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Cardioprotective activity of *Piper betle* juice in isoproterenol induced hypertrophic rat models

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ABSTRACT

Impaired electrolytes (preferably Na^+ , K^+ , Cl^- , HCO_3^-) is one of the key fundamental complications associated with cardiac hypertrophy (enlarged heart) mediated heart failure. *Piper betle* (*P. betle* - family Piperaceae) due to its potent antioxidant activity is used in various pharmacological applications. The present study evaluates the cardioprotective (anti-hypertrophic) potential of aqueous (juice) extract of *P. betle* in restoring electrolyte homeostasis, thereby its ability to curb the fundamental progression of hypertrophy. Isoproterenol (ISO - 10 mg/kg b.w., i.p., 7 days) induced cardiac hypertrophic rats were developed that were simultaneously treated with the standard drug losartan (50mg/kg b.w., orally, 7days) and aqueous juice extract of *P. betle* (30mg/kg b.w., orally, 7 days). The biochemical estimation of glucose, protein, cholesterol, cardiac marker enzymes like SGOT, SGPT, LDH, enzymatic antioxidants namely SOD, catalase, GPx and electrolytes (Na^+ , K^+ , Cl^- and bicarbonate) in serum were statistically performed along with the histopathological analysis of left ventricles (H&E stain). The present study showed increased levels of glucose, protein, cholesterol, cardiac marker enzymes, reduced enzymatic antioxidants with reduced electrolytes during cardiac hypertrophy (ISO) that were reflected in a histopathological analysis by thickened abnormal myocardial architecture which was reversed similar to normal in plant juice administered rats. Hence, this study demonstrated the beneficial effect of *P. betle* (aqueous juice) on electrolytes (Na^+ , K^+ , Cl^- , HCO_3^-), thereby imposing an interventional effect against cardiac hypertrophy.

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INTRODUCTION

Cardiac hypertrophy (CH) is the clinical condition in which the heart is enlarged due to a constitutive uncontrolled state of the adaptive response of cardiomyocytes towards vigorous functional stress. CH is characterized by the thickening of the ventricular wall, increased heartbeat and enlargement (mostly left ventricle) that leads to diastolic and systolic dysfunctions. It has become one of the major causes of morbidity and mortality in the world (Zhao *et al.*, 2017). The two types of cardiac hypertrophy, namely physiological and pathological, are due to aggressive exercise (as seen in athletes mostly), pressure, volume, overload and endocrine disorders, respectively (Yan *et al.*, 2015).

In India, the mortality rate in cardiovascular diseases (CVD) has been increased by 59% from 23 million (1990) to 37 million (2010) (Prabhakaran *et al.*, 2016). The treatment for CH is usually based on the electrocardiographic (ECG) or echocardiographic (ECHO) or magnetic resonance imaging (cardiac MRI) that estimates left ventricular mass (Buss *et al.*, 2012). The treatment options involve surgical procedures, lifestyle changes and medication, which include β -blockers, Calcium channel blockers and anti-hypertensive drugs (Zwieten, 2000). Among the various mechanism of pathophysiology underlying CH, electrolytes being the fundamental player of the cardiac electrophysiological events is impaired during cardiac hypertrophy, primarily potassium. Supplements to improvise the electrolyte imbalances are suggested for treating cardiac hypertrophy and CVDs (Wang *et al.*, 2005).

Piper betle belongs to the Piperaceae family, widely distributed in the tropical and sub-tropical regions of the world. The leaves of *P. betle* are used as carminative and wound healing in the traditional system of medicine (Atiya *et al.*, 2017). Phytochemical investigation of the plant revealed that the presence of protein, amino acids, saponins, tannins, flavonoids and glycosides (Rekha *et al.*, 2014).

The plant showed various therapeutic uses such as anti-bacterial (Hoque *et al.*, 2012), antioxidant, anti-diabetic, anti-malarial and anti-inflammatory (Alam *et al.*, 2013; Arambewela *et al.*, 2005). This study aims to evaluate the cardio-protective (anti-hypertrophic) activity of aqueous extract (juice) of *Piper betle* by biochemically investigating the effects of *P. betle* juice upon restoring the electrolyte balance (Na^+ , K^+ , Cl^- , HCO_3^-).

