IJBPAS, October, 2024, 13(10): 4708-4729

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF NOVEL FORMULATION CONTAINING *MOMORDICA DIOICA* EXTRACT AGAINST EXPERIMENTALLY INDUCED CARDIAC FIBROSIS IN RATS

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Received 18th July 2023; Revised 20th Sept. 2023; Accepted 1st Dec. 2023; Available online 1st Oct. 2024

https://doi.org/10.31032/IJBPAS/2024/13.10.8267

ABSTRACT

In this investigation, the effectiveness of cubosomes filled with fruit extract from *Momordica dioica* against experimentally induced cardiac fibrosis in rats will be assessed. Male Sprague Dawley rats were given twice-weekly doses of deoxycorticosterone acetate (DOCA), 1% NaCl in drinking water, and uninephrectomy to promote cardiac fibrosis. The experimental rats were split into four groups of eight each, with the groups named Sham Control, Surgical Control, Model Control, and Treatment Control (Formulation dose: 104 mg/kg). After the treatment protocol was followed, anthropometric, hemodynamic, biochemical, ex-vivo Langendorff investigation, and antioxidant parameters were carried out. The histology of the heart stained with Masson's trichrome was also investigated. *Momordica dioica* fruit extract-loaded cubosomes had cardio-preventive benefits by reducing blood pressure, preserving normal ECG patterns, and improving left ventricular functions, as demonstrated by a substantial reduction in LVEDP. The therapy groups have higher serum creatinine levels and lower levels of urea and blood urea nitrogen. The activity of antioxidant enzymes was also increased. Additionally, cardiac homogenate from animals that had been treated

had less collagen I and III. By looking at the animal hearts that had been stained with Masson's, this protective effect was further demonstrated.

Keywords: *Momordica dioica*, Novel Cubosomes formulation, cardiac fibrosis

INTRODUCTION:

Ayurveda entails a scientific tradition of harmonious living, and its origin can be traced from ancient knowledge contained in Rigveda and Atharvaveda. Ayurveda is a traditional healthcare system of Indian medicine since time immortal. Ayurvedic several drugs have been exploited for the treatment and management of various diseases in human beings. Several drugs have been developed and practiced from Ayurveda since ancient times to modern practice as 'tradition to trend.' The potential of Ayurvedic medicine needs to be explored further with current scientific validation approaches for better therapeutic leads **[1]**.

Ayurveda, the traditional Indian medicine, is gaining popularity because it effectively treats many chronic illnesses. Most patients start taking traditional pharmaceuticals as soon as their diagnoses are made. Therefore, ayurveda therapies are often used with or after conventional medical procedures. To properly comprehend food, spices, and medicinal plants' potential effects, one must thoroughly understand how they work. Indian spices and herbs used in Ayurveda medicine are widely used in society without causing any harm. Still, using more potent medication derived from a single plant, sometimes in the form of teas or pills, is a cause for worry. While it is true that ayurvedic medications are made from natural herbs, how they are administered—considering the needs of the individual and their particular illness conditions—determines how safe they are **[2]**.

Momordica dioica is a cucurbitaceous, perennial climbing creeper (known as kakrol, spiny gourd, or teasle gourd). It has a wide geographic distribution in Bangladesh and India and is indigenous to Asia. For thousands of years, it has been utilized as a vegetable with high nutritional content and as a preventative and therapeutic agent for many ailments. While it contains considerable amounts of certain chemicals with better nutritional value than many other regularly consumed vegetables, kakrol is still considered an underused vegetable **[3]**. Recently, there has been considerable interest in the genus Cucurbita. This endemic to the Americas plant species has been used in traditional medicine worldwide to treat gastrointestinal disorders, intestinal parasites, and other clinical ailments. Their nutritional and phytochemical makeup has been increasingly linked to these therapeutic effects.

Carotenoids, terpenoids, tocopherols, phenols, sterols, saponins, functional carbohydrates,

fatty acids, and polysaccharides are those chemical components that exist in greater quantity. Yet, due to its well-known biological properties, cucurbitacin, a family of triterpenoids, has recently attracted much attention **[4]**. Many Phyto-constituents are present in the various plant sections of *M. dioica*, including alkaloids, flavonoids, steroids, flavonoids, triterpenoi ds, glycosides, ursolic acid, minerals, vitamins, and high levels of fibre. They can treat conditions including asthma, excessive salivation, lizard bite irritation, elephantiasis, fever, mental and digestive issues, and preserve skin health. According to traditional knowledge, a spine gourd is essential for treating several diseases, including digestive problems, urinary problems, and bleeding **[5]**.

Novel herbal drug delivery systems include liposomes, phytosomes, niosomes, proniosomes, transferosomes, microspheres, ethosomes, nanoparticles, etc. A novel approach to drug delivery overcomes the limitations of traditional medication delivery methods. The innovative drug delivery method can improve herbal medicines' solubility, bioavailability, and effectiveness. It also enhances the stability of medications, prevents toxicity, and protects against chemical and physical drug degradation. This is the fundamental idea underlying the incorporation of innovative medication delivery techniques into herbal medicines **[6]**.

Cardiac fibrosis, caused by the build-up of extracellular matrix proteins that are not contractile, is a major cause of death worldwide and worsens the cardiovascular disease. Heart failure and a higher incidence of arrhythmia are strongly correlated with cardiac fibrosis. Despite its widespread occurrence, no effective treatments currently exist to prevent or reverse cardiac fibrosis. This is primarily because the cell types and signalling pathways involved are complicated. The ongoing study has aimed to comprehend the mechanisms underlying cardiac fibrosis and generate new approaches to addressing the development of scar tissue **[7]**. New methods, such as direct cellular reprogramming and molecular targets, like noncoding RNAs and epigenetic regulators, bring potential treatment possibilities for fibrosis to light **[8]**.

Scientific groups have long regarded the study of medicinal plants for the creation of new medications. Recently, various plants and Phyto molecules have been developed to treat various illnesses, including cardiovascular diseases **[9]**.

Due to the fact that herbal remedies are more efficient, safe, and have fewer side effects than conventional therapies and are more readily available, affordable, and easily accessible, they have grown in popularity in

recent decades. The need for new treatment options has risen to combat this multifaceted illness as a result of the consequences of present treatment options not being satisfactory enough **[10]**.

The current study aimed to investigate the quality evaluation of cubosomes loaded with MD extract. Cubosomes was created in this work, and the resulting formulation was standardised using advanced methodologies based on phytochemical screening, physicochemical assessment, and pharmacological evaluation.

MATERIALS AND METHODS

Collection of Herb

The fruits of Momordica dioica Roxb. was collected from local market of Anand. It is Authentified by Dr. R. R. Acharya, Head & Research Scientist (Veg.), Main Vegetable Research Station, Anand agricultural university, Anand - 388 110, Gujarat (India) against a voucher specimen AAU/ MVRS/EST/53/2021 on Dated 10/05/2021. The fruits used for the studies were sun dried.

Drug and chemical

All of the chemicals used in the investigation were from Merck Chemicals in Ahmedabad and were of analytical quality. The reagents and standards included with the kits which were purchased from I-chem, Jeev Diagnostic Private Limited, Surat were utilized for biochemical tests as necessary.

Experimental Protocol

Wistar male rats weighing 250 $\pm$ 10 grammes were utilized for the research. According to the guidelines of the committee for purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, the experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Anand Pharmacy College (APC) (Protocol no. APC/2022-IAEC/2213). (Appendix C) According to Rule 5(a) of the Breeding and Experimentation on Animal Control and Supervision Rules 1998 (Registration No. 277/CPCSEA 24th 2000), the Animal Housing Facility of APC is registered. Animals were housed in a climate-controlled space that was intended to keep the temperature at 22° ± 3°C and the relative humidity at 30% to 70%. The light/dark cycle was regulated with the help of automated timers those was set to provide a daily photoperiod of 12 hours of light followed by 12 hours of darkness. Temperature and relative humidity in the animal room used for the study was recorded once daily.

