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RESEARCH ARTICLE

The potential impression of fructo-oligosaccharides and zinc oxide nano composite against nicotine influenced cardiovascular changes

Monalisha Paul[1], Chaitali Kundu[1], Rudranil Bhowmik[2], Sanmoy Karmakar[2], Sandip K. Sinha[3], Nilanjana Chatterjee[4*]

Abstract

Nicotine is easily transported to heart through blood and it increase the risk of coronary artery diseases by twofold besides developing a hyper-coagulable state (through platelets aggregation or fat deposition in artery), increased heart rate and blood, free radicals' generation, increased cardiac stress, etc. Nutritional therapy establishes nutrients' health potential without side effects. Fructo-oligosaccharides (functional soluble fiber) are capable of producing a higher amount of short-chain fatty acid (SCFA: fermented product) in the GI tract than other fibers. Zinc is an abundant metal for physiological and biological activities. A new fructo-oligosaccharides coated zinc nanocomposite (ZnOFNC) applied against nicotine tempted heart abnormalities. This scientific study was needed to establish a new nanocomposite, ZnOFNC (FOS coted zinc oxide nanocomposite), applied against nicotine entice heart abnormalities. ZnONPs and ZnOFNC synthesized through the green synthesis method were applied on an animal model. Required animals were (male albino rats, 100–120 gm bw) divided into six groups on the basis of toxic drugs (Nicotine) and therapeutic drugs (ZnOFNC, FOS, ZnONPs and vitamin C) treated for 15 days. The estimated parameters were biochemical parameters, electrocardiogram, platelets count and histological study. All the parameters of the ZnOFNC applied group result were satisfied with the control group than FOS, ZnONPs and vitamin C applied groups. Our result led us to conclude that the ZnOFNC may have the anti-nicotinic capability to protect the heart from nicotinic toxicity.

Keywords: Zinc oxide nanoparticle, Fructo-oligosaccharides, Nano composite, Soluble dietary fiber, Vitamin C, Short chain fatty acid.

Introduction

World Health Organization (WHO 2000 to 2025 and Siddiqi *et al.,* 2020) reported that in coronary heart disease (CHD) 7 million (21%) of deaths occur due to direct tobacco consumption. Other plants of the Solanaceae family, like *Solanum melongena* (eggplant), *S. tuberosum* (Potato), *Lycopersicon esculentum* (Tomato), etc. are contain nicotine in a very lower concentration. But nicotine is a principal alkaloid of *Nicotiana tabacum* plant that is synthesized in its roots and accumulates in the leaves (Roemer *et al*., 2012). Hukkanen *et al*., (2005) reported that after tobacco consumption the cotinine (a metabolised form of nicotine) level in the bloodstream is highly increased up to 500 ng/mL where its normal level is 10 ng/ml (non-smokers). High concentration of nicotine increases twofold the risk of coronary artery diseases along with other physiological disorders (Benowitz and Gourlay, 1997). Tobacco (Nicotine) is also responsible for developing a hyper-coagulable state (through platelets aggregation or fat deposition in an artery), secretes catecholamines from adrenal glands leading to increased heart rate with increased blood pressure, hyperlipidemia, reactive oxygen species (ROS) generation, increased cardiac tissue toxicity etc. Nicotine is one of the strong blockers of A-type K^+ channels, which is a potent inhibitor of cardiac contraction. It may contribute to increasing the capacity of nicotine to induce arrhythmias which in turn causes increased interstitial

[1]Department of Human Physiology with Community Health, Vidyasagar University, Paschim Medinipur, West Bengal, India.

[2]Department of Pharmaceutical Technology, Jadavpur University Kolkata, India.

[3]Department of Human Physiology and Community Nutrition, Vidyasagar University, Paschim Medinipur, West Bengal, India.

[4]Faculty of Zoology Department, Ramananda College, Bishnupur, Bankura, West Bengal, India.

