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Nagapashana Pishti - A Potent Cardio Tonic

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ABSTRACT

Nagapashana (Serpentine), a hydrous silicate of Magnesium (Mg6 (Sio10) OH8), is an important mineral drug used in Ayurveda, often in the form of *Pishti* (fine powder of *Nagapashana*). It is a *Hrudya Dravya* (cardiotonic). It is particularly indicated in *Hrud Dourbalya*a, a condition associated with weakness of cardiac muscles. Cardiovascular disease is a leading cause of death in developed and developing countries. The present study was conducted to evaluate the cardio tonic activity of *Nagapashana Pishti* on Wistar rats. In the in-vivo study, Doxorubicin (2mg/kg) for 7 days was used to induce cardiac damage and the cardio tonic effect of *Nagapashana Pishti* at a dose of 150 and 300 mg/kg.b.w, was compared with standard drug Digoxin. Biochemical assays like serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), lactate dehydrogenase (LDH), Total Cholesterol (TC), Triglyceride (TG) and Creatine kinase Monoenzyme B (CKMB) were done together with histopathology of heart tissue and ECG analysis. The *in-vivo* study revealed that *Nagapashana Pishti* (300mg/kg) was relatively more effective due to decreased QT and ST interval in ECG, significantly reduced levels of serum CKMB, SGOT, SGPT, TC and LDH and improvement in myocardial tissue.

Key words: *Nagapashana Pishti, Cardio Tonic Activity, In-Vivo, Digoxin.*

INTRODUCTION

Cardiovascular diseases are currently the leading cause of death for men and women both in developed and developing countries, and is caused by disorders of the heart and blood vessels, and includes coronary heart disease (heart attacks), cerebrovascular disease (stroke), hypertension, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure.[1] Cardiotonic are drugs used to increase the efficiency and improve the contraction of the heart muscle, which leads to improved blood flow to all tissues of the body. Cardiotonic drugs increase the force of the contraction of the muscle (myocardium) of the heart. Most known cardiotonic agents belong to conventional medicine, while those in complementary systems of medicine remain inadequately studied. The most common cardio tonics include cardiac glycosides (digoxin), catecholamine while others include ACE inhibitors, angiotensin receptor blockers, diuretics and digitalis which lack specificity in their action and produce adverse effects like dysrhythmias, hypotension, and bronchospasms.[2] Thus the objective of the present study is to evaluate an Ayurvedic mineral based drug, *Nagapashana*, which has potent cardiotonicactivity and multiple actions that are beneficial in conditions associated with cardiac damage with no known adverse effects.

In Ayurveda, cardiovascular diseases are the diseases of the *Hrudaya* which is considered to be heart in this context. It is one of the three *Marmas,* vital points. If *Hrudaya* doesn't function properly, the other organs and tissues are also affected and damaged due to lack

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of, or inadequate supply of nutrition and oxygen. The medicines or herbs that give strength to *Hrudaya* are called *Hrudya Dravyas.*[3]

Ayurveda is based on a pharmacoepia that consists almost entirely of herbo-mineral drugs. *Nagapashana* was first mentioned in Ayurvedic texts under the heading *'Sikatha Varga'* - a group of silicate compounds.[4],[5] It is famous as *Jaharmohara* in Hindi. *Nagapashana* is useful in Hrid-daurbalya (as a cardiotonic), *Chardi* (vomiting), *Daha* (burning sensation), *Visuchika* (colicky abdominal pain), *Shwasa* (respiratory disorders), *Hrudroga* (a wide spectrum of cardiovascular disorders), *Kasa* (cough) and *Raktaarshas* (bleeding heamarroids). In Ayurvedic thought, it is considered as *Hrudya*, helps to strengthen the brain and liver, improves bodily strength (*Balya*), pacifies increased *Pitta Dosha* (one among the three main elements in Ayurveda), and is a potent aphrodisiac (*Veerya Vardhaka*).[6],[14]

This study attempted to evaluate the cardiotonic activity of *Nagapashana Pishti* experimentally in Wistar albino rats.

Materials and Methods

Collection of the drug

The raw material *Nagapashana* was collected based on suitable *Grahya Lakshana* (and Mineralogical properties from *Amruthkesari* Depot, and preparation was done at Department of Rasashastra, RAMC, Bangalore.