MATERIALS AND METHODS

Chemicals

All the chemicals were purchased from Hi-media laboratories, India. Isoproterenol was purchased from Sigma-Aldrich. The standard drug losartan (50 mg tablets) was purchased commercially from a local pharmacy, India. The biochemical estimation kits were purchased from Arkay Healthcare Pvt. Ltd and from Agappe Pvt. Ltd., India.

Plant collection and extraction

P. betle was identified and authenticated by the Botanical Survey of India (BSI/SRC/5/23/2019/Tech./2694) and the leaves of *P. betle* were collected during the month of December 2019 from the local area of Krishnagiri district, Tamil Nadu, India. The collected leaves were shade dried and ground to a coarse powder,

extracted using cold maceration with distilled water for about 72 hours and then filtered to prepare the aqueous (juice) (Alam *et al.*, 2013), which was subjected to further studies.

Procurement of animal

Male Wistar albino rats (100-200g) were procured after the approval by the Institutional Animal Ethics Committee (IAEC) and the Ethical Clearance (CPC-SEA/No 421/2018/IAEC).

The animals were acclimatized under standard laboratory conditions for 3 days with controlled temperature ($29^\circ \pm 5^\circ\text{C}$), humidity ($55\% \pm 5\%$), and 12 hours of light/dark cycles.

Experimental design

Experimental rats were grouped into 4 categories (n=6 per group). Isoproterenol was administered to rats for inducing cardiac hypertrophy with the simultaneous treatment of aqueous juice of *P. betle* and the standard drug losartan for a week as follows:

Group 1 — Normal (control rats received saline),

Group 2 — Isoproterenol (10mg/kg b.w., i.p., 7days) (Mohan and Bloom, 1999),

Group 3 — Isoproterenol + Losartan (50mg/kg b.w., oral., 7days) (Shahinfar *et al.*, 1999),

Group 4 — Isoproterenol + aqueous leaf juice of *P. betle* (30mg/kg b.w., oral., 7days) (Khatun *et al.*, 2018).

In the end, the serum and heart tissue (left ventricle) were isolated for further biochemical and histopathological analysis.

Hypertrophic indices

The hypertrophic indices such as heart weight – HW, bodyweight – BW, HW/BW ratio were measured (Planavila *et al.*, 2013).

Biochemical estimation

Serum glucose was assayed using glucose oxidase method (AUTOSPAN Liquid Gold Glucose Kit), serum total protein by Lowry's method (Lowry *et al.*, 1951), serum albumin using Bromocresol green endpoint assay method (AUTOSPAN), estimation of serum total cholesterol using POD-PAP enzymatic endpoint assay (AUTOSPAN), estimations of SGOT and SGPT activities by modified IFCC method (Microlyn) and Electrolytes (Sodium Potassium, Chloride and Bicarbonate) (Tiagha and ., 2015) and determination of LDH activity by optimized kinetic assay method (AUTOSPAN) followed by estimation of SOD (Kakkar *et al.*, 1984), catalase (Sinha, 1972) and glutathione peroxidase (Rotruck *et al.*, 1973).

Histopathological analysis

The excised heart tissues were cleaned and preserved in 10% formalin immediately until tissue processing (left ventricle) as transverse, 5 μ m thick paraffin sections. The sections were stained with hematoxylin and eosin and the cellular configuration of the heart tissues were examined by scanning the stained slides (40 X) (Mahmoud *et al.*, 2015).

Statistical analysis

Data obtained were expressed as mean \pm standard deviation. Statistical analysis was performed by using the Student t-test in SPSS.25 statistical package and values of $P < 0.05$ were considered as significance level (Cochran and Snedecor, 1946).

RESULTS AND DISCUSSION

P. betle an edible and potent antioxidant herb (Prabu *et al.*, 2012) is studied enormously in treating various diseases, but its intervening effect towards CH is less explored. Besides the increased necrosis and cellular inflammation, electrolyte imbalance plays a fundamental key role in the progression of CH (Urso *et al.*, 2015).

Effect of *P. betle* on hypertrophic index

CH characterized by thickened left ventricle due to increased protein synthesis could contribute to the increased heart weight that serves as the primary phenotypic index of CH as indicated by the increased HW/BW ratio in ISO administered rats when compared to normal and plant juice administered groups (Yang *et al.*, 2016) as shown in Table 1.