***Corresponding Author:** Nilanjana Chatterjee, Faculty of Zoology Department, Ramananda College, Bishnupur, Bankura, West Bengal, India., E-Mail: nilchat@gmail.com

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collagen of blood vessels leading to myocardial infarction (Wang *et al*., 2000 and; Miyauchi *et al*., 2005). Any nutritional therapy establishes nutrients against physiological side effects. In our regular diet, we intake dietary fibre (DF is a non-digestible carbohydrate) in the forms of soluble (SDF) and insoluble (IDF). After fermentation in the GI tract, DF releases SCFA, which is of great physiologically important (Gavin *et al.,* 2019). Various clinical reports proved that dietary fiber supplements are more effective on coronary heart disease, stroke, hypertension, diabetes, gastrointestinal disorders, obesity, and even cancer development (Anderson *et al*., 2009). Dietary fiber like fructo-oligosaccharide (FOS) reduces some factors of cardiac diseases like oxidative stress components (Superoxide dismutase, Catalase, malondialdehyde, etc.), serum cholesterol level (Triglycerides, low-density lipoproteins, very low-density lipoprotein, high-density lipoprotein percentage), blood sugar level, and inflammation of the endothelium layer. They also reduce the risk of high blood pressure and stroke (Reyes *et al.,* 2010). On the other hand, nanotechnology is an alternative of conventional as well as advanced avenues in the biomedical field. Several inorganic metal nanoparticles like Mn, Fe, Cu, Zn, etc., are used in biomedical science. Among these Zn is very essential for the human body and has a wide range of biological activities. Zinc oxide nanoparticles (ZnONP) could save the cells from oxidative stress, decrease the levels of free radicals and play a vital role against cancer and they have anti-inflammatory, antimicrobial activity, antidiabetic activity (Atef *et al*., 2016, Mishra *et al*., 2017).

Our present study was focused on establishing a new nanocomposite in between ZnONPs and FOS (ZnOFNC) and conducting its Nano characterization through different methods. At the same time, we have tried to develop a comparative observation with ZnOFNC and the FOS, ZnONPs and vitamin C individual effects against nicotine tempted cardiovascular abnormalities through biochemical estimations (like ROS, ECG, lipid profile, platelet count and histological study), which helped to understand the condition of heart against nicotine toxicity.

Material and Methods

Synthesis and Characterization of ZnONPs and ZnOFNC

In aqueous solution of zinc acetate **(**0.025 M), NaOH (0.1 M) solution was mixed drop by drop under constant stirring with maintenance of pH (11) balance and then kept it at room temperature for 24 hours. The solution was centrifuged at 2,000 rpm for 10 minutes. The NaOH was removed by discarding the supernatant water. The pellets were washed thrice and then dried at 80°C then collected as ZnONPs (Mandal and Bhattacharjee, 2024; Moharekar *et al.,* 2014).

For ZnOFNC synthesis, zinc acetate and sodium hydroxide were used as substrate and reducing agent, respectively. O.5% (w/v) zinc acetate was thoroughly mixed with 3.8% (w/v) aqueous solutions of FOS. To this solution, 0.1 M NaOH solution was added for upholding the pH at 11 and the solution was continuously stirred for 24 hours. Then, the mixture was centrifuged at 2,000 rpm for 10 minutes and the precipitate was for the removal of NaOH (Abdelmigid *et al.,* 2022, Naiel *et al.,* 2022 and; Moharekar *et al.,* 2014). Finally, the ZnOFNC pallets were collected and dried at 80°C. The synthesized sample was characterized through the Fourier transform infrared (FTIR), X-ray diffraction (XRD) spectrum and scanning electron microscope (SEM).

Experimental Design

Animal handling

Adult Wistar strain male albino rats having body weight between 100 to 120 gm are used in this experiment. All the animals were divided into Six groups and in each group, 5 animals were required. Standard diet (Hindusthan Lever Ltd, India) and water were supplied as their requirement. The total experimental period was 15 days and before the application, the experimental animals were acclimatized for 15 days.

