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Effect of hydroethanolic extract of *Piper betle* in isoproterenol induced cardiac hypertrophy

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ABSTRACT

Cardiac hypertrophy (CH) is a condition in which myocardial mass is increased beyond the normal range due to irreversible fibrotic events that lead to various complication like ventricular chamber dilation, thinning of the internal walls and extensive myocardial damage. In this study, the cardioprotective potential of the hydro-ethanolic extract of *Piper betle* (*P. betle*) was evaluated against cardiac hypertrophy induced by isoproterenol in male albino Wistar rats. Isoproterenol (10 mg/kg b.w., i.p., 7 days) induced cardiac hypertrophy in experimental rats which were simultaneously treated with the standard drug losartan (50 mg/ kg b.w., oral., 7 days) and hydro-ethanolic extract of *P. betle* (200 mg/kg b.w., oral., 7 days). Biochemical estimations revealed increased levels of glucose, protein, albumin, lipid profiles (total cholesterol, HDL and triglycerides), urea, creatinine, cardiac maker enzymes (SGOT; SGPT and LDH), reduced enzymic antioxidants (SOD, CAT, GPx) and serum were observed during CH which were reciprocated to normal when treated with plant extract. Histopathological analysis of the heart tissue (left ventricles) showed repairment of cellular architecture with reduced stiffened cell layers and necrosis in plant extract administered rats thereby indicating the anti-hypertrophic potential of *P. betle*.

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INTRODUCTION

Cardiac hypertrophy is an enlargement of the heart due to cellular stress, hypertension and other valvular diseases (Samak *et al.*, 2016). There are two types of hypertrophy, namely physiologic and pathologic hypertrophy. In the case of physiologic hypertrophy, the mass of heart rarely exceeds 500g. Pathologic hypertrophy might exceed 500g but usually never exceeds 1000g (Badeer, 1964) . Cardiac hypertrophy is influenced by both external and internal factors which included the hormone regulation, namely Angiotensin II, Aldosterone, Norepinephrine and insulin.

Piper betle (Family - Piperaceae) is used in the ancient medicinal system. Its leaves possess several biochemical and pharmacological activities as anticancer, antioxidant, antidiabetic, gastroprotective, cytotoxic, antiplatelet, wound healing, chlorophyllase activity, oral hygiene and anti-asthmatic attributes. This study aims to evaluate the anti-hypertrophic (cardioprotective) potential of the *P. betle* leaves (Shah *et al.*, 2016) .

MATERIALS AND METHODS

Chemicals

All the chemicals were purchased as the analytical grade from Hi-Media Laboratories, India. Isoproterenol (Isoprenaline Hydrochloride) was pur-

chased from Sigma- Aldrich. The reference drug losartan (50mg tablets) was purchased commercially from a local pharmacy, Coimbatore, India. The protein, glucose, cholesterol, triglyceride, albumin estimation kits were purchased from Arkray Healthcare Pvt Ltd., India. The SGOT, SGPT and LDH kits were purchased from Agappe Diagnostics Ltd., India. The urea and Creatinine kits were obtained from Pariksha Neochem Pvt. Ltd., India.

Plant authentication and preparation of hydroethanolic extract of *P. betle*

P. betle leaves were collected from Krishnagiri district, Tamil Nadu, India, during the month of January and authenticated (BSI/SRC/5/23/2019/Tech/2690) at Botanical Survey of India, Southern Region Centre, Coimbatore, India. The hydroethanolic extract of *P. betle* was prepared in 50:50 ratio by cold maceration (72 hours), filtered and dried using controlled temperature in water bath (T *et al.*, 2015) where 152 g of crude hydroethanolic extract was obtained whose yield was 30.4% which was subjected to further analysis (Azwanida, 2015) **.**

Quantitative analysis of hydroethanolic extract of *P. betle*

With the large scale prepared various quantitative analysis were performed to evaluate the concentration of different functional groups present in the given sample namely protein, glucose, carbohydrates, flavonoids, tannins, total phenols (Kumar *et al.*, 2018)**.**

Free radical scavenging assay of hydroethanolic extract of *P. betle*

DPPH radical scavenging activity and nitric oxide radical scavenging activity of the hydroethanolic leaf extract was performed to assay the free radical scavenging activity the leaf extract. Different range of concentration of the extracts namely 100, 200, 300, 400 ,500 (μg/ml) were used to calculate the percentage (%) inhibition (Seo *et al.*, 2014).