Effect of *P. betle* on glucose, total protein and total cholesterol

ISO elevates glucose, protein and cholesterol levels by impairing insulin activity and AMPK activities. Decreased albumin (hypoalbuminemia), considered as risk factors for cardiac hypertrophy is observed in ISO, which could be attributed to the inhibitory effects of ISO upon albumin (Doss and Kuberapandian, 2019). Similarly, in this study, raised glucose, protein and cholesterol levels and decreased albumin (hypoalbuminemia), as shown in Table 2, were observed in the hypertrophic groups were reduced after treatment with the plant extract and losartan.

Effect of *P. betle* on serum cardiac marker enzymes

Increased cellular stress with decreased antioxidants is indicated by the elevated levels of SGOT, SGPT and LDH during CH, thereby imposing risk upon cardiac function. Similar results were observed in most studies, but LDH in a recent study

was reported with decreased levels in ISO treated groups due to metabolic alterations of AMPK. So, the relationship between AMPK-LDH warrants further investigation in terms of CH (Doss and Kuberapandian, 2019; Long, 2006; Doss *et al.*, 2018). Cardiac markers enzymes, namely SGOT, SGPT, LDH, were increased in serum of isoproterenol induced rats and were reduced by the administration of aqueous (juice) extract of *P. betle* as shown in Table 3.

Effect of *P. betle* on enzymic antioxidants

Eugenol is a reported anti-hypertrophic phyto compound (Choudhary *et al.*, 2006). Similar compounds such as isoeugenol, chavibetol, hydroxychavicol (Vikash *et al.*, 2012) present in this plant are potent antioxidants and cardioprotective compounds, which could be attributed to the anti-hypertrophic potential of *Pbetle* juice which requires further investigations. Free radicals trigger the cellular stress underlying the metabolic and cellular alterations involved in the pathophysiology of CH (Widowati *et al.*, 2013; Maulik and Kumar, 2012). ISO being cardiotoxic induces oxidative stress marked by the decreased levels of enzymic antioxidants, which upon treatment using *Pbetle* is involved in the restoration of SOD, CAT and GPx when compared to ISO administered rats (Doss and Kuberapandian, 2019; Wexler, 1979; Wu *et al.*, 2009). Decreased enzymic antioxidants (SOD, catalase, GPx) and increased level of lipid peroxidation were seen in the isoproterenol induced rat models. Administration of aqueous (juice) extract of *P. betle* showed increased levels of SOD, catalase and GPx, as shown in Table 4.

Effect of *P. betle* on serum electrolytes

ISO administration caused decreased levels of serum electrolytes, which can be considered as a risk factor for CH that may progress to ventricular arrhythmias and even myocardial infarction (Brembilla-Perrot *et al.*, 1993; Okin *et al.*, 2012). To our knowledge, *Pbetle* is reported to have beneficial effects upon Na^+/K^+ , Ca^{2+} and Mg^{2+} pumps in erythrocytes (Chitra and Vidya, 2006) but its effect upon serum electrolytes in association with CH pathology is first reported in this study. ISO administration resulted in reduced serum levels of sodium, potassium, chloride and bicarbonate as indicated in Table 5 and their ranges were brought to near normal values when treated with plant extract and losartan.

Histopathological analysis of left ventricle

Cardiotoxicity induced by ISO due to the increased free radicals and metabolic shifts causes cellular stress that results in increased myocardial degen-

Table 1: Effect of *Piper betle* on the hypertrophic index (HW/BW ratio)

Parameter	Group I	Group II	Group III	Group IV
Heart weight (HW - mg)	325 ± 2.51	366 ± 2.64 ^{a*}	328 ± 2.08 ^{b*}	345 ± 3.45 ^{c*}
Bodyweight (BW - g)	101.33 ± 0.70	104 ± 1.41 ^{a*}	105.5 ± 0.07 ^{b*}	102.5 ± 4.56 ^{c*}
HW/BW ratio	3.2 ± 0.036	3.5 ± 0.06 ^{a*}	3.1 ± 0.07 ^{b*}	3.36 ± 1.01 ^{c*}

Values are explicit by Mean ± SD of 6 samples. Group comparison:- Normal (I) Vs. ISO (II); b- ISO (II) Vs. Losartan (III); c- ISO(II) Vs. Aqueous (juice) extract of *Piper betle* (VI). Statistical significance is denoted by*.