Animal grouping

Group I-control group group II- Nicotine treated group-applied dose was 3.5 mg/kg body weight (ip) treated for 15 days. Nicotine purchase from Sigma Aldrich. Group III- Nicotine + FOS treated (2 g/kg body wt: Indian GRAS assessment, 2015, Costa *et al*., 2015, Jain *et al*., 2018) group. Group IV- Nicotine + ZnONPs treated (10 mg/kg body wt.) group (Mohamed *et al*., 2019). Group V- Nicotine + Vitamin C treated (5 mg/kg body wt: Nair and Jacob. 2016 and FDA. 2005) group group VI- Nicotine + ZnOFNC treated (80 mg/kg body wt.) group (established dose). FOS, vitamin C, ZnONPs and ZnOFNC were applied oral gavage administration after 6-hour latter of nicotine treatment (i.p) daily.

Tissue supernatant and serum preparation

End of the treatment, the animals were survived overnight with fasting condition. The next morning in, between 9 am to 11 am sacrificed them by cervical dislocation. The blood samples were collected by cardiac puncture technique in EDTA container and the blood samples are centrifuged again at 3000 rpm for 10 minutes for the separation of plasma and it was utilized for the lipid profile estimation. Before homogenization of heart were washed by 0.5 M PBS and the blood clots. Then homogenate with chilled 0.05 M PBS (phosphates buffered saline, pH 7 and 9g1-1NaCl) solution and centrifuged for 1000 rpm for 5 minutes (at 4°C) and the supernatants were used for ROS measurement (Maity *et al.,* 2013).

Estimated Parameters

Liver enzyme and C-reactive protein estimation methods

At first serum glutamic oxaloacetic transaminase (SGOT) buffer was prepared by mixing of 0.1 M PBS, 0.1 M L-aspartate and 2 mM α-ketoglutarate. On the other hand, serum

glutamic pyruvic transaminase (SGPT) buffer was prepared by 0.1 M PBS, 0.1 M DL- alanine and 2 mM α ketoglutarate. Then 20 µL serum was added to 100 µL of individual SGOT and SGPT buffer solution followed by incubation at 37°C for 30 minutes. After that 100 µL of 2,4-dinitrophenylhydrazine was mixed with the buffer solution and incubated at 25°C for 20 minutes. Then 0.5 mL of 0.4 N NaOH solution was added with that mixed buffer solution. After 5 min of NaOH mixing, we took the OD value at 540 nm. In the case of serum alkaline phosphatase (ALP) estimation, 50 µL of blood serum was added with 0.5 mL of alkali buffer substrate (0.05 M glycine buffer containing 5.5 mM PNPP, pH 10.5) and incubated for 30 minutes at 37°C. Then 5 mL of NaOH (0.02N NaOH) was added with the mixture and measured OD value at 400 nm, after 5 minutes. ALP activity was determined as µmol/mg protein (Bowers *et al.,* 1966). Simultaneously we measured C-reactive protein (CRP) data by using Elabscience ELISA Kit.

Serum lipid profile estimation method

The serum lipid profile estimated by kit method. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were estimated by Coral Cholesterol Biochemistry Kit Private Ltd.

Platelet counting method

Platelet diluting fluid was prepared with 0.2 mL of neutral formaldehyde and 0.1 g of brilliant cresyl blue in 100 mL of deionized water. RBC counting chamber of the hemacytometer was used for total platelets counting. Then the blood samples (anticoagulant mixed) were diluted to 1:200 dilutions and the number of cells/cu.mm of whole blood (Brahimi *et al*., 2009).

Oxidative stress and antioxidant markers

- *SOD activity estimation*

About 1-mM of diethylene-triamine penta-acetic acid was added with tris-buffer (50 mM, pH 8.2), then 10 µL of heart tissue supernatant was added with the aliquot followed by 0.2 mM pyrogallol (10 mM pyrogallol added in 10 mM HCl) was added with in above mixture. OD values were observed at 420 nm (Mahapatra *et al*., 2009).