FTIR analysis of hydroethanolic extract of *P.betle*

The crude obtained from the hydroethanolic leaf extract of *P. betle* was characterized by FTIR spectrum to study the conformational changes and the presence variations functional groups contributed from carbohydrates, lipids, proteins and other components of the plant (Wei *et al.*, 2015) **.**

Procurement of animals

Male albino Wister rats weighing 100-200g procured and ethical clearance for handling of experimental animals was obtained from the Institutional Animal Ethics Committee (CPSCEA/NO.422/2018/IAEC) at the PSG Institute of Medical Science and Research (PSG IMS & R), Coimbatore. The animals were acclimatized under standard laboratory conditions for 3 days with controlled temperature (29° $\pm$ 5°C), humidity (55% $\pm$ 5%), and 12 hours of light/dark cycles**.**

Experimental groups

The experimental rats were divided into four groups (n= 3 animals each group). In each group, Cardiac hypertrophy was induced to albino wistar rats using isoproterenol and simultaneous treatment using the hydroethanolic plant extract and the reference drug, and losartan was done, as shown in Table 1,Table 2 shows,

After the end of the experimental treatment period (7 days), the grouped animals were sacrificed under mild anaesthesia. Blood was collected by cardiac puncture method, and the serum sample was separated by centrifugation at 5000 rpm for 20 min (Doss and Kuberapandian, 2019) . Then, immediately, the heart tissues were excised for histopathological analysis.

Hypertrophic indices

The status of cardiac hypertrophy was assessed using the hypertrophic indices, body weight (BW), Heart weight (HW), HW/BW ratio (Sánchez-Campos *et al.*, 1999) .

Biochemical parameters

Serum Glucose was assayed by using Glucose oxidase method (Auto span Liquid Gold Glucose Kit), Serum Total Protein by Lowry's Method, Serum Albumin by using Bromo cresol green end point Assay method (Auto span), Estimation of Serum cholesterol by using POD-PAP enzymatic end point assay (Auto span) ,Estimation of SGOT by modified method (Microlyn) ,Estimation of urea by modified Berthelot Method, Estimation of Creatinine by optimized Kinetic Jaffe's Method and Determination of LDH activity by optimized Kinetic method (Auto span) followed by Estimation of Superoxide dismutase (SOD),catalase and glutathione peroxidise (GPx) (Mahmoud *et al.*, 2015) .

Histopathological analysis

The excised hearts were preserved in 10% formalin in paraffin until the tissues were processed as transverse, 5μm thick paraffin, left ventricular sections. The dyes such as haematoxylin and eosin (H & E) were used to stain these sections, and they were magnified (40X) for analysing the cellular architecture of the heart tissues (Gudbjarnason *et al.*, 1964).

Statistical analysis

Data obtained from the results were expressed as

Table 1: Experimental groups of induction and treatment of cardiac hypertrophy

GROUPS	EXPERIMENTAL ANIMALS
Group 1	Normal control rats
Group 2	Isoproterenol (10 mg/kg b.w., i.p., 7 days) (Saxena and Panjwani, 2014)
Group 3	Isoproterenol + Losartan (50 mg/kg b.w., oral., 7 days) (LIN and LIN, 2009)
Group 4	Isoproterenol + Hydroethanolic leaf extract of P. betle (100 mg/kg b.w., oral., 7 days) (Hoff, 2000)

Table 2: Peak table of FTIR analysis of hydroethanolic extract of P. betle

Type	Absorbance frequency (Cm-1)	Intensity	Remarks and Assignment
Amines	3873.06	W	N-H stretch
Carboxylic acids	3410.15		Broad OH stretch
Alkanes	2931.80		C-H stretch
Phosphine	2368.59		P-H stretch
Amines	1620.21	WM	N-H bend
Sulphate	1401.18		S=O
Alkyl halides	1280.73	VS	C-F stretch
Ethers	1049.28	MS	=C-O-C symmetrical
Aromatic compounds(p-disubstitued)	871.82		CH bond
Aromatic compounds(monosubstituted)	777.53		CH bond
Alkyl halides	570.93		C-Br stretch

mean ± SD. Statistical analysis was performed using Student 't' test in SPSS software (version 16.0) and the P-value < 0.05 was considered statistically significant (Depre *et al.*, 1998) .