Preparation of the extract

The sun-dried *Momordica dioica* Roxb fruit was put through a mechanical grinder to make powder. Using a Soxhlet extractor, 900 mL of petroleum ether and 900 mL of methanol were used to progressively extract the powdered material (100 g). Filtering the

extracts was done using Whatman filter paper. After being reduced in a rotary evaporator, the extracts were vacuum dried, and they were kept in storage at a temperature of 4-5ºC until they were needed **[11]**.

Preliminary Phytochemical Analysis of Extract

The MD fruit extract was next subjected to an initial phytochemical screening procedure to check for both secondary and primary metabolites. The phytochemical screening of the petroleum ether and methanol extracts was carried out using established procedures for the identification of alkaloids, glycosides, steroids, saponins, proteins, carbohydrates, flavonoids, tannins, and terpenoids **[12, 13]**.

Preperation of MD extract loaded cubosomes

Cubosome preparation generally involves either the top-down or bottom-up approaches. To prevent cubosomes from aggregating, both of them require the application of a suitable stabilizer. It involves two main stages. Then, combine the lipid that forms cubosomes with the proper stabilizer to make the bulk viscous cubic aggregates. Second, cubosomes were finally produced by using high energy as a high-pressure homogenizer or sonication to the created viscous cubic aggregates in an aqueous media **[14]**. The top-down approach is the most widely used method for cubosome synthesis **[15]**. Cubosomes created via the top-down method, however, have been found to remain durable against aggregation for up to a year **[16]**. GMO was melted and 1.5 gram of MD extract were combined. The mixture was added dropwise to warmed water containing Poloxamer 407 while being continuously agitated using a magnetic stirrer set to 1500 rotations per minute and 70 °C for an hour. After an hour, place the solution for 48 hours. Put the solution through a high-speed homogenization procedure at 1500 rpm for an hour at 70 °C to form cubosomes that are filled with MD extract **[17]**.

Conversion of liquid cubosomes into solid cubosomes

For the creation of powder for formulation development, only cubosomal dispersion was chosen. To the cubosomal dispersion, several adsorbents were separately added to generate a dry mass, which was then physically combined in a small mortar and pestle. These adsorbents included lactose, Syloid, Aeroperl 300, and starch 1500. The dry powder was then dried by air **[18]**.

Characterization of Cubosomes

Generated MD-loaded cubosomes were examined using transmission electron microscopy, a Fourier transform infrared spectroscopy, zeta potential, particle size (PS) and a stability investigation **[18, 19, 20]**.

Surgical procedure to induce Cardiac

fibrosis

We utilized male albino Wistar rats that weighed 250 ± 10 g. Using the DOCA-salt Hypertension model, cardiac fibrosis is generated in rats. Rat was intraperitoneally injected with a combination of ketamine (50 mg/kg) and xylazine (10 mg/kg) before being uni nephrectomised (Unix) (i.p.) To lessen the danger of infections and improve organ visibility, the fur on the dorsal midline to slightly left was removed. Around 2 cm of the skin was removed in a vertical incision. The left kidney was removed together with the fat pad and adrenal gland from the body after the renal artery was located and ligated using 22 mm sutures. Thereafter, each animal was placed in its cage to prevent cannibalism and infection. Infections in animals are monitored in the surgical areas. Every fourth day for 28 days, a dosage of 25 mg/kg of DOCA was given subcutaneously after being prepared in olive oil. In the drinking water, salt was administered at a dosage of 1% w/v **[21, 22]**.

Treatment Procedure

Animals were randomized based on baseline parameter in four groups with eight animals each as follows:

Group, I underwent the surgical procedure with no uninephrectomy, no deoxycorticosterone acetate (DOCA), and no salt in the water.

Group II underwent the Unix procedure and was not received DOCA or 1% Saline water.

Group III was uninephrectomized and DOCA was given in multiple doses via subcutaneous route, while the animals will have free access to 1% saline water and normal food.

Group IV underwent the Unix procedure as in the model group and received DOCA subcutaneous (s.c.) and 1% Saline water. It was received Novel herbal formulation (104 mg/kg) dissolved in CMC (0.5% Carboxymethyl cellulose) for 28 days

Initial and final body weights of the animals were noted for each group throughout the whole investigation. Biopac Student Lab measured and evaluated the blood pressure and the ECG on the 28th day, which marked the completion of the treatment (MP-36 Biopac Systems, Inc.). After that, under anesthesia with Ketamine (50 mg/kg) + Xylazine (10 mg/kg), blood samples were taken from the retro-orbital plexuses in clear, dried centrifuge tubes. The serum was separated through centrifugation at 10,000 rpm for 6 minutes, then kept at -22 °C before analysis **[21, 22]**.

Anthropometric analysis

Comparisons between the control and treatment groups were made using anthropometric measures. This involved the initial, intermediate, and final analyses using body weight. After euthanizing the animal, the remaining criteria were carried out. They assessed tibia length, kidney weight, and heart weight.

Estimation of hemodynamic parameters

On the first and fourteenth days, as well as at the conclusion of the research period, blood pressure was monitored using a non-invasive technique with a tail-cuff utilising a Biopac Student Lab (MP-36 Biopac Systems, Inc) **[23]**.

Electrocardiography: The Biopac Student Lab (MP-36 Biopac Systems, Inc.) and 10 leads—instead of V1, V2, V3, V4, V5, and V6—I, II, III, aVR, aVL, aVF, and precordial VM—were used to record the electrocardiograms. Animals that had undergone the previously mentioned aesthesia had baseline recordings were taken before the experiment began, as well as 1-minute intervals every 5 minutes while the therapy was being administered. We took into account the type of modifications (ST-segment elevation or depression, and T wave inversion). The changes discovered in the final trace captured five minutes before the sacrifice will be carefully examined in particular because they will be the closest to any changed plasma biochemical profiles right away after collection **[24]**.

Biochemical parameters

The measurement of serum creatinine using the modified Jaffe technique **[25, 26]**. The calculation of blood glucose using the enzymes glucose oxidase and peroxidase **[27]**, and the estimation of urea using the ureease-L-glutamate dehydrogenase method are used for the evaluation of biochemical parameters **[28, 29]**.

Heart homogenate parameters

The separated cardiac tissues were divided into minute pieces and homogenized in an ice-cold Tris-hydrochloride buffer (0.1M, pH 7.4). The homogenate was spun in a cooled centrifuge at 1000 g for 10 min at 4˚C. Malondialdehyde was detected in the supernatant using the Slater and Sawyer technique as a marker of lipid peroxidation **[30, 31]**. Superoxide dismutase (SOD) and reduced glutathione (GSH) levels were assessed as antioxidant enzymes **[32, 33, 34]**.

Langendorff isolated perfused heart preparation

The Langendorff method was used to do perfusion on isolated hearts. After thoracotomy, the hearts were removed and fastened to the aortic cannula. Hearts were perfused with modified Krebs-Henseleit buffer, which included bovine albumin (0.1 %w/v), $CaCl_2$ (1.5 mM), $MgSO_4$ (1.66 mM), KCl (4.7 mM), KH_2PO_4 (1.18 mM), $NaHCO_3$ (24.88 mM), NaCl (118 mM), Na-pyruvate (2 mM) and glucose (5.55 mM). Before usage, the buffer was filtered using a 0.45 m membrane filter. The Langendorff perfusion equipment was quickly attached to the cannulated heart (flow rate of buffer: 9.7 ± 0.5 ml/min; carbogen (95% O_2 and 5% CO_2), and temperature: 37˚C). A polyethylene tube attached to the pressure transducer and a latex balloon filled with

50% methanol was introduced into the left ventricle of the isolated heart. After adjusting the diastolic BP to between 5 and 6 mmHg, 30 minutes later, several parameters were assessed.