Table 2: Effect of *P.betle* on the serum levels of glucose, total protein, albumin and total cholesterol in experimental rats

Groups	Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dL)	Total cholesterol (mg/dl)
Group I	95.25 ± 1.60	6.36 ± 1.52	4.293 ± 1.369	115.005 ± 1.28
Group II	133.25 ± 0.750 ^{a*}	6.60 ± 1.51 ^a	3.106 ± 1.620 ^{a*}	160.61 ± 1.52 ^{a*}
Group III	134.06 ± 1.42 ^{b*}	6.58 ± 1.05 ^b	3.862 ± 1.537 ^{b*}	141.22 ± 1.64 ^b
Group IV	115.43 ± 1.90 ^c	6.40 ± 0.42 ^c	4.104 ± 0.832 ^{c*}	148.59 ± 4.36 ^{c*}

Values are explicit by Mean ± SD of 6 samples. Group comparison:- Normal (I) Vs. ISO (II); b- ISO (II) Vs. Losartan (III); c- ISO(II) Vs. aqueous (juice) extract of *Piper betle* (VI). Statistical significance is denoted by*.

Table 3: Effect of *P.betle* on the serum levels of SGOT, SGPT, and LDH in experimental rats

Group	SGOT (U/L)	SGPT (U/L)	LDH (μkat/l)
Group I	6.610 ± .56	9.320 ± 0.52	85.086 ± 2.65
Group II	8.660 ± .83 ^a	10.900 ± 1.41 ^a	115.34 ± 1.49 ^a
Group III	7.125 ± .33 ^b	10.505 ± .73 ^b	100.62 ± 8.80 ^b
Group IV	7.610 ± .43 ^c	9.705 ± .93 ^c	94.350 ± 1.25 ^{c*}

Values are explicit by Mean ± SD of 6 samples. Group comparison:- Normal (I) Vs. ISO (II); b- ISO (II) Vs. Losartan (III); c- ISO (II) Vs. Aqueous (juice) extract of *Piper betle* (VI). Statistical significance is denoted by*.

Table 4: Serum levels of enzymatic antioxidants in experimental rats

Groups	SOD	Catalase	GPx
Group I	47.79 ± 1.91	5.81 ± 1.23	8.04 ± 1.51
Group II	26.53 ± 2.58 ^a	2.83 ± 0.86 ^a	8.045 ± 0.86 ^a
Group III	32.83 ± 2.42 ^b	5.02 ± 0.51 ^{b*}	8.02 ± 0.28 ^b
Group IV	43.32 ± 2.06 ^c	4.42 ± 1.64 ^{c*}	7.73 ± 1.06 ^c

Values are explicit by Mean ± SD of 6 samples. Group comparison:- Normal (I) Vs. ISO (II); b- ISO (II) Vs. Losartan (III); c- ISO(II) Vs. Aqueous (juice) extract of *Piper betle* (VI). Statistical significance is denoted by*.

Table 5: Serum levels of electrolytes of experimental rats

Groups	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
Group I	153.23 ± 1.65	7.42 ± 0.48	182.66 ± 6.11	33.28 ± 2.38
Group II	150.88 ± 1.24 ^{a*}	4.70 ± 0.16 ^{a*}	154.80 ± 2.69 ^{a*}	29.62 ± 1.44 ^{a*}
Group III	157.37 ± 1.37 ^b	6.60 ± 0.45 ^{b*}	161.67 ± 1.53 ^b	31.12 ± 2.29 ^b
Group IV	153.89 ± 1.56 ^c	6.83 ± 0.10 ^{c*}	167.55 ± 1.63 ^{c*}	32.22 ± 2.86 ^c

Values are explicit by Mean ± SD of 6 samples. Group comparison:- Normal (I) Vs. ISO (II); b- ISO (II) Vs. Losartan (III); c- ISO(II) Vs. Aqueous (juice) extract of *Piper betle* (VI). Statistical significance is denoted by*.