RESULTS AND DISCUSSION

Phytochemical evaluation of *P. betle*

In this study, the preliminary qualitative analysis of various extracts of *P. betle* indicates the presence of significant compound like alkaloids, Flavonoids, phenol, protein, amino acids and carbohydrateand their quantification is depicted in Figure 1 .

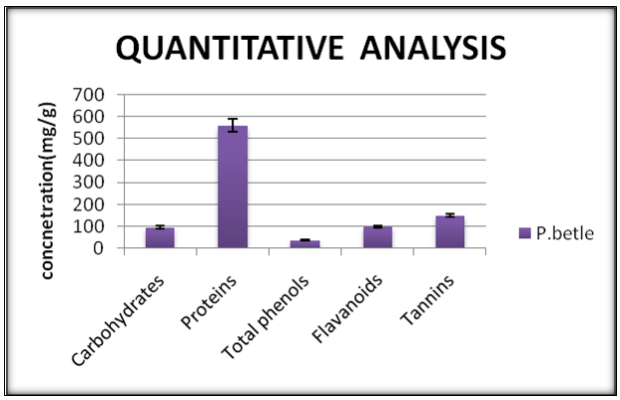


Figure 1: Quantitative analysis of hydroethanolic extract of P. betle

Antioxidant activity of hydroethanolic extract of *P. betle*

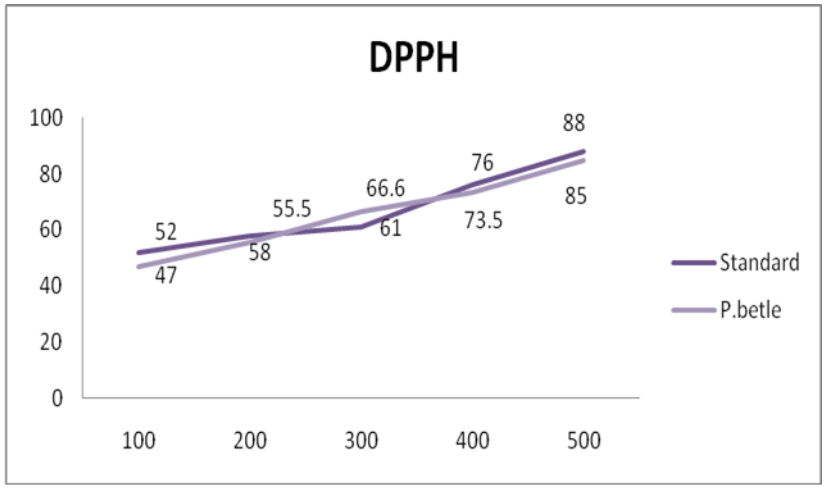
The DPPH and NO- scavenging assay showed that the hydroethanolic extract of the sample has an effective antioxidant potential against these free radicals, as shown in Figure 2a and Figure 2b.

DPPH Radical scavenging activity

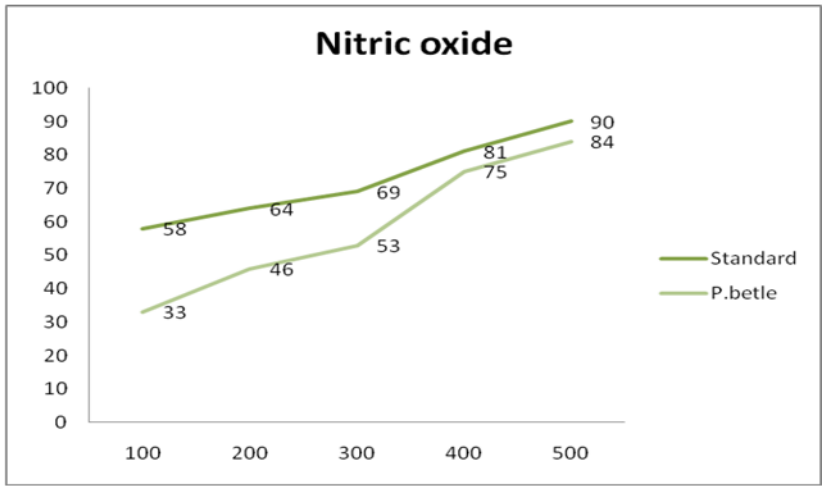
Nitric oxide scavenging activity

The data shows that the hydroethanolic leaf extract of *Piper betle* (L)has potent antioxidant activity which may be due to the phytochemicals which are richly present in *Piper betle* (L) which was confirmed by qualitative analysis and other methods.

