VPML group** **Figure 4d: STV group** **Figure 4e: STV+VPML group**

Figure 5: Effect on AST, ALT, and ALP levels in cyclophosphamide-induced cardiotoxicity.



All values are mean ± SEM, n=6, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to Normal control, ###P< 0.001 compared to Cyclophosphamide control and 000P<0.001 when compared to VPML (9mg/kg).

All the treatment groups, such as VPML 9 mg/kg p.o, STV 50mg/kg p.o, and STV + VPML, showed an

Table 2: Effect of drugs on serum level of AST, ALT, and ALP in cyclophosphamide-induced cardiotoxicity.

TREATMENT	SERUM IN IU/L		
	AST	ALT	ALP
Normal control	46.87±4.19	30.58±3.04	46.24±3.39
CYP	213.94±6.26***	147.80±5.65***	217.51±4.37***
VPML	106.01±1.44***###	82.55±6.44***###	156.46±3.71***###
STV	126.79±2.79***###	102.19±5.26***###	179.68±5.58***###
STV+VPML	90.86±1.50***###000	55.86±7.55**###000	132.79±2.87***###000

All values are mean ± SEM, n=6, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to Normal control, ###P< 0.001 compared to Cyclophosphamide control and 000P<0.001 when compared to VPML (9mg/kg).

extremely significant (p≤0.001) decrease in the QT segment compared to the CYP control group.

STV+VPML showed an extremely significant (p≤0.001) decrease in the QT segment compared to the VPML-treated group. **(Figure 2) (Table 1)**

Effect on RR interval

The CYP control group demonstrated an extremely significant (p≤0.001) increase in RR interval compared to the normal control group.

All the treatment groups, such as VPML 9mg/kg p.o, STV50mg/kg p.o, and STV+VPML, showed extremely significant (p≤0.001) decrease in RR interval compared to the CYP control group.

STV+VPML showed an extremely significant (p≤0.001) decrease in RR interval compared to the VPML-treated group. **(Figure 3) (Table 1)**

Effect on PR interval

The CYP control group demonstrated a highly significant (p≤0.001) increase in PR interval compared with the normal control group.

All the treatment groups, such as VPML 9mg/kg p.o, STV 50mg/kg p.o, and STV+VPML, showed extremely significant (p≤0.001) decrease in PR interval compared to the CYP control group.

STV+VPML found a moderately significant (p≤0.01) decrease in PR interval compared to the VPML-treated group. **(Figure 3) (Table 1)**

Representative electrocardiographic (ECG) tracings of the normal control **(Figure 4a)**, toxic control **(Figure 4b)**, VPML-treated **(Figure 4c)**, STV-treated **(Figure 4d)**, and combined STV+VPML-treated groups **(Figure 4e)** are shown below. **(Figure 4)**

3.2. Effect of hemodynamic parameters:

Effect on AST

The CYP control group demonstrated an extremely significant (p≤0.001) increase in serum AST level compared to the normal control group.

All the treatment groups, such as VPML 9mg/kg p.o, STV 50mg/kg p.o, and STV+VPML, showed extremely significant (p≤0.001) decrease in serum AST level compared to the CYP control group.

STV+VPML showed a moderately significant (p≤0.01) decrease in serum AST level compared to the VPML-treated group. **(Figure 5) (Table 2)**

Effect on ALT

The CYP control group demonstrated an extremely significant (p≤0.001) increase in serum ALT level compared to the normal control group.

All the treatment groups, such as VPML 9mg/kg p.o, STV 50mg/kg p.o, and STV +VPML, showed extremely significant (p≤0.001) decrease in serum ALT level compared to the CYP control group.

STV+VPML showed an extremely significant (p≤0.001) decrease in serum ALT level compared to the VPML-treated group. **(Figure 5) (Table 2)**

Effect on ALP

The CYP control group demonstrated an extremely significant (p≤0.001) increase in serum ALP level compared to the normal control group.

All the treatment groups, such as VPML 9mg/kg p.o, STV 50mg/kg p.o, and STV +VPML, showed extremely

significant (p≤0.001) decrease in serum ALP level compared to the CYP control group. STV+VPML showed an extremely significant (p≤0.001) decrease in serum ALP level compared to the VPML-treated group. **(Figure 5) (Table 2)**

Effect on CKMB

The CYP control group demonstrated an extremely significant (p≤0.001) increase in serum CKMB level compared to the normal control group.