Left ventricular end-diastolic pressure (LVEDP), which is a measurement of relaxation, was measured together with dp/dt max (rate of maximum LV pressure increase), dp/dt min (rate of minimum LV pressure decline), and other parameters. A physiological recording system and Biopac recording device collected the data (MP-36 Biopac Systems, Inc., USA) **[35]**.

Histopathology of Heart:

The tissue was fixed and embedded in paraffin before being cut into serial pieces after being stored in a 10% buffered neutral solution. Masson's trichrome staining was used to determine the degree of myocardial fibrosis in the sections, and photomicrographs of the staining were made after the myocardial was stained in red and collagen in blue. The sections were then viewed under a light microscope [36].

Statistical Analysis:

The results were shown as mean SEM. Using Graph Pad Prism version 6.01, statistical analysis was conducted using one-way ANOVA and Dunnett's post hoc test. At $p \leq 0.05$ and $p \leq 0.0001$, data were deemed statistically significant.

RESULTS AND DISCUSSION:

Phytoconstituents

Soxhlet extractions were used to create the petroleum ether extract (5.03 g, 5.03%, oily-viscous yellowish brown that solidifies at 28^0 C) and the methanolic extract (16.45 g, 16.45%, semi-solid dark brown) from the *Momordica dioica* fruits (100 g). Following a phytochemical investigation, steroids, and terpenoids were discovered in the petroleum extract. Alkaloids, glycosides, steroids, terpenoids, proteins, tannins, saponins, flavonoids, and phenol were discovered in methanolic extracts.

Characterization of Cubosomes

The optimized MD loaded cubosomes indicated spherical-polyangular cube - shaped structure with mean particle size, entrapment efficiency, and zeta potential were 241.06±2.16 nm, 89.26 ± 0.021 %, and −49.4 ± 2.00 mV, respectively. The polydispersity index (PDI) value of the cubosomal dispersion was 0.12 ± 0.04. In vitro drug release of optimized MD loaded cubosomes was 85.96 ± 0.063%. The investigation proposed with the controlled sustained release of MD extract to enhance its bioavailability and extend its activity. The sachet formulation of MD extract was chemically stable at room temperature storage conditions.

Effect of MD extract loaded cubosomes on Anthropometric Parameters

Table 1 displays the impact of cubosomes containing MD extract on body weight, heart weight, tibia length, heart weight to

body weight ratio, and heart weight to tibia length ratio. There were no significant changes between the Unix group and the Model group compared to the sham group for the heart weight to body weight ratio. However, the heart weight to bodyweight ratio in the Treatment group was much lower than in the Model group. No significant variations were identified between the Unix group and Model group and the Sham group in the heart weight to tibia length ratio. Significant changes between the Treatment group and the Model group could not be detected.

Effect of Cubosomes loaded with *Momordica dioica* extract on Haemodynamic parameters

DOCA salt treatment in rats caused a rapid increase in systolic BP (measured by tail cuff) which reached a plateau of approximately (143.8 ± 0.370 mmHg) above baseline within 28 days post-surgery, While only surgery without DOCA salt treatment in rats caused a rapid increase in systolic BP (measured by tail cuff) which reached a plateau of approximately 133.7 ± 0.263 mmHg) above baseline within 28 days post-surgery, also in the sham group without Uninephrectomy without DOCA salt treatment in rats systolic BP is normal (125.7 ± 0.778 mmHg), and in rats treated with Novel formulation systolic BP is decreased (136.2 ± 1.093 mmHg) as compare to model control group within 28 days post-surgery **(Figure 1)**. When all the groups' systolic blood pressure values were examined, it became clear that there were significant disparities between the Unix group and the Sham group as well as the Model group and the Sham group. Additionally, there were noticeable variations between the Treatment group and the Model group.

On the other hand, DOCA salt treatment in rats caused a rapid increase in diastolic BP (measured by tail cuff) which reached a plateau of approximately (100.5± 0.90 mmHg) above baseline within 28 days post-surgery, While only surgery without DOCA salt treatment in rats caused a rapid increase in diastolic BP (measured by tail cuff) which reached a plateau of approximately (100.5 ± 0.64 mmHg) above baseline within 28 days post-surgery, also in the sham group without Uninephrectomy without DOCA salt treatment in rats diastolic BP is normal (100.5 ± 0.86 mmHg), and in rats treated with Novel formulation diastolic BP is decreased (92.69 ± 2.095 mmHg) as compare to model control group within 28 days post-surgery. No significant differences were discovered when the diastolic blood pressure readings of all the groups, i.e., Unix group with Sham group and Model group with Unix group and Sham group, were examined. Additionally, the Treatment group displayed significant

changes when compared to the Model group **(Figure 2)**.

While DOCA salt treatment in rats resulted in a quick rise in mean arterial pressure (as determined by a tail-cuff), the level of this rise plateaued at about (114.4 ± 0.366 mmHg) above baseline after 28 days following surgery. While only uninephrectomy without DOCA salt treatment caused a significant increase in mean arterial pressure (measured by tail cuff) in rats, which peaked at about (111.2 ± 0.081 mmHg) above baseline within 28 days post-surgery, mean arterial pressure is also normal in the sham group without uninephrectomy without DOCA salt treatment in rats (108.4 ± 0.47 mmHg), and in rats treated with novel formulation mean arterial pressure is decreased (107.2 ± 1.077 mmHg) as compare to model control group within 28 days post-surgery. The Mean arterial pressure levels of all the groups were compared i.e., Unix group with Sham group and Model group with Unix group and Sham group both showed significant differences. Additionally, there were noticeable variations between the Treatment group (107.2 ± 1.077 mmHg) and the Model group **(Figure 3)**.

Effect of Cubosomes loaded with *Momordica dioica* extract on Electrocardiogram

When the baseline analysis was conducted, the ECGs obtained from all the groups were all normal. Sham group maintained normal ECG patterns throughout the research period indicating the normal cardiac structure remained unchanged. The ECG of the Model group and the Unix group, however, differed during the mid-term analysis. These groups displayed abnormal ECGs with tall and prolonged QRS complexes, which are seen when the myocardium thickens abnormally, as in cases of Left ventricular dysfunction or hypertrophy, where the electrical activity must pass through a larger mass of myocardium. As a result, it takes longer for the electrical activity to travel through the entire heart, extending the time for the QRS complex. Along with a tall and extended QRS complex, the ECG pattern also showed deep T wave inversion on the 28th day of the term, which may indicate myocardial ischemia or myocarditis. And using a novel herbal formulation as treatment, the hypertrophic alterations that appeared in the ECG patterns were reversed **(Figure 4)**.

Effect of Cubosomes loaded with *Momordica dioica* extract on left Ventricular Function

Comparing the model group to the sham group, the Left Ventricular End Diastolic Pressure (LVEDP) increased significantly ($p<0.01$) **(Table 2)**. When compared to the model group, treatment with MD-loaded cubosomes caused a significant ($p<0.0001$) decrease in LVEDP. Both +dp/dt max and -

dp/dt min significantly increased in both model and Unix group rats. In comparison to model control animals, the rats treated with MD-loaded cubosomes displayed a significant ($p<0.0001$) reduction in $+dp/dt_{max}$ and $-dp/dt_{min}$. When compared to the sham group, the Coronary flow rate (CFR) decreased significantly ($p<0.0001$) in the model and Unix groups **(Table 2)**. When compared to the model group, the use of MD-loaded cubosomes caused a significant ($p<0.0001$) increase in CFR.

Effect of Cubosomes loaded with *Momordica dioica* extract on Biochemical Parameters

Serum glucose levels should be between 50 and 135 mg/dl. No significant differences were identified in the glucose levels of all the groups, i.e., the Unix group (130.6 ± 2.92), the Sham group (124.3 ± 1.91), and the Model group (145.7 ± 1.38) between the Unix group and Sham group. Additionally, there were noticeable differences between the Treatment group (136.9 ± 9.046) and the Model group **(Figure 5)**.