FTIR analysis:



2a



2b

Figure 2: (2a) DPPH scavenging activity of hydroethanolic extract of P. betle (2b) NO^- scavenging activity of hydroethanolicextract of P. betle

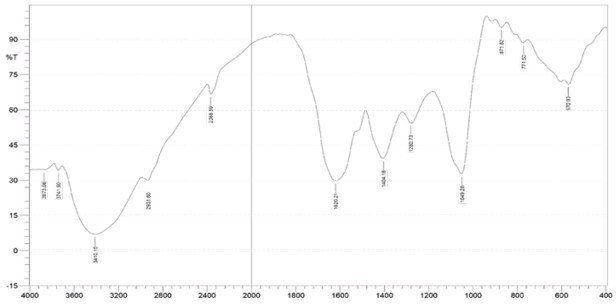


Figure 3: FTIR analysis of hydroethanolic extract of P. betle

Each peak corresponded to different functional groups, and this sample shows that the sample is rich in alkyl halides, aromatic compounds which possess specific medicinal attributes as shown in Figure 3

Hypertrophic indices

The heart weight /body weight ratio [HW/BW] as evident fromTable 3 was found to be increased in the isoproterenol administered rats (group II) when compared to control rats (group I), demonstrating an increase in the heart size. However, losartan treated rats (group III) showed a reduction in the HW/BW ratio when compared to isoproterenol administered rats similar to the reduced HW/BW ratio in plant extract administered rats (Sánchez-Campos *et al.*, 1999) (Lipke *et al.*, 1997)

Effect of hydroethanolic extract *of P. betle* on serum glucose, total protein and albumin

During cardiac hypertrophic condition the level of glucose, total protein was found to be increased

Table 3: Effect of P. betle on the HW/BW ratio in the control and experimental rats

PARAMETER	GROUP I	GROUP II	GROUP III	GROUP IV
Heart weight HW) (mg)	410 ±8.99	453.3± 12.47a*	411± 6.97b*	415± 12.47c
bodyweight (BW)(g)	102± 2.05	100 ±5.35a*	110 ±3.63b	103±2.44c*
HW/BW	4.02± 4.38	4.52± 2.33a*	3.72 ±1.92b*	4.02±5.11c*

Table value defines the mean ± SD of 3 samples per group. Group comparison a-Normal Vs ISO; b-ISO Vs Drug; c-Iso Vs Plant extract. Statistical significance($p<0.05$) indicated by *

accompanied by reduced albumin levels in serum (Al-Ahmad *et al.*, 2001) (Sánchez-Campos *et al.*, 1999) when compared to normal group (Group I) (Witteveen *et al.*, 1975) . After the oral administration of hydroethanolic extract and losartan the levels of glucose, total protein and cholesterol were restored in experiment animals similar to normal (Group I). Table 4 shows,

Effect of hydroethanolic extract *of P. betle* on lipid profile

In this study, lipid profile studies revealed increased serum total cholesterol (Sánchez-Campos *et al.*, 1999) and triglycerides (Shen and Qian, 2006) with reduced HDL cholesterol in isoproterenol administered hypertrophic rats. These effects were reciprocated when treated with plant extract and losartan as shown in Table 5.

Effect of hydroethanolic extract *of P. betle* on urea and creatinine

Urea and creatinine levels are likely to be increased during cardiac hypertrophy (Maruta *et al.*, 1997) because an increase in polyamine synthesis from ornithine is associated with cardiac hypertrophy urea level as shown in Table 6 significantly increase in isoproterenol induced (group II) (Tappayuthpijarn *et al.*, 1982) . Oral administration of plant hydroethanolic extract and losartan restored the level in the experimental animal when compared to normal (group I).

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

Cardiac markers enzyme is an important indication of cardiac hypertrophy. Administration of ISO lead to a significant increase in the level of the SGOT, SGPT, LDH (Snedecor *et al.*, 1986) in cardiac hypertrophic rat compared with the normal control rats, simultaneously when it is treated with losartan and hydroethanolic rats for a period of 7 that showed a significant decrease in their levels as shown in Table 7 .