Preparation of the drug

Shodhana of *Nagapashana* was done by *Nirvapa* (heating red hot and quenching in milk) method with *Godugdha* for 21 times and 14 days *Bhavana* (trituration) with *Gulab Arka* (rose distillate) till it attained the *Pishti* (fine powder).[6]

Pharmacological Studies

Nagapashana Pishti in gum acacia was subjected for the following pharmacological studies;

1. Acute oral toxicity (OECD 423)
2. In-vivo evaluation of cardiotonic activity of *Nagapashana Pishti*

Animals

The animal experiments were conducted at Invivo Bio sciences laboratory, Bangalore, India, and approved by the IAEC and care of laboratory animals were taken as per the guidelines of CPCSEA.

Acute toxicity studies

The acute toxicity study was performed in female Sprague Dawley rats by using fixed dose method as per OECD guideline 420. In this study, a sighting dose of 300 mg/kg was used, and no adverse signs were found. Subsequently additional doses of 1000 and 2000 mg/kg were used. Total of 5 animals were used for each dose. Animals were observed for 24hrs and parameters like alertness, irritability, fearfulness, touch response, spontaneous activity, pain response, defecation, urination, etc. were recorded. Animals were observed till 14 days for any mortality. No clinical signs/adverse effects or death occurred in any of the doses tested.

In-vivo evaluation of cardio tonic activity of *Nagapashana Pishti*

Group Allocation

SN	Group	Treatment	Dose
1.	Control	Control	-
2.	Positive	Doxorubicin	2mg/kg
3.	Standard	Doxorubicin + Digoxin	2mg/kg + 100mcg/kg
4.	Single dose test	Single dose *Nagapashana*	2mg/kg + 150mg/kg
5.	Double dose test	Double dose *Nagapashana*	2mg/kg+ 300mg/kg

Treatment protocol

Doxorubicin 2 mg/kg, i.p administered for 7 days to induce cardiac toxicity in all groups except normal control. The other groups were receiving their respective treatment along with doxorubicin. Animals were divided into 5 groups, (n=6). Treated for 14 days.

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1. Group I Normal control: Normal control received water
2. Group II Disease control: Disease control treated with doxorubicin (2 mg/kg, i.p. for 7 days)
3. Group III Standard: Standard control treated with digoxin (100 µg/kg, p.o., per day) along with doxorubicin (2 mg/kg, i.p. for 14days)
4. Group IV Low Dose: Low dose of *Nagapashana Pishti* treated (150 mg/kg, p.o., per day) along with doxorubicin (2 mg/kg, i.p. for 14days)
5. Group V High Dose: High dose of *Nagapashana Pishti* treated (300 mg/kg, p.o., per day) along with doxorubicin (2 mg/kg, i.p. for 14 days) Treatments were carried for 14 days.

During treatment period several general observations were made which include mortality rate and body weight. At the end of treatment period, blood samples were collected under fasting conditions.

Histopathology of heart was performed at the end treatment period to study cardiac damage occurred due to doxorubicin.

Parameters measured

After 24 hrs of last dose following parameters were measured:

- ECG
- Serum biomarkers like: CKMB, LDH, TC, TG, SGOT and SGPT.
- Histopathology of transverse section of heart evaluation of cardiac enzymes

Data Compilation and Statistical Analysis

Averages of all the data were compiled and SEM were calculated. All the data were compiled using one-way ANOVA followed by Dennett's multiple comparison tests.

P values <0.05 were considered as statistically significant.

RESULTS

Acute oral toxicity Study (OECD 423)

Nagapashana Pishti was subjected for acute oral toxicity study as per OECD 423 guidelines. It was administered up to a dose of 2000 mg/kg, p.o. (Limit test). The *Pishti* didn't show any changes in behavioural parameter like alertness, fearfulness, irritability. No changes in neurological profile like spontaneous activity touch response, pain response and No changes in autonomic profile like urination, defecation. After 14 days of observation, No mortality was shown in *Nagapashana Pishti* treated animals. Hence, 150mg/kg and 300mg/Kg doses were selected for experimental study.