The serum creatinine range should be between 0.2 and 0.8 mg/dl. There were no significant changes between the creatinine levels of the Unix group (0.731 ± 0.035), the Sham group (0.566 ± 0.076), and the Model group (0.908 ± 0.018) with the Unix group and the Sham group. Additionally, there were significant differences between the Treatment group (0.775 ± 0.056) and the Model group **(Figure 6)**.

BUN levels should be between 7 and 20 mg/dl. There were no discernible variations between the BUN levels of all the groups, including the Unix group (56.25 ± 2.95), the Sham group (48.08 ± 2.84), and the Model group (68.2 ± 2.36) with the Unix group and Sham group. Additionally, there were noticeable variations between the Treatment group (30.95 ± 7.654) and the Model group **(Figure 7)**.

Effect of Cubosomes loaded with MD extract on Heart homogenate parameters

Malondialdehyde (MDA) levels in Heart homogenate were compared across all groups, i.e., Unix group (0.154 ± 0.0074) with Sham group (0.0960 ± 0.0245) and Model group (0.204 ± 0.0174) with Unix group and Sham group both showing significant differences. Additionally, there were significant differences between the Treatment group (0.0515 ± 0.0068) and the Model group **(Figure 8)**.

The levels of glutathione (GSH) in the heart homogenate of each group were compared, i.e., the Unix group (5.581 ± 0.221), the Sham group (10.67 ± 0.328), and the Model group (1.925 ± 0.083), with both the Unix group and Sham group exhibiting significant differences. Additionally, there were significant differences between the Treatment group (4.23 ± 0.272) and the Model group **(Figure 9)**.

The levels of Superoxide Dismutase (SOD) in Heart Homogenate of all the groups were compared, i.e., Unix group (28.83 ± 3.68) versus Sham group (57.4 ± 8.091) and Model group (18.06 ± 3.10), with Unix group and Sham group both showing significant differences. Additionally, there were substantial differences between the Treatment group (33.42 ± 2.23) and the Model group **(Figure 10)**.

Effect of Cubosomes loaded with MD extract on Histopathology

The Masson's trichome staining technique was used for the histology. It is particular to collagen fibres. With reddish-brown cells, the collagen fibres are dyed blue. The myocardial in the sham group was seen to be nearly normal in integrity, with unaltered fibres and no remarkable collagen deposition. Small regions of collagen deposition were visible in the Unix group. In the model group, there were numerous patches of the infarcted area, many of which had collagen fibres in their place. There were fewer minor infarcted areas and less collagen deposition in the treatment groups compared to the model group **(Figure 11)**.

Table 1: MD extract loaded cubosomes on Anthropometric Parameters

Groups	Body Weight (BW) (g)	Heart Weight (HW) (g)	Tibia Length (TL) (cm)	HW/BW	HW/TL
SHAM	245 ± 6.042	1.12 ± 0.102	5.06 ± 0.0748	0.003098 ± 0.000112	0.2244 ± 0.06933
UNIX	245 ± 2.55	1.2 ± 0.0316	4.84 ± 0.0979	0.003268 ± 0.00014	0.2377 ± 0.09066
MODEL	248.8 ± 3.826	1.14 ± 0.0244	4.96 ± 0.1364	0.003262 ± 0.000174	0.1574 ± 0.008101
TREATMENT	339.2 ± 12.74 (****)	0.76 ± 0.0509 (**)	4.88 ± 0.1428	0.00212 ± 0.000137 (***)	0.152 ± 0.00826

Results were presented as mean ± SEM. One-way ANOVA was used to determine the significant statistical difference between the groups followed by Dunnett's Post-hoc test by using graph pad prism 6.01. Where, ***p<0.001, ****p<0.0001 vs. Model. n=6 for Sham, Unix, Model, Treatment

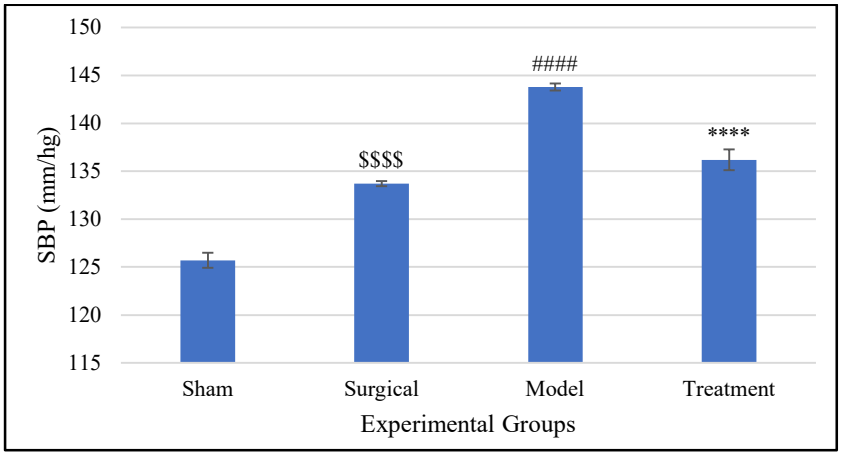


Figure 1: Effect of Cubosomes loaded with MD extract on Systolic Blood Pressure
Results were presented as mean ± SEM. One-way ANOVA was used to determine the significant statistical difference between the groups followed by Dunnett's Post-hoc test by using graph pad prism 6.01. Where, $$$$p<0.0001, ####p<0.0001 vs. Sham; ****p<0.0001, vs. Model. n=6 for Sham, Unix, Model, Treatment

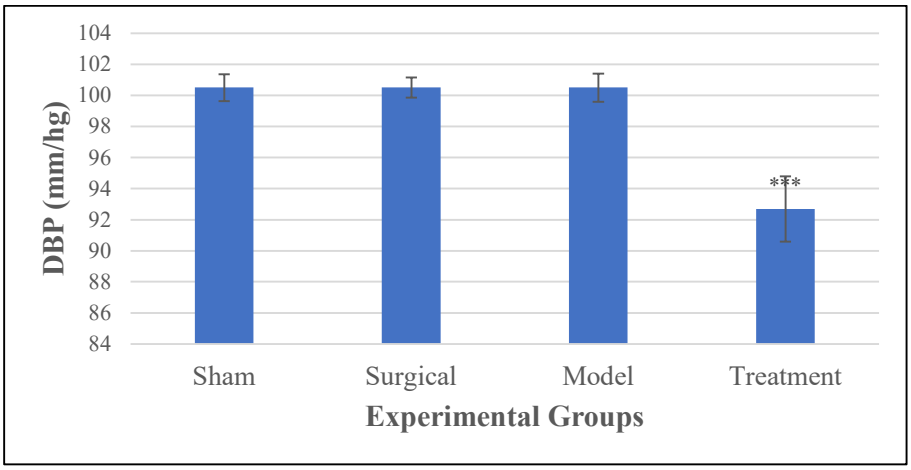


Figure 2: Effect of Cubosomes loaded with MD extract on Diastolic Blood Pressure
Results were presented as mean ± SEM. One-way ANOVA was used to determine the significant statistical difference between the groups followed by Dunnett's Post-hoc test by using graph pad prism 6.01. Where, ***p<0.001, vs. Model. n=6 for Sham, Unix, Model, Treatment.