Effect of hydroethanolic extract of *P. betle* on enzymic antioxidants

Regulated antioxidant system is essential for successful therapy to treat cardiac hypertrophy. In isoproterenol induced cardiac hypertrophic rats, the antioxidant enzymes catalase, SOD, GPx were significantly decreased with that of the normal group (Cullen *et al.*, 1971) . Oral administration of the hydroethanolic extract of *Piper betle* and losartan restored those levels in experimental animals (Group III&IV) similar to that of normal control (Group I) as shown inTable 8 .

Histopathological observations

Histopathological analysis of the heart tissue (left ventricle) revealed the hypertrophic impact of isoproterenol on myocardial architecture which was restored in normal on treatment with losartan and hydroethanolic extract of *P. betle* (Doss *et al.*, 2018) as shown in Figure 4

In this study,Figure 4A, Group I (NORMAL) exhibited clear intact myofibril arrangement of heart tissue. Figure 4B, GROUP II (ISO) administered showed the degenerated myofibril network with presence of inflammatory cell infiltration and thickened cellular architecture (Doss *et al.*, 2018) .Figure 4C, GROUP III (ISO+ Losartan) treatment - reorganized myofibril arrangement was seen with and decrease the cellular thickening and infiltration. Figure 4D, GROUP 1V (ISO+ *P. betle* extract) showed reduced cell necrosis, stiffening and improved myofibril arrangement when compared to group II but similar to that of normal.

CONCLUSIONS

The present study concludes that the hydroethanolic extract of *P.betle* show a significant effect upon the altered biochemical parameters (glucose, protein, cholesterol, urea, creatinine, SGOT, SGPT, LDH, SOD, CATALASE, GPx) thereby indicating the antihypertrophic potential of the plant extract which may be due to the phytochemical compound eugenol

Table 4: Effects of hydroethanolic extract of Piper betleon glucose, protein and albumin in serum

GROUPS	SERUM GLUCOSE (mg/g)	SERUM PROTEIN (mg/g)	ALBUMIN (mg/dl)
GROUP I	94.70 ±1.43	7.35± 0.89	5.58 ±1.01
GROUP II	133.3 ± 2.20a*	7.51± 0.79a*	5.028 ± 0.92a
GROUP III	163.33± 1.76b*	7.39 ±0.79b	5.430 ± 6.45b*
GROUP IV	144.43 ±1.22c	7.09 ± 0.84c*	5.75 ± 0.60c*

Table value defines the mean ± SD OF 3 samples per group. Group comparison a-Normal Vs ISO; b-ISO Vs Drug; c-Iso Vs Plant extract. Statistical significance (p<0.05)indicated by *

Table 5: Effect of Hydroethanolic Extract on serum lipid profile

GROUPS	SERUM CHOLESTEROL (mg/dL)	HDL (mg/dL)	TRIGLYCERIDE (mg/dL)
GROUP I	123.4 ±1.576	110.4 ± 0.979	81.70 ± 1.44
GROUP II	236.3 ± 0.790a*	37.76 ±1.978a*	110.1 ± 1.978a*
GROUP III	177.73 ±7.813b	87.93 ±1.359b	93.9 ±3.103b*
GROUP IV	216.1± 6.238c	84.76± 1.776c*	89.68 ± 2.25c*

Table value defines the mean ± SD OF 3 samples per group. Group comparison a-Normal Vs ISO; b-ISO Vs Drug; c-Iso Vs Plant extract. Statistical significance(p<0.05) indicated by *

Table 6: Effects of Hydroethanolic Extract of P. betleOn Urea and Creatinine in Serum

GROUPS	SERUM UREA (mg/dl)	SERUM CREATININE (mg/dl)
GROUP I	91.93 ±1.65	3.11± 0.215
GROUP II	98.43 ± 2.57a	6.12 ±0.230a*
GROUP III	74.86 ± 1.490b*	4.31 ± 0.170b*
GROUP IV	80.57 ±1.58c*	5.19 ± 0.2c*

Table value defines the mean ± SD OF 3 samples per group. Group comparison a-Normal Vs ISO; b-ISO Vs Drug; c-Iso Vs Plant extract. Statistical significance(p<0.05) indicated by *

Table 7: Effects of Hydro Ethanol Plant Extract of Cardiac Markers Enzyme

GROUPS	SERUM SGOT(IU/l)	SERUM SGPT(IU/L)	SERUM LDH
GROUP I	3.916 ± 0.65	4.180 ±0.336	82.90 ± 2.67
GROUP II	4.89 ± 0.24a*	5.25 ±0.357a*	115.2 ±2.02a*
GROUP III	4.89 ± 0.502b*	4.95 ± 0.405b*	102.63 ±.016b
GROUP IV	3.91 ± 0.80c*	4.91 ±0.80c*	90.53 ± 2.56c*