Effect of *Nagapashana Pishti* on Cardiac Enzymes in Doxorubicin Induced Heart damage Model - An In-vivo Study

Administration of doxorubicin for 7 days (2mg/kg, i.p.) cause severe elevation of cardiac bio-markers due to cardiomyopathy. The level of Creatine kinase Monoenzyme B (CKMB) in normal group was found to be 45.91±0.927(IU/L). CKMB level was significantly raised in disease control 174.76±1.953 (IU/L). Digoxin treated group showed highly significant reduction in CKMB level, whereas *Nagapashana Pishti* 150 and 300 mg/kg treated groups showed decreased CKMB level in dose dependent manner 61.53±1.779*** (IU/L), 167.89±2.555* (IU/L) & 121.72±1.689*** (IU/L) respectively. The level of Serum Glutamic Oxaloacetic Transaminase (SGOT) in normal group was found to be 43.59±1.540 (IU/L). SGOT level was significantly raised in disease control 189.31±2.187 (IU/L). Digoxin treated group showed highly significant reduction in SGOT level, whereas *Nagapashana Pishti* 150 and 300 mg/kg treated groups showed decreased SGOT level in dose dependent manner 37.77±1.890*** (IU/L), 172.57±4.896** (IU/L) &79.77±2.669*** (IU/L) respectively. The level of Serum glutamic pyruvic transaminase (SGPT) in normal group was found to be 20.23±0.555(IU/L). SGPT level was significantly raised in disease control 46.15±0.239 (IU/L). Digoxin treated group showed highly significant reduction in SGPT level, whereas *Nagapashana Pishti* 150 and 300 mg/kg treated groups showed decreased SGPT level in dose dependent manner 22.03±0.799*** (IU/L), 42.08±0.433* (IU/L) &29.53±0.672*** (IU/L) respectively. The level of Lactate De-Hydrogenase (LDH) in normal group found to be 73.63±1.121(IU/L).

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LDH level was significantly raised in disease control 166.00±2.050(IU/L). Digoxin treated group showed highly significant reduction in LDH level, whereas *Nagapashana* 150 and 300 mg/kg treated groups showed decreased LDH level in dose dependent manner 82.4±1.754***(IU/L), 161.68±2.334** (IU/L) &129.14±1.271*** (IU/L) respectively. The level of Total Cholesterol (TC) in normal group found to be 81.53±0.579 (IU/L). TC level was significantly raised in disease control 161.95±3.716 (IU/L). Digoxin treated group showed highly significant reduction in TC level, where as *Nagapashana* 150 and 300 mg/kg treated groups showed decreased TC level in dose dependent manner 90.07±0.822***(IU/L),148.30±3.402** (IU/L) & 82.23±0.829*** (IU/L) respectively. The level of Triglyceride in normal group found to be 93.42±0.605 (IU/L). TG level was significantly raised in disease control 280.43±2.677 (IU/L). Digoxin treated group showed highly significant reduction in TG level, whereas *Nagapashana* 150 and 300 mg/kg treated groups showed decreased TG level in dose dependent manner 102.93±1.786***(IU/L),176.75±1.848** (IU/L) & 82.12±1.235*** (IU/L) respectively. The above findings were suggested that there was significant increase in cardiac marker level in disease control group while in digoxin and *Nagapashana* treated groups shown reduction in cardiac marker levels significantly, compared to disease control group.

Table 1: Effect of *Nagapashana Pishti* on Cardiac Enzymes.

SN	Groups	CKMB (U/L)	LDH (IU/L)	SGOT (IU/L)	SGPT (IU/L)	TC (mg/dl)	TG (mg/dl)
1.	Normal	45.91±0.927	73.63±1.121	43.59±1.540	20.23±0.555	81.53±0.579	93.42±0.605
2.	Disease	174.76±1.953	166.00±2.050	189.31±2.187	46.15±0.239	161.95±3.716	280.43±2.677
3.	Digoxin	61.53±1.779***	82.4±1.754***	37.77±1.890***	22.03±0.799***	90.07±0.822***	102.93±1.786***
4.	*Nagapashana Pishti* 150mg/kg	167.89±2.555*	161.68±2.334**	172.57±4.896**	42.08±42.08*	148.30±3.402**	176.75±1.848**
5.	*Nagapashana Pishti* 300mg/kg	121.72±1.689***	129.14±1.271***	79.77±2.669***	29.53±0.672***	82.23±0.829***	81.53±1.235***