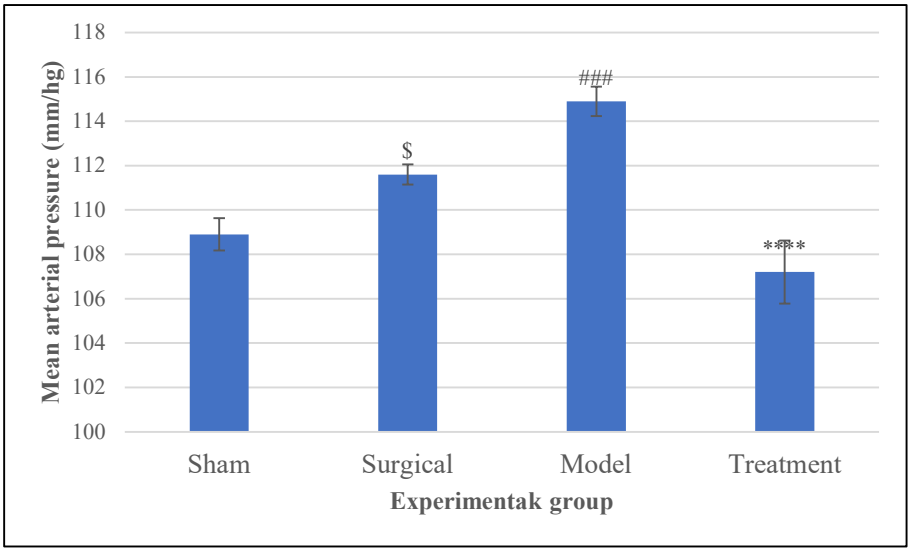
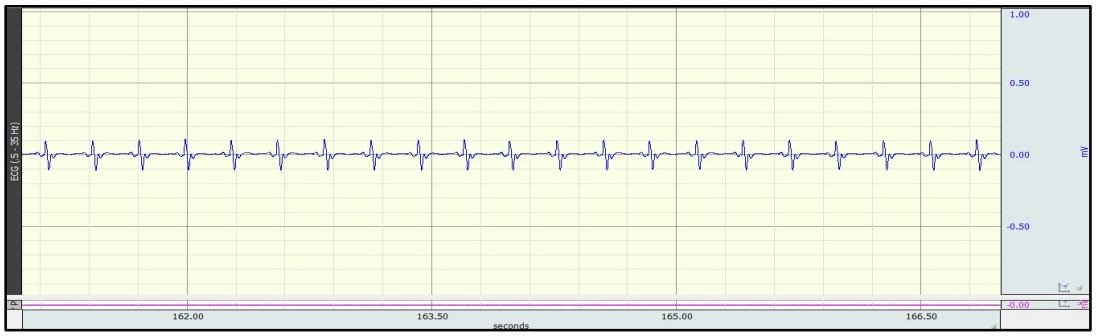
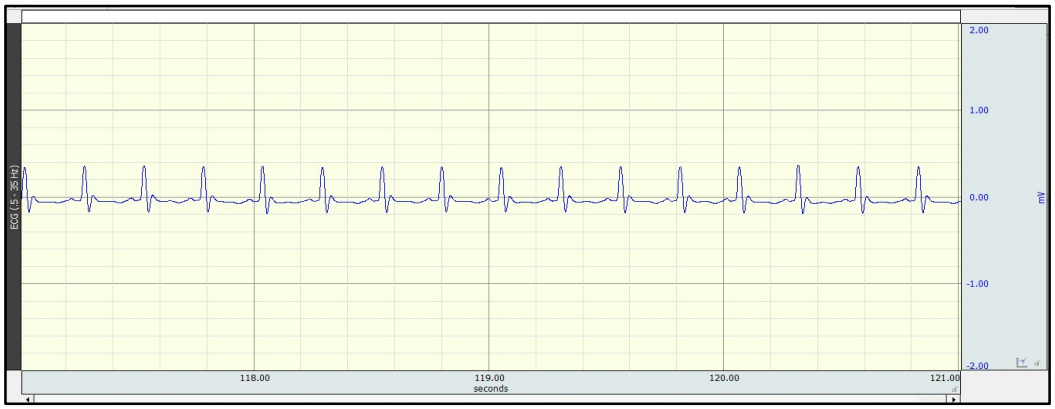


Figure 3: Effect of Cubosomes loaded with MD extract on Mean Arterial Pressure
Results were presented as mean ± SEM. One-way ANOVA was used to determine the significant statistical difference between the groups followed by Dunnett's post-hoc test by using graph pad prism 6.01. Where, $$$$p<0.0001, ####p<0.0001 vs. Sham; ****p<0.0001, vs. Model. n=8 for Sham, Unix, Model, Treatment



(A)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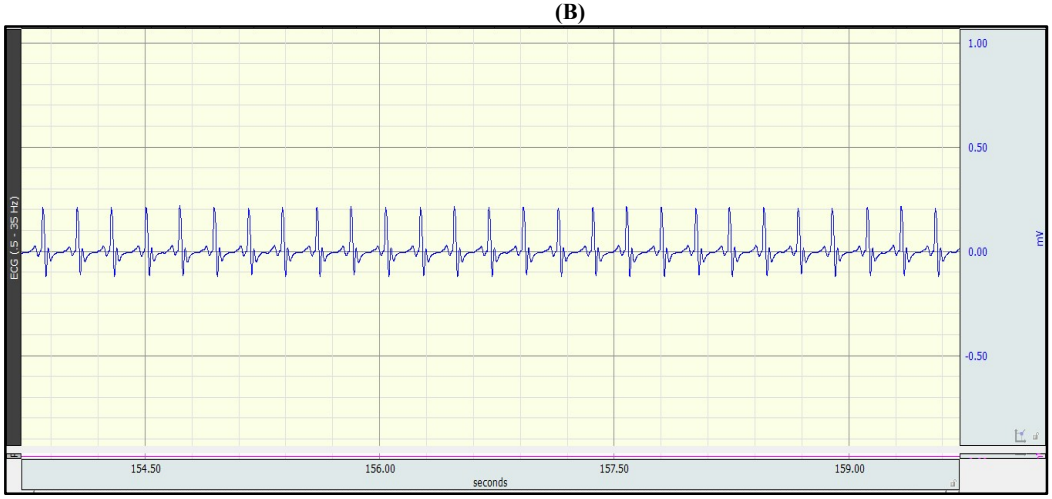
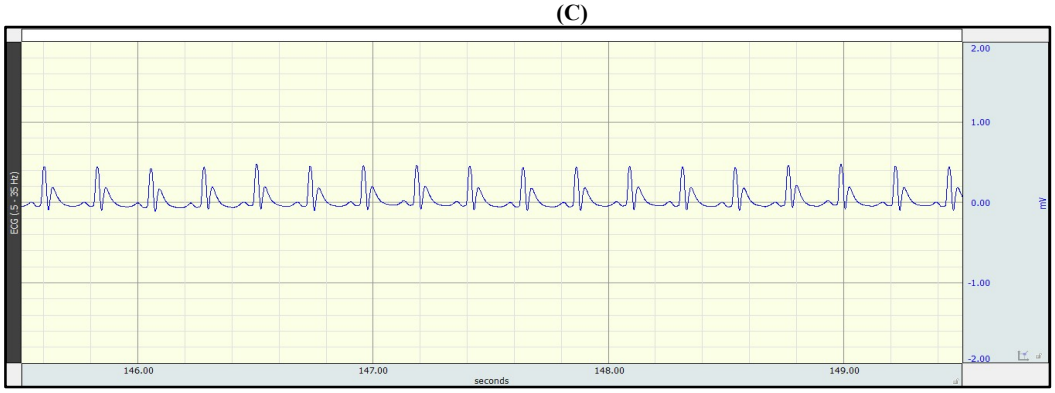
2.00
1.00
0.00
-1.00
-2.00
mV
118.00
119.00
120.00
121.00
seconds