- *CAT activity determination*

About 50 µL of cardiac tissue supernatant was added to aliquot mixture which was prepared by mixing with 0.05M tris-buffer + 5 mM EDTA (pH 7.0) + 10 mM H_2O_2 (in 0.1 M potassium PBS, pH 7.0) and measured the OD value with time duration at 240 nm (Mahapatra *et al.,* 2009).

- *Measurement of lipid peroxidation (MDA)*

In 2 mL 0.375 and 15% thiobarbituric acid and trichloroacetic acid were added thoroughly and mixed well. After that 200 µL of tissue supernatant and 1.0 mL distilled water were added. Then the supernatant solution was boiled on a water bath at 95°C for 20 minutes (the solution get pink color) followed by cooled it under tap water. In the next step 3 mL of n-butanol was added with the above-mixed solution to stop the reaction and OD value was taken at 532 nm (Baliga *et al*., 2018).

- *Measurement of lactose dehydrogenase (LDH)*

Substrate reagent (1-mL) which was prepared by 100 mL Glycine reagent, 0.1N NaOH and 3.2 g lithium lactate used for incubation at 37°C for 5 minutes. Then 10 mL NAD solution which prepared by 2.62 mM nicotinamide,15 mL distilled water and 0.01 w/v % NAD was added with substrate reagent. At the same time 10 µL heart tissue sample was also added with the mixture then incubate it at 37°C for 15 minutes. Ketone reagent (0.5 mL) was added with the mixture and again incubated it for 15 minutes in room temperature. Last of all 0.4 N NaOH was added with it and measured the OD value at 440 nm. LDH activity was express as U/mg protein (Young *et al*., 1975).

Histological estimation

Haematoxylin and eosin (H/E) staining method was used for histological analysis (Titford, 2009).

ECG data acquisition

The animals (before sacrificed) were anesthetized by ketamine-xylazine, (IP, 0.1 mL/100 gm, where 87 mg ketamine/kg of body weight and 13 mg xylazine/kg bodyweight were mixed thoroughly) (Van-Pelt. 1977). After that wait for 10 minutes to stabilize the normal physiology and then ECG electrodes were placed and connected with a bioelectric amplifier (BIOPAC System INC. MP36) system for the recording of ECG with respect to control groups.

Quantitative estimation of plasma short chain fatty acids through GC technique

The full scan mode of GC technique m/z range was 30 to 150. Such absorption picks like 55, 57, 60 and 74 of m/z values were received on the base on retain time. That was proved that in plasma samples SCFAs (acetic acid, propionic acid and butyric acid) were present. The result developed on the basis of retention time and those compounds variations depend on the partition coefficients of the compounds of Plasma samples (Zhang *et al*., 2019).

Statistical Analysis

After collecting all the data, statistical analysis was adopted by mean ± Standard error of mean (SEM), and then applied one-way ANOVA for observation of normality, sample independence and variance equality in different group with the help of Origin 6.0 professional software.

Results and Discussion

Characteristics of ZnONPs and ZnOFNC

The FTIR spectrum absorption peaks of ZnONPs and ZnOFNC

were 743, 595, 489, 638.64 and 527.110 cm^{-1} which may confirm the presence of ZnO stretching. The FTIR spectrum of the ZnOFNC shows such common groups like the hydroxyl group, carboxyl group, aldehyde group, and alkyne group which are normally found in the FOS spectrum (Lambertz *et al*., 2017). But in the case of ZnOFNC new strong O=C=O stretching group was found at 2346.58 cm^{-1} which may help to bind the FOS and ZnO and form a nanocomposite. The ZnONPs particle's SEM image represents a rod-shaped structure with an average size of 24 nm. ZnOFNC particle's SEM image showed a spherical shape and the average size was 45 nm which indicates a strong composite structure. The X-ray diffraction technique of ZnONPs showed such important picks like 719.459, 314.419, 288.269, 274.862, 218.007, and 189.762 A° Debye Scherrer method was applied for nano size calculation from XRD data. The average ZnONPs particle size after XRD analysis was 33.41nm. The absorption picks of The XRD study of ZnOFNC, important picks were 310.05, 285.323, 278.992, 221.014, 191.515, 173.716 and 169.683 A° the average particle size of ZnOFNC was 23.28 nm (Figure 1).