Table 2: ECG Interpretation

SN	Groups	Heart Rate	RR Interval	QRS Interval
1.	Normal	312.95±12.552	0.17915±0.0006	0.01918±0.002315
2.	Disease	401.13±37.46	0.7074±0.2368	0.02866±0.002951
3.	Digoxin	270.516±2.281***	0.2174±0.00093***	0.0155±0.000428**
4.	*Nagapashana Pishti* 150mg/kg	274.26±2.479**	0.2174±0.00093**	0.01483±0.000477***
5.	*Nagapashana Pishti* 300mg/kg	301.683±10.309***	0.1856±0.00084***	0.01829±0.002558*

Effects of *Nagapashana Pishti* on ECG in Doxorubicin Induced cardiac toxicity

ECG was recorded in all the groups after 24hrs of the last dose. Disease control group showed prolonged RR & QT intervals, with abnormalities in P wave, QRS complex and T wave compared with normal ECG. Digoxin & *Nagapashana* treated groups showed improvement in some ECG parameters compared with disease control group.

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ECG pattern in Control indicates normal ECG. (Results are shown in Figure: 1)

ECG pattern in the Disease Control indicates cardiac toxicity showing various parameters like. (Results are shown in Figure: 2)

- Enlarged QRS complex
- Elevated ST segment
- Prolonged RR interval
- Prolonged QT interval

ECG Pattern in the Standard Control showing (Results are shown in Figure: 3)

- Well defined P wave, QRS complex &T wave.
- ST segment, RR interval, QT interval are improved compare to disease control group.

ECG pattern in *Nagapashana Pishti* 150mg/showing (Results are shown in Figure: 4)

- Decreased RR & QT interval
- Slightly reduced in P wave
- Showing improvement in elevated ST segment & enlarged QRS complex compare to disease control group.

ECG pattern in *Nagapashana* 300 mg/kg showing (Results are shown in Figure: 5)

- Showed significant improvement in all ECG parameters compare to disease control.
- Decrease prolongation of QT and ST interval.