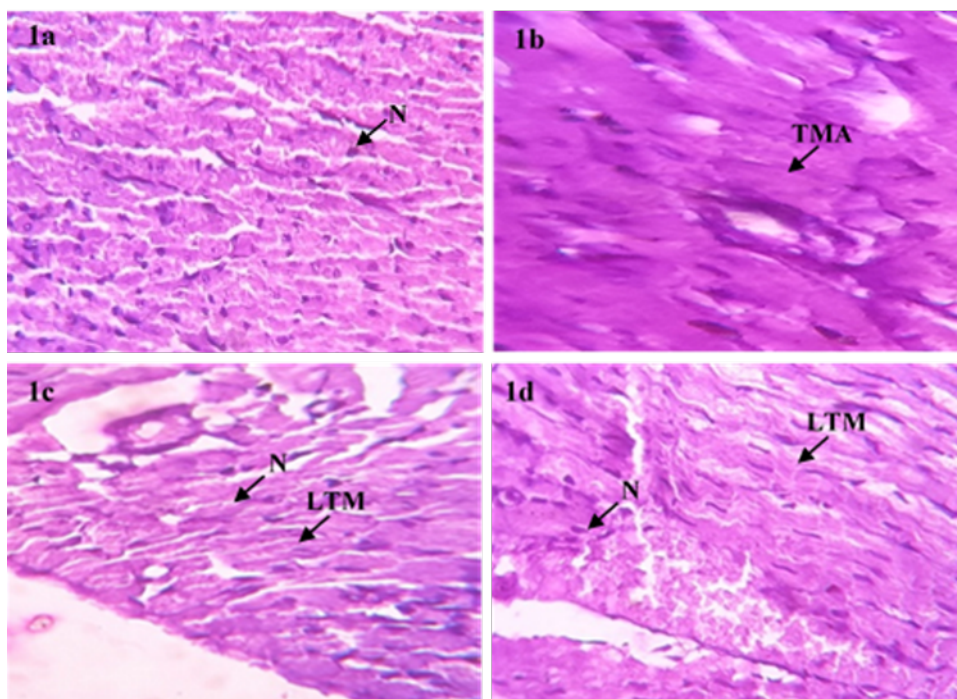


Figure 1: Histopathological analysis of heart tissue (left ventricle) experimental animals using hematoxylin-eosin stain.

eration with reduced cardiomyocytes (Doss and Kuberapandian, 2019; Padma *et al.*, 2013). In CH, the enlargement of the heart is depicted by thickened ventricle (preferably left ventricle) with distorted myocardial architecture, as shown similarly in this study.

The strong antioxidant, anti-inflammatory potentials and metabolic restoration ability (herein via serum electrolytes) showed reduced inflammatory and degeneration of the heart tissue (left ventricle). In the isoproterenol administered group Figure 1(b), the enlargement of heart tissue was observed by the presence of thickened cellular architecture with distorted myofibrils, as shown in Figure 1.

In the aqueous (juice) extract administered rats administered group Figure 1(d), reduced cellular thickening and necrosis were observed similar to the standard drug losartan administered rats Figure 1(c) that showed better myofibrillar rearrangement similar to the control group Figure 1(a).

Figure 1(a) represents an organized myocardial network of nucleated (N) cells (group 1 – normal). Figure 1(b) indicates the thickened myocardial architecture (TMA) with invisible nucleated cellular structure as a marked feature of cardiac hypertrophy. Figure 1(c) and Figure 1(d) indicate the presence of visibly nucleated cells with rejuvenating striated heart tissue organization and less thickened myocardial architecture (LTM) when compared to

normal and hypertrophic tissues.

CONCLUSION

The present study primarily concludes that the *Piper betle* aqueous (juice) extract possesses the ability to reverse the cellular effects induced by isoproterenol induced cardiac hypertrophy animal model. This has been confirmed by both biochemical and histopathological studies in this work. It is shown to restore electrolytes to near-normal levels. Further studies are required to check the electrophysiological benefits of *P. betle* and to isolate its bioactive compounds like chavibetol, hydroxychavicol, isoeugenol for checking its anti-hypertrophic effect. As *P. betle* possesses effect upon restoring electrolyte balance, this study also suggests that it can be extended to the treatment of blood pressure and other electrolyte mediated complications.

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Conflict of interests

The authors declare that they have no conflict of interest for this study.

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