Table value defines the ± SD OF 3 samples per group. Group comparison a-Normal Vs ISO; b-ISO Vs Drug; c-Iso Vs Plant extract. Statistical significance(p<0.05) indicated by *

Table 8: Effect of Hydroethanolic Extract of P. betle on Enzymic Antioxidants

GROUPS	CATALASE(IU/L)	SOD(IU/L)	GPx (IU/L)
GROUPS I	35.66 ± 6.02	42.07 ± 1.265	7.97 ± 0.539
GROUPS II	11 ± 2.00a	27.37±1.832a*	4.76 ± 0.150a*
GROUPS III	14.5 ± 1.513b	34.37 ±2.05b*	8.65 ± 0.406b
GROUPS IV	14 ± 2.00c	40.52 ± 1.60c*	8.38 ± 0.174c*

Table value defines the mean ± SD OF 3 samples per group. Group comparison a-Normal Vs ISO; b-ISO Vs Drug; c-Iso Vs Plant extract. Statistical significance(p<0.05) indicated by *

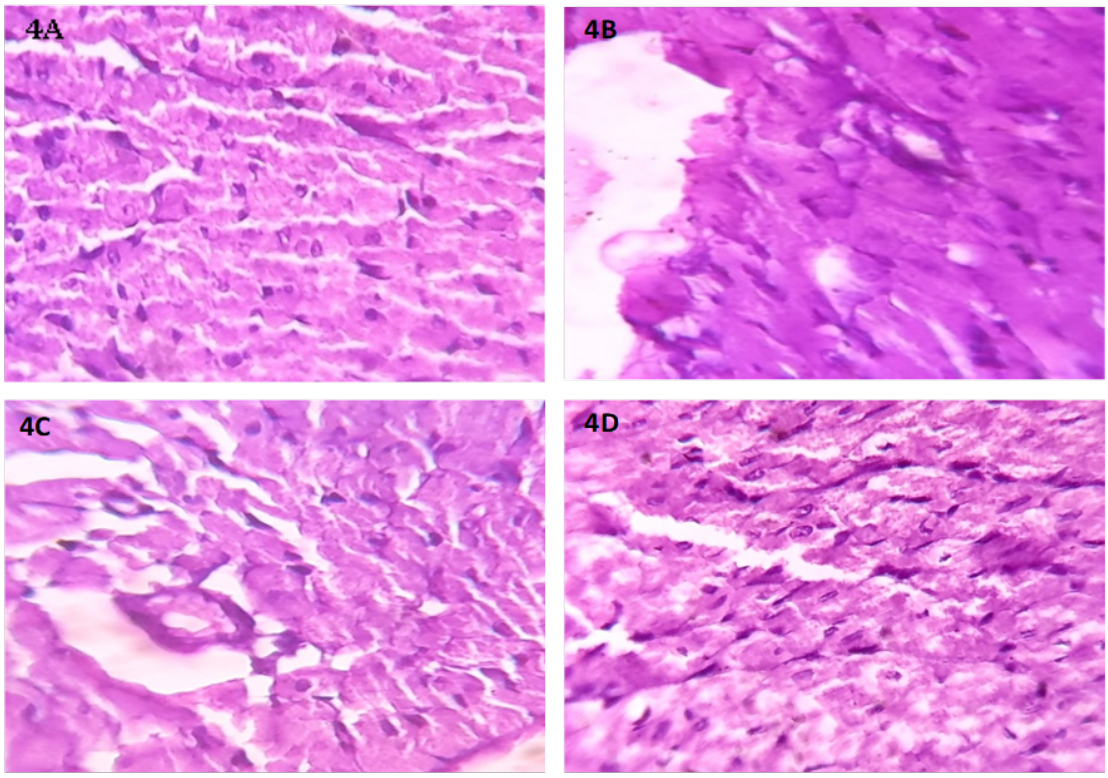


Figure 4: Histopathological observation of left ventricular heart tissue sections

and hydroxylchavicol present in it that require further studies to isolate and characterize the specific bioactive compound such as eugenol which is responsible for cardioprotective activity (anti hypertrophic activity).

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