ECG Images

Figure 1: ECG of Normal control

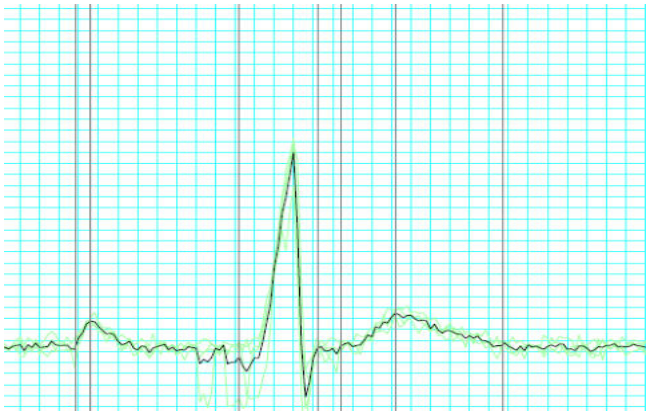


Figure 2: ECG of Disease control

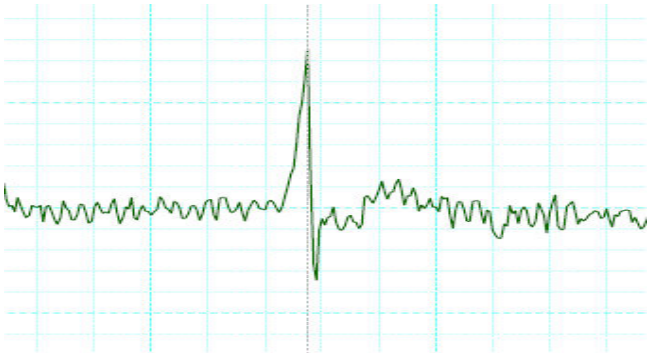


Figure 3: ECG of Standard treatment

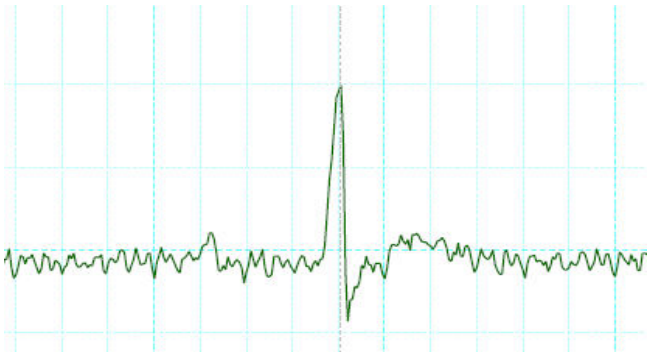


Figure 4: ECG of single dose of *Nagapashana*

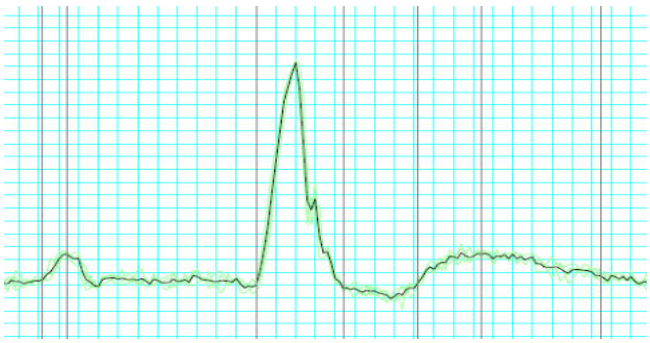
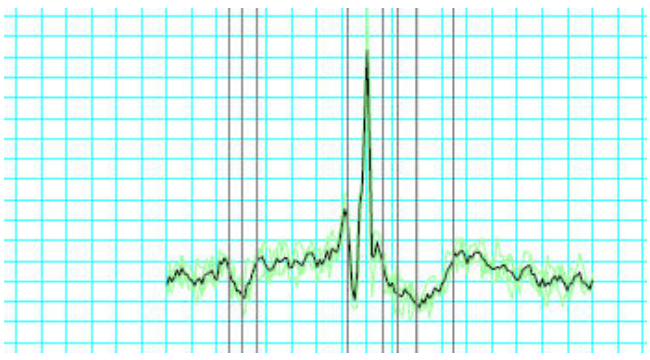


Figure 5: ECG of Double dose of Nagapashana



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Effect of *Nagapashana Pishti* on Histopathology of Cardiac Tissues in Doxorubicin

Induced Congestive Heart Failure Model - An Invivo Study Histopathological studies were carried out in normal, doxorubicin, standard and treated groups. Normal group: Showed clear integrity of myocardial membrane, without loss of myofibrils and cellular infiltration and with a clear intercalated disk, nuclei and myocytes. It also showed intact arrangement of the cardiac muscle fibres and muscle fibres showed intact integrity of myocardial cell membrane with striations. Results are shown in Figure: 6.

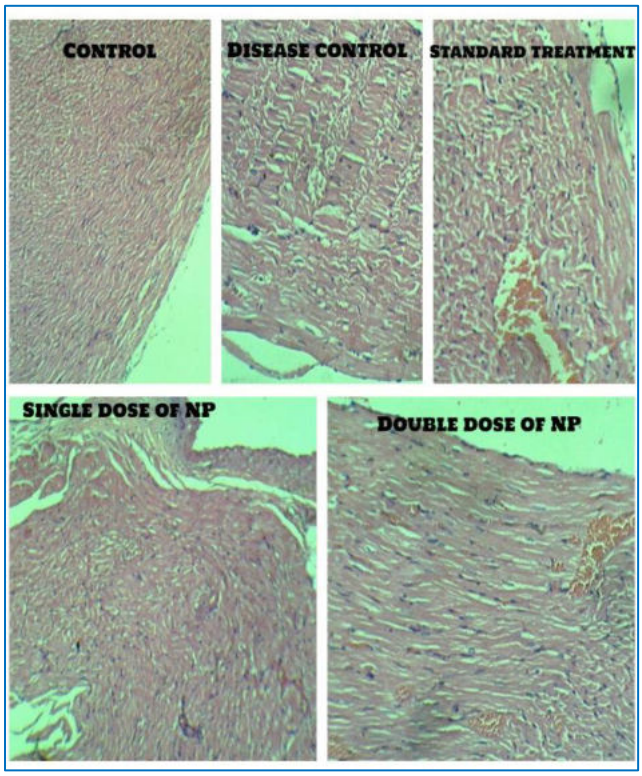

Figure 6: Histopathology images

Doxorubicin treated group: Resulted in cardiac muscle necrosis, cellular infiltration, vascular dilation, homogenous cytoplasm, fibrosis in focal areas. Results are shown in Figure: 7 Digoxin treated group: Showed normal architecture features of myocardium, without necrosis, cellular infiltration as well as vasculature was regenerated, decreased fibrosis in focal area and reduced vascular dilation compare to disease control group. All the Parameters like infiltration, fibrosis of focal areas, necrosis, etc were comparatively less in standard group. Results are shown in Figure: 6