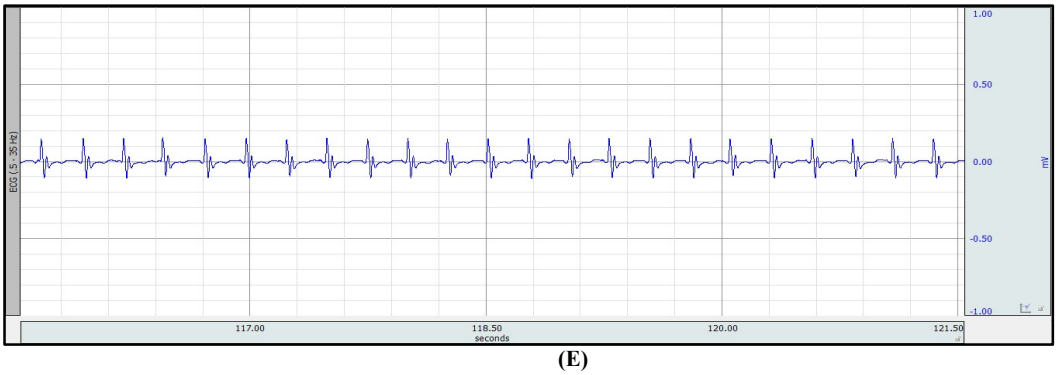
(B)


ECG (.5 - 35 Hz)
1.00
0.50
0.00
-0.50
mV
154.50
156.00
157.50
159.00
seconds

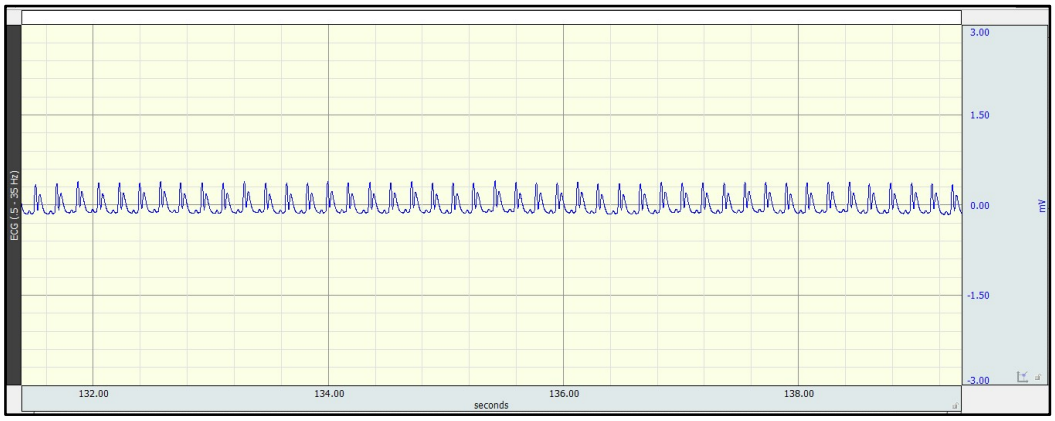
(C)


ECG (.5 - 35 Hz)
2.00
1.00
0.00
-1.00
-2.00
mV
146.00
147.00
148.00
149.00
seconds

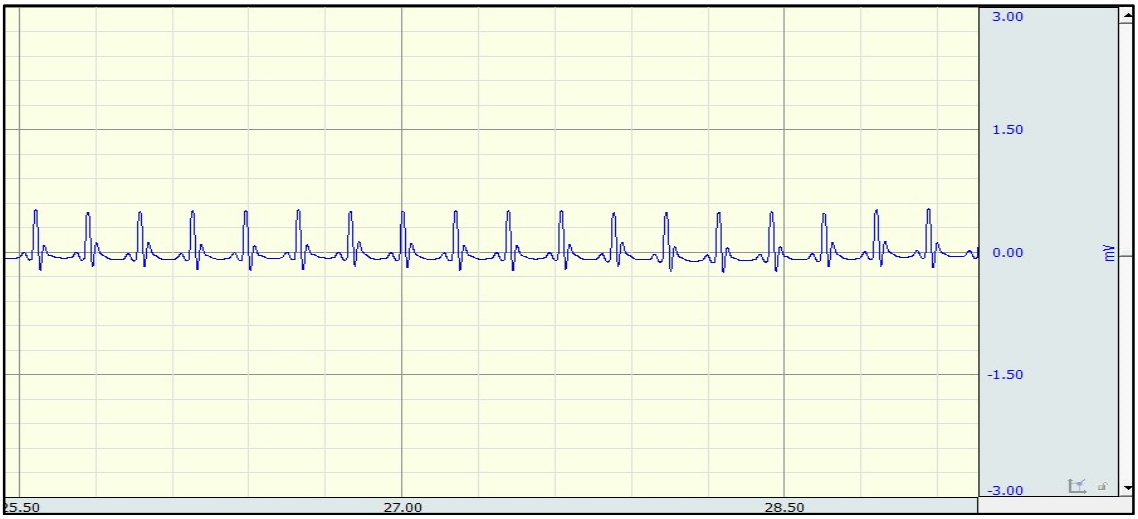
(D)


ECG (.5 - 35 Hz)
1.00
0.50
0.00
-0.50
-1.00
mV
117.00
118.50
120.00
121.50
seconds

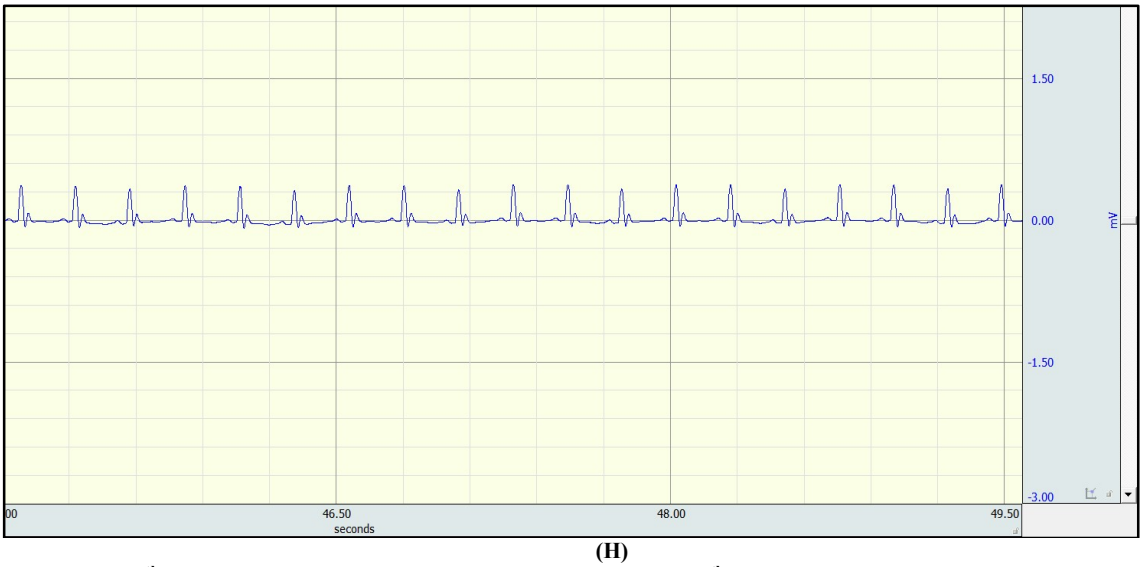
(E)



(F)



(G)



(H)

Figure 4: (A) 0th day Electrocardiography of Sham control anmials (B) 28th day Electrocardiography of Sham control anmials (C) 0th day Electrocardiography of Surgical control anmials (D) 28th day Electrocardiography of Surgical control anmials (E) 0th day Electrocardiography of Model control anmials (F) 28th day Electrocardiography of Model control anmials (G) 0th day Electrocardiography of Treatment control anmials (H) 28th day Electrocardiography of Treatment control anmials

Table 2: Cubosomes loaded with Momordica dioica extract on Left Ventricular Function Parameters