Nanoparticles are microscopic materials with sizes ranging from 1 to 100 nm that have a that have a less toxic effect on our system (Chithrani *et al*., 2006). Several studies reported that 14 to 50 nm-sized particles are the most efficient biomolecules that enhance cellular uptake, such as cell-penetrating peptides, which easily bind with receptors (Jiang *et al*., 2008 and Conde *et al*., 2012). From the above characterization study, it was proved that ZnONPs and ZnOFNC were successfully synthesised, and their particle sizes were below 50 nm. Several pilot researches suggest that ~50 nm is the ideal size for nano particals to achieve the highest cellular uptake in certain cells (Chithrani *et al*., 2006).

Measurement of Serum Liver Enzyme and C-Reactive Protein

Nicotine-treated animals, SGOT, SGPT, and ALP levels were significantly ($p < 0.05$) higher than the control group. The levels of all these above enzymes were significantly ($p < 0.05$) different in ZnONP and vitamin C treated groups compared to group II group I. However, in the FOS-treated group, the above enzyme level was insignificant with the nicotine-treated group. ZnOFNC may be capable of reducing the SGOT, SGPT, and ALP levels to the words control group. CRP is also an inflammatory biomarker for CVD, indicating the inflammatory reactions in the heart tissue or vessels. The present study showed that the CRP level of nicotine nicotine-treated group (155 ± 6 mg/l) of animals was significantly ($p < 0.05$) higher than group I (80 ± 4 mg/l). Similarly, for FOS in the treated group the results were insignificant with the nicotine-treated group and significantly ($p < 0.05$) higher than the control group. On the other hand, the CRP levels of ZnONP (119 ± 4.5 mg/l) and vitamin C (120 ± 4 mg/l) treated groups were significantly ($p < 0.05$) different in comparison to nicotine and control groups. Whereas, the CRP level of ZnOFNC treated group (85.1 ± 4 mg/l) was significantly ($p < 0.05$) altered with group II which was nicotine-treated (Figure 2).

High SGOT enzyme is responsible for myocardial necrosis and induced cardiometabolic risk factors (metabolic syndrome, abdominal obesity, insulin resistance, and diabetes). High glutamic pyruvic transaminase (SGPT) increased cardiovascular risk related to non-alcoholic fatty liver disease (NAFLD), myocardial infection, cardiometabolic risk factors, chronic alcoholism or structural heart disease (Aisah *et al*., 2021; Goessling *et al*., 2008; Whitfield *et al*., 2002). High serum alkaline phosphatase (ALP) levels are associated with vascular calcification, atherosclerotic disease, and an increased risk of cardiovascular events (Wang *et al*., 2018). CRP is an inflammatory biomarker that indicates inflammatory reactions in the heart tissue or vessels (Jana *et al*., 2010; Nelson, 1990; Shakhanbeh, 2001). In the present study, it was observed that after nicotine treatment, GOT, GPT, and ALP levels were significantly higher than normal. Jensen *et al*. (2012) reported that nicotine is involved in the general fibrogenic process that governs fibrosis and fibrosis-related diseases, focusing on the cellular mechanisms that have implications for multiple organ systems. On the other hand, the FOS-applied group (group III) results were insignificant compared to the nicotine-treated group. ZnONPs slightly normalised those parameters, but that change was not satisfying. But the ZnOFNC-treated group significantly ($p < 0.05$) reduced the SGOT, SGPT, ALP, and CRP levels to normal. Some studies have found that SCFAs can prevent or recover liver injury, acetate normalises elevated GOT, GPT, and ALP, and butyrate decreases excessive inflammation (Arisoylu, 2016; Campisano *et al*., 2019; Chen and Vitetta, 2020).Similarly, zinc supplementation appears to be effective at maintaining liver function (Hosui *et al*., 2018; Kechrid and Bouzerna, 2004). On the basis of the above references and our observation, it may be possible that zinc and SCFA in the ZnOFNC supplement actively work against elevated SGOT, SGPT, ALP, and CRP levels in experimental animals.