Nagapashana Pishti 150 mg/kg: Showed slightly improvement in arrangement of the cardiac muscle fibres with increase in integrity of myocardial cell membrane and myofibrils. Results are shown in Figure: 6

Nagapashana Pishti 300 mg/kg: Showed significantly increase in arrangement of the cardiac muscle fibres with increase in integrity of myocardial cell membrane and myofibrils. Results are shown in Figure: 6.

DISCUSSION

Cardiovascular disease is one of the primary causes for hospitalization and death worldwide. In which heart is unable pump the sufficient blood properly and which results into reduced blood supply to vital organ.[7] Various cardio tonic drugs are useful in low output failure condition, which may occur due to myocardial ischemia, myocardial infarction, cardiac hypertrophy, cardiomyopathy, congenital heart defects, heart valve defects, etc. But these cardio tonic drugs have low therapeutic index with high side effects. Hence the research is focused on mineral based drug. Along with herbs our ancient scholars were also well aware of the medicinal value of metals, minerals, aquatic products and gems etc. which are found in nature. *Rasaouṣadhis* have unique place in Ayurvedic therapeutics because of their qualities like *Alpamātropayogitvāt* (used in less dose), *Arucher-Aprasangata* (no incidence of bad taste) and *Kṣipramārogayadāyitvat* (fast acting).[8]

Thus the objective of present study is to evaluate the mineral based drug which having potent cardiotonic activity. Some of the *Rasoushadhis* like *Prabakaravati, Yakutirasa, Hrudayaranava Rasa, Sangeyashma Pishti, Akikapisti* is mentioned to treat *Hrudroga* in Ayurveda.

Nagapashana is Serpentine, a hydrous silicate of Magnesium (Mg6 (Sio10) OH8).[9] It is mentioned under *Sikathavarga* - silicate compounds,[4],[5] It is famous as *Jaharmohara* in Hindi. It acts on *Hrudaya Dourbalya*, It consists of magnesium and silicate mainly, along with other. Hence the present study was undertaken to evaluate cardiotonic activity of *Nagapashana Pishti* against doxorubicin induced

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congestive cardiac failure in rats. Raw *Nagapashana* was collected, and *Pishti* was prepared by classical method. Acute toxicity study was performed as per OECD 423 guidelines and *Nagapashana Pishti* didn't show any lethal effect up to a dose of 2000 mg/kg. Hence, (150 mg/kg) and (300 mg/kg) doses were selected for experimental study.

In-vivo cardiotonic activity was evaluated in failed heart. Doxorubicin 2 mg/kg., i.p. for 7days leads to cardiomyopathy. Doxorubicin is a potent chemotherapeutic agent using for the treatment of cancers, which shows drastically decrease in weight, chronic cardiac side effects like congestive cardiac failure and cardiomyopathy. Clinical and laboratory findings of overall long term treatment with doxorubicin showed marked tachycardia, hypotension, cardiac dilation, ventricular failure due to elevation in serum enzymes such as CKMB, SGOT, SGPT, TC, TG and LDH levels. The level CKMB, SGOT, SGPT, TC, TG and LDH levels significantly increased in disease control group compared to normal. The digoxin and *Nagapashana* treated groups showed decrease in CKMB, SGOT, SGPT, TC, TG and LDH levels, which are near to normal values. *Nagapashana* 300 mg/kg showed more effective reduction in the above cardiac markers than *Nagapashana* 150 mg/kg. Doxorubicin which causes cardio toxicity results in changes in ECG pattern. ECG was recorded in all the groups after 24 hrs of the last dose. Disease control group showed prolonged RR & QT intervals, with abnormalities in P wave, QRS complex and T wave compared with normal ECG. Digoxin & *Nagapashana* treated groups showed improvement in all the ECG parameters compared with disease control group. Myocardial damage and toxicity of doxorubicin on architecture of heart was estimated by histopathological examination of heart tissue. Administration of doxorubicin leads to cardiac toxicity due to increased oxidative stress. An imbalance between endogenous myocardial oxidants and free radical production has been suggested to play major role in pathogenesis of CVD.[10] Myocardial damage and toxicity of doxorubicin on architecture of heart was estimated by histopathological examination of heart tissue. Normal group showed clear integrity of myocardial membrane, without cellular infiltration and loss of myofibrils and with a clear intercalated disk, nuclei and myocytes. Doxorubicin treated group showed cardiac muscle necrosis, cellular infiltration, homogenous cytoplasm, vascular dilation, fibrosis in focal areas. Animals treated with *Nagapashana* showed improvement in damage architecture of cardiac muscles. Digoxin treated group also showed improvement in the architecture of cardiac muscles. *Nagapashana* 300 mg/kg showed more effective improvement in architecture of the heart muscle than *Nagapashana* 150 mg/kg.C