GROUPS	Coronary flow rate (ml/min)	LVEDP (mmHg)	+dp/dt max (mmHg)	-dp/dt min (mmHg)
Sham	13.32 ± 0.3917	4.338 ± 0.6359	271 ± 4.213	202.3 ± 1.953
Unix	10.74 ± 0.3709 ($$$$)	6.87 ± 0.3926 ($$)	308.2 ± 3.103	218.4 ± 2.06
Model	6.74 ± 0.6615 (####)	12.13 ± 1.856 (###)	331.7 ± 1.297 (##)	232.9 ± 2.556 (#)
Treatment	12.64 ± 0.103 (****)	2.602 ± 0.854 (****)	244.2 ± 19.3 (****)	180.6 ± 12.26 (****)

Values are expressed as Mean ± SEM. Values are statistically evaluated using one-way ANOVA analysis followed by Bonferroni Post-hoc test using GraphPad prism 8.01. Significant values were compared with $$p<0.01 and $$$$p<0.0001 sham vs. Unix; #, ##, ### and #### p<0.05, p<0.01, p<0.001 and p<0.0001 sham vs. model control respectively; ****p<0.0001 model control vs. treatment

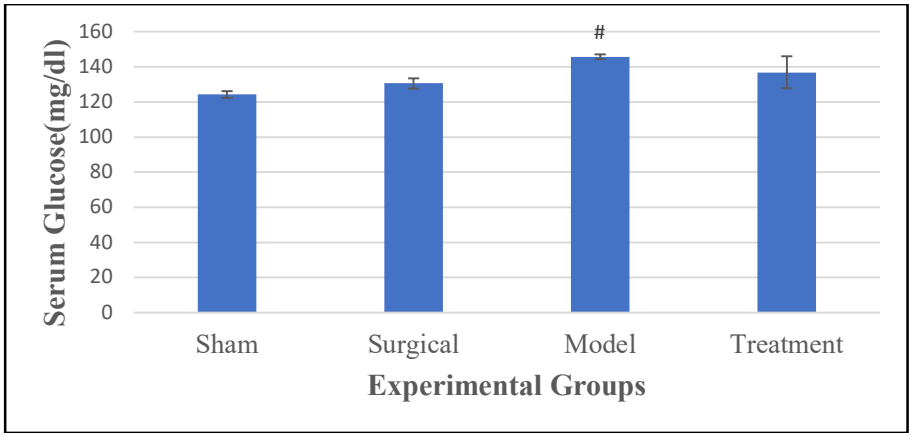


Figure 5: Effect of Cubosomes loaded with MD extract on Serum glucose
Results were presented as mean ± SEM. One-way ANOVA was used to determine the significant statistical difference between the groups followed by Dunnett's Post-hoc test by using graph pad prism 6.01. Where, #p<0.05 vs. Sham. n=6 for Sham, Unix, Model, Treatment

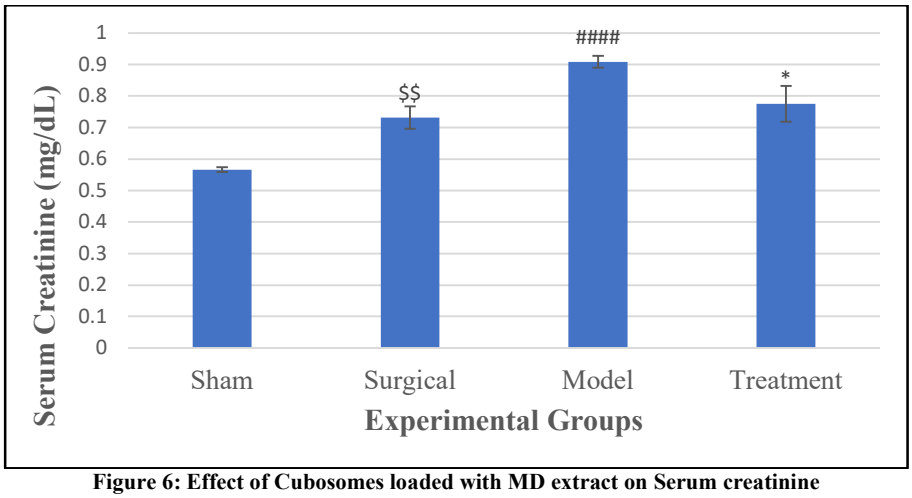


Figure 6: Effect of Cubosomes loaded with MD extract on Serum creatinine
Results were presented as mean ± SEM. One-way ANOVA was used to determine the significant statistical difference between the groups followed by Dunnett's Post-hoc test by using graph pad prism 6.01. Where, $$p<0.01, ####p<0.0001 vs. Sham; *p<0.05, vs. Model. n=6 for Sham, Unix, Model, Treatment

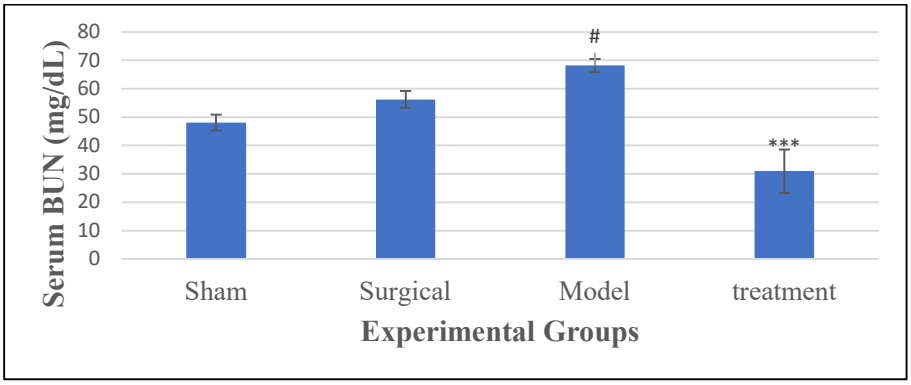


Figure 7: Effect of Cubosomes loaded with MD extract on Serum BUN
Results were presented as mean ± SEM. One-way ANOVA was used to determine the significant statistical difference between the groups followed by Dunnett's Post-hoc test by using graph pad prism 6.01. Where, #p<0.05 vs. Sham ***p<0.001, vs. Model. n=6 for Sham, Unix, Model, Treatment

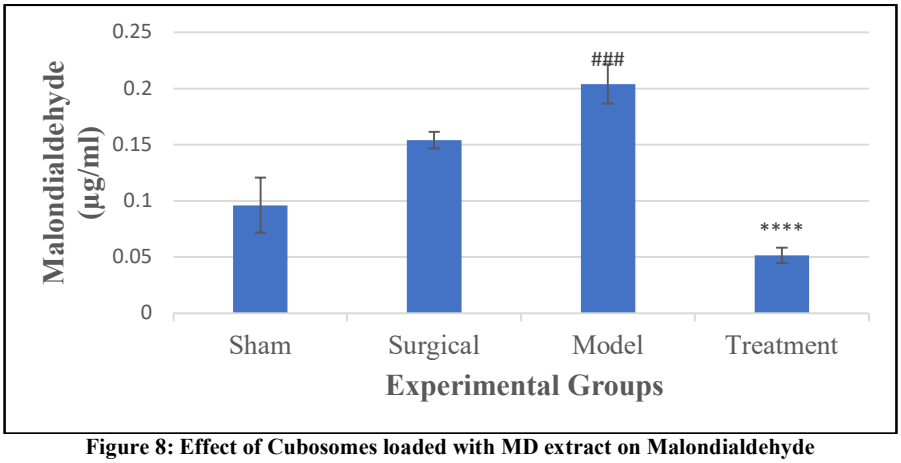


Figure 8: Effect of Cubosomes loaded with MD extract on Malondialdehyde
Results were presented as mean ± SEM. One-way ANOVA was used to determine the significant statistical difference between the groups followed by Dunnett's Post-hoc test by using graph pad prism 6.01. Where, ###p<0.001 vs. Sham ****p<0.0001 vs. Model. n=6 for Sham, Unix, Model, Treatment