Serum Lipid Profile

Current study represents that serum TC, TG, LDL and VLDL level of nicotine (group II) and FOS treated group (group III) were significantly ($p < 0.05$) high but HDL level was significantly ($p < 0.05$) low compare to control group. But ZnONPs treated groups (group IV) shown such efficacy on TG, HDL and VLDL lipid parameters and that was significantly ($p < 0.05$) differ than group II and insignificant with control group. Similarly, vitamin C (group IV) represent its efficacy only on serum VLDL level. But the result was not satisfied with control group. On the other hand, serum TC, TG, LDL and VLDL levels of ZnOFNC treated group (group VI) were significantly ($p < 0.05$) low than nicotine treated group and HDL level equally increased. Each lipid profile parameters of group VI were insignificant with control group (Figure 2).

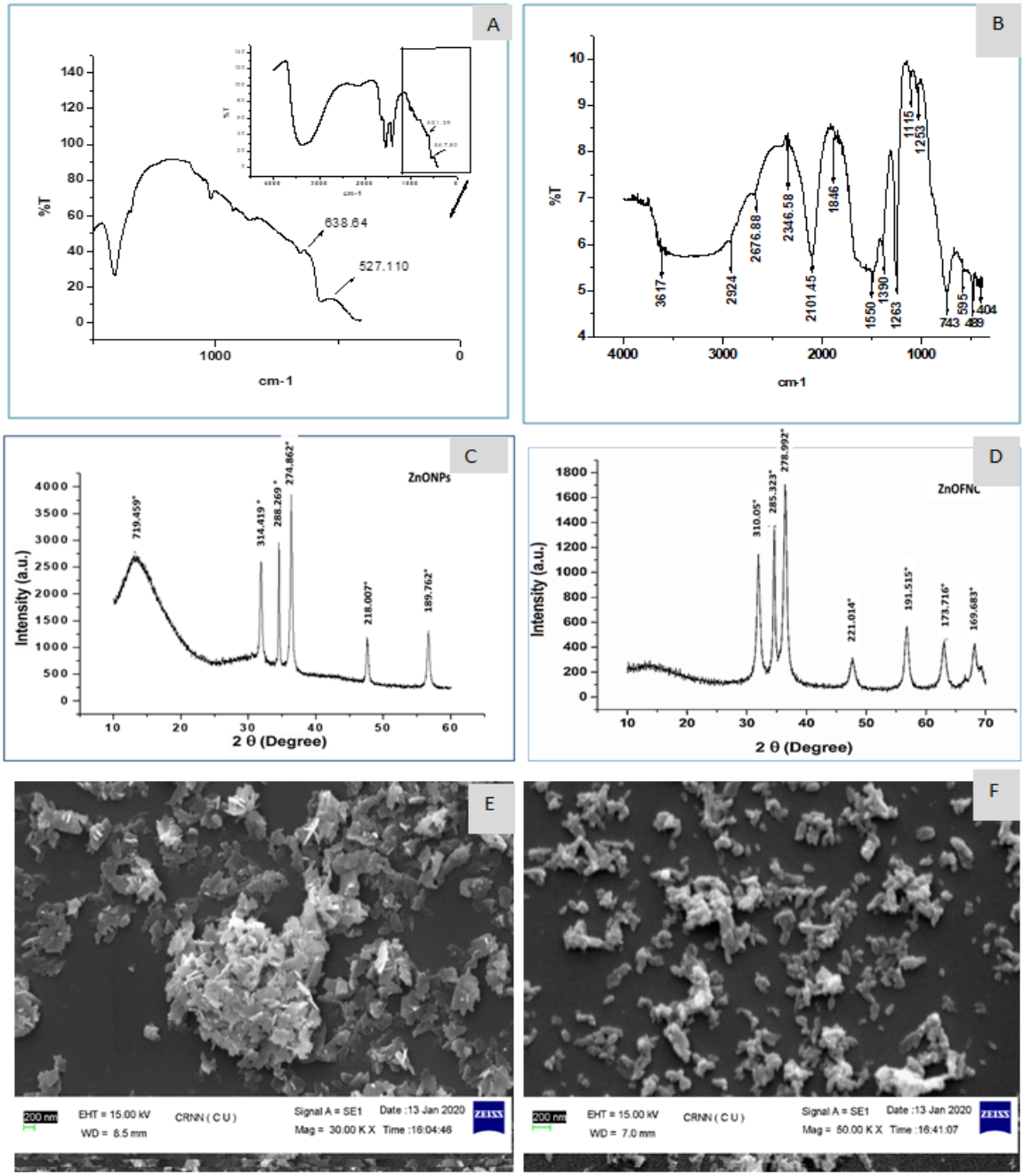

Figure 1: 'A', 'B' image shows FTIR spectrum of ZnONPs and ZnOFNC, respectively. Here ZnONPs represent two important picks 638.64 cm-1 and 527.110 cm-1 and ZnOFNC shown one important pick at 2346.58 cm^{-1}. 'C', 'D' image shows XRD spectrum of ZnONPs and ZnOFNC, respectively. ZnONPs shown impotent pick at 31.880 °, 34.920 °, 35.80 °, 46.870°, 56.280°, 64.80 ° and 67.280 ° and ZnOFNC represent such pick like 13.300°, 31.400°, 34.5260°, 36.400°, 47.660°, and 56.690° were mostly important for nanoparticle size calculation. 'E,F' represent SEM image of ZnONPs and ZnOFNC, respectively.