Exact correlation may not be possible between Ayurvedic and modern description of heart diseases. But a reasonable comparison can be done on the basis of symptomatology.

Hridrogavikruti - *Vyanavayu* controls all the activities of *Hridaya* including its rhythm. Disturbance of *Vyanavayu* gives rise to various *Hridaya Vikrutis.* Disturbance in contraction and expansion of muscles and palpitations, may be due to *Vata* and *Pitta*, and *Mandata* due to *Kapha*. It is very difficult to explain the exact mode of action of drug. But on the basis of certain principles and theories, an attempt has been made here to describe the probable mode of action of *Nagapashana Pishti* as cardio tonic. According to Ayurveda, the pharmacodynamics of a drug can be evaluated through its *Panchabhoutika* constitution and *Rasapanchaka*. *Nagapashana* has *Gunas* like *Ruksha, Ushna, Hrudya, Medhya, Balya* and *Vishagna*. It is *Tridoshahara*, mainly *Pittanashaka* and has *Samasheethoshna Veerya*. It strengthens the heart and brain, also improves *Ojas* and acts as *Vishahara*. By all these properties and because of *Balya* and *Hrudya* action, *Nagapashana* acts as *Hrudya*, gives strength to cardiac muscles and helps to improve the pumping capacity of heart, which can be correlated to cardio tonic. Since it is *Ojovardaka* also, it may help in preventing the occurrence of Cardiac diseases. ***Gulab*** is having the properties of *Tikta, Katu, Kashaya, Madhura Rasa, Laghu, Snigdha Guna, Sheetha Veerya, Madhura Vipaka* and *Tridoshagna*. It acts as brain tonic and is useful in heart diseases and bleeding

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disorders. These *Gunas* also gets imbibed with *Nagapashana* and acts as *Hrudya*.[11] ***Godugdha*** is *Madhura Rasa, Anabhishyandi, Snigdha, Guru, Sheetha Veerya, Madhura Vipaka,* and *Vata Pitta Nashaka.* Cow's milk is *Rasayana*, *Balavardaka, Raktapittahara, Medhya* and good for heart, increases life span and aphrodisiac. By these properties, it produces little moisture in *Srotas, Doshas, Dhatus* and *Malas*, which helps to improve the blood flow and muscle tonicity.[11]

M**agnesium** is the main component of cardiac glycosides and ACE inhibitors, which are the best choice of treatment in cardiovascular diseases, as it coordinates the activity of the heart muscle and the nerves that initiate the normal heartbeat by improving the blood flow and decreases the heart's work load.[12],[13] As *Nagapashana* is a hydrous magnesium silicate, which contains maximum percentage of magnesium, hence may be considered as cardio tonic, which can be correlated as *Hrudya*.

Conclusion

It is concluded that *Nagapashana Pishti* possesses cardiotonic activity. *Nagapashana* treated groups showed improvement in RR & QT interval with normal P wave, QRS complex & T wave in ECG and it also showed reduction in CKMB, SGOT, SGPT, TC, TG and LDH. *Nagapashana* treated groups showed improvement in architecture of cardiac muscle. Hence, it is concluded that *Nagapashana Pishti* hascardiotonic activity. Where *Nagapashana* 300 mg/kg showed highly significant cardiotonic activity than *Nagapashana* 150 mg/kg.

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