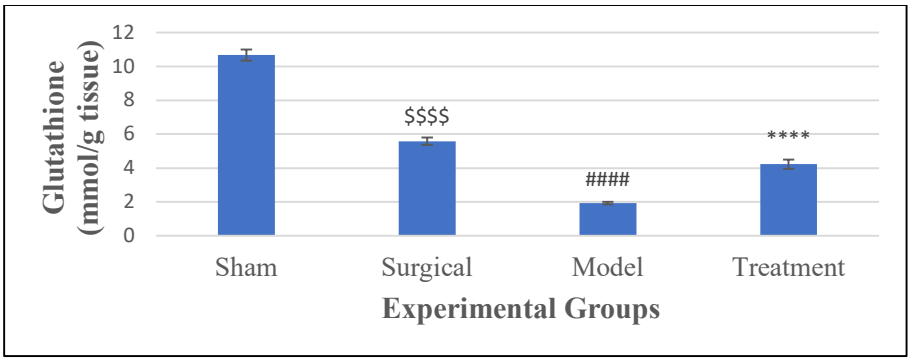


Figure 9: Effect of Cubosomes loaded with MD extract on Glutathione
Results were presented as mean ± SEM. One-way ANOVA was used to determine the significant statistical difference between the groups followed by Dunnett's Post-hoc test by using graph pad prism 6.01. Where, ####p<0.0001, $$$$p<0.0001vs. Sham ****p<0.0001 vs. Model. n=6 for Sham, Unix, Model, Treatment.

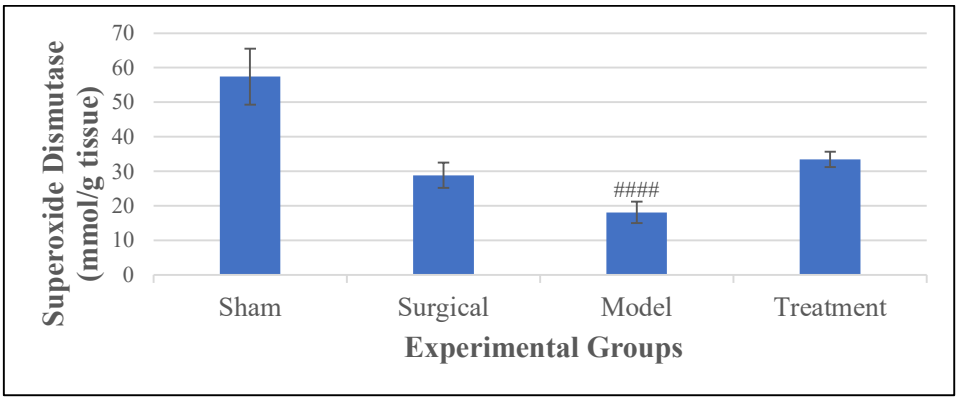


Figure 10: Effect of Cubosomes loaded with MD extract on SOD

Results were presented as mean ± SEM. One-way ANOVA was used to determine the significant statistical difference between the groups followed by Dunnett's Post-hoc test by using graph pad prism 6.01. Where, ####p<0.0001, vs. Sham. n=6 for Sham, Unix, Model, Treatment

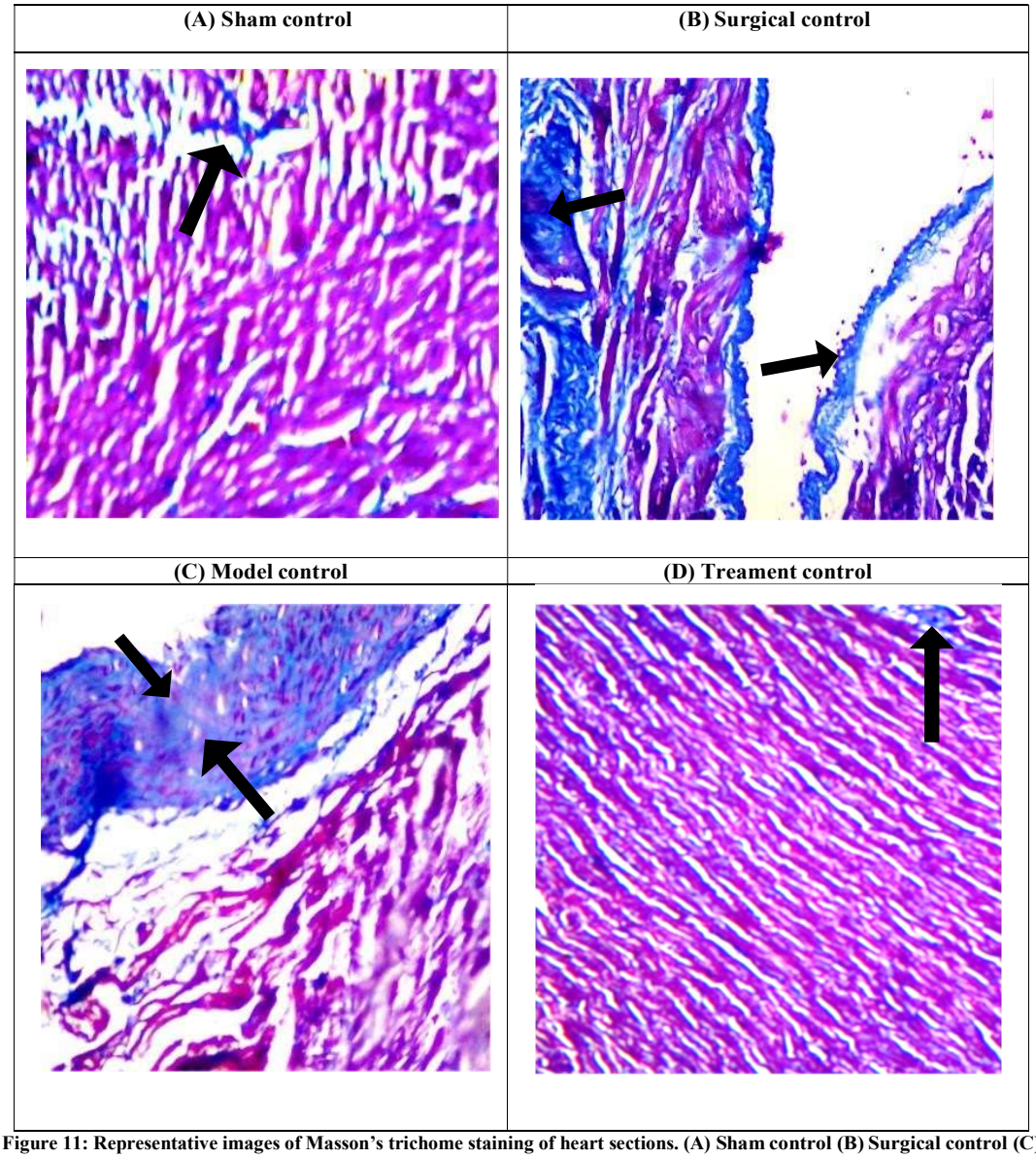


Figure 11: Representative images of Masson's trichome staining of heart sections. (A) Sham control (B) Surgical control (C) Model control (D) Treament control. Blue staining indicates the deposition of collagen and red staining indicates the myocardium

CONCLUSION:

Cubosomes containing MD extract have outstanding cardioprotective and anti-fibrotic properties demonstrated by stable blood pressure, a normal ECG pattern, normal left ventricular function, and maintenance of membrane integrity. It works by maintaining normal levels of blood urea nitrogen, creatinine, and glucose.

ACKNOWLEDGMENTS

We would like to extend our acknowledgement to Dr. Vaishali T. Thakkar, Research Coordinator and Professor, Anand Pharmacy College, Anand for constant support and guidance. We also like to acknowledge Ms. Anjali B. Patel, Ms. Navdha J. Vyas and Mr. Chetan Sojitra, Assistant Professor, Anand Pharmacy College, Anand, for giving direction during research. We would like to thanks Anand Pharmacy College, Anand for giving platform to carry out the research.

FINANCIAL DISCLOSURE

None

ETHICS STATEMENT

None

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