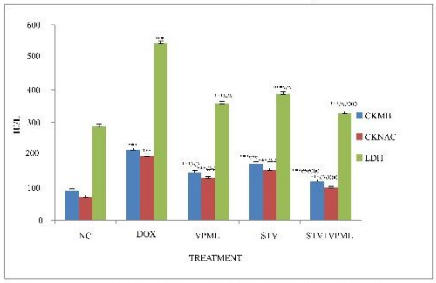
All the treatment groups, such as VPML 9mg/kg p.o, STV 50mg/kg p.o, and STV+VPML, showed extremely significant (p≤0.001) decrease in serum CKMB level compared to the CYP control group.STV +VPML showed an extremely significant (p≤0.001) decrease in

Table 3: Effect of drugs on serum level of CK-MB, CK-NAC and LDH in cyclophosphamide-induced cardiotoxicity.

Treatment	Serum level in IU/L		
	CKMB	CKNAC	LDH
Normal control	70.68±4.22	72.07±3.85	296.90±4.56
CYP control	223.62±5.73***	193.79±3.49***	676.22±4.10***
VPML	130.80±2.87***###	144.11±6.33***###	364.91±3.44***###
STV	144.62±5.12***###	165.94±6.89***###	385.16±6.37***###
STV +VPML	101.05±2.62***###000	110.79±5.25***###000	321.43±5.07***###00

All values are mean ± SEM, n=6, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to Normal control, ###P< 0.001 compared to Cyclophosphamide control and 000P<0.001 when compared to VPML (9mg/kg).

Figure 6: Effect on CKMB, CKNAC, and LDH levels in cyclophosphamide-induced Cardiotoxicity



All values are mean ± SEM, n=6, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to Normal control, ###P< 0.001 compared to Cyclophosphamide control and 000P<0.001 when compared to VPML (9mg/kg).

serum CKMB level compared to the VPML-treated group. **(Figure 6) (Table 3)**

4. Discussion:

The current study systematically compared the combined cardioprotective effect of stevioside (STV), a natural glycoside of *Stevia rebaudiana* (Asteraceae) leaf with established antihypertensive, antihyperglycemic and hepatoprotective properties, with cyclophosphamide (CYP)-induced cardiotoxicity. CYP, via its active metabolites phosphoramide mustard and acrolein, induces oxidative stress and causes endothelial capillary injury, which results in protein/erythrocyte extravasation, myocardial oedema, interstitial haemorrhage, microthrombi formation, and cardiomyocyte necrosis, respectively[6,9].

We have found that CYP causes myocardial injury, as evidenced by significant increases in serum cardiac injury biomarkers, such as lactate dehydrogenase (LDH), creatine kinase-NAC (CK-NAC), creatine kinase-MB (CK-MB), aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase. At the same time, CYP generated typical electrocardiographic changes such as bradycardia (reduced heart rate) and conduction delays (prolonged RR, PR, QT intervals and QRS complex duration) as it had a known pathophysiology of hemorrhagic myocarditis and fibrosis[11].

Biochemical and electrophysiological derangements were significantly reduced with individual treatments using VPML and STV versus CYP controls. Nevertheless, the STV+VPML combination proved to be more cardioprotective, with much higher restoration of biomarker levels and ECG parameters compared to VPML monotherapy. This increased activity is probably due to complementary effects: VPML stabilizes cardiac electrophysiology by inhibiting L-type Ca^{2+} channels and slowing their re-opening, and STV is an antioxidant of CYP-mediated reactive oxygen species on top of its known potentiation of calcium channel blockade. This synergistic pharmacodynamic effect indicates that STV increases VPML bioavailability or efficacy, possibly via pharmacokinetic alteration, which requires transporter interactions (P-glycoprotein inhibition by VPML) and molecular pathways (e.g., MAPK, cAMP signalling) to be investigated further.

Clinical implications of these findings have the potential to be applied in adjunctive cardioprotection in CYP-based chemotherapy regimens due to CYP criticality in lymphoma, leukaemia, and transplantation regimens, despite CYP 10-28% incidence of cardiotoxicity in high doses. Natural origin and safety profile of STV further increase translational potential. To facilitate clinical use, future research should clarify the exact mechanisms of interaction, the best dose schedule to use, and long-term safety in chronic models.

5. Conclusion

The current study has shown that stevioside (50 mg/kg, p.o.) + verapamil (9 mg/kg, p.o.) offers greater cardioprotection against cardiotoxicity induced by cyclophosphamide in rats than verapamil alone through significant improvements in serum cardiac injury biomarkers (LDH, CK-MB, CK-NAC, AST, ALT, ALP).

This synergistic effect indicates a possible pharmacodynamic interaction allowing an increase in the therapeutic effect. This combination therapy has potential clinical use in reducing the dosage of verapamil and preventing the negative effects of this agent in case of chemotherapy using cyclophosphamide and preserving the cardioprotective effects. More pharmacokinetic, molecular, and clinical research is justified to confirm these results to be used in therapy.

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