High cholesterol increases blood concentration and induces fatty deposition in blood vessels, and these deposits grow, making it difficult for enough blood to flow through the arteries. Sometimes, fat deposition is a cause of CVD, a heart attack, or a stroke. Nicotine increased lipolysis and increased FFA in blood vessels, reduced HDL levels, and induced harmful effects on the cardiovascular system (Rao and Emmanuel, 2013). Current monitoring of this study has recognised that the nicotine-treated group significantly ($p < 0.05$) increased TC, TG, LDL, and VLDL and reduced HDL levels. Sincerely, it was found that the ZnOFNC-treated group (group VI) was capable of reducing hyperlipidic conditions (Figure 2). According to certain observations, SCFA reduced the TG level, increasing fatty

acid uptake and fatty acid oxidation (; Arash *et al*., 2022; Lei *et al*., 2019; Yu *et al*., 2020). Similarly, zinc supplementation significantly reduced total cholesterol, LDL cholesterol, and triglycerides, and zinc increased HDL-c levels as well (Maia *et al*., 2015; Ranasinghe *et al*., 2015). According to the above research and present study, such possibilities have been developed that ZnOFNC increased SCFA formation through gut bacterial fermentation. So, it may be possible that the ZnOFNC supplement zinc and SCFA actively work against the hyperlipidaemic condition of experimental animals.

Platelets count

The platelets count of nicotine treated group (1012.2 ± 12 $10^{3}/mm^{3}$) significantly ($p < 0.05$) high than control group (816 ± 11 $10^{3}/mm^{3}$). Alternatively, the platelet counts of group VI (Nicotine+ ZnOFNC: 820 ± 10 $10^{3}/mm^{3}$) was low than nicotine treated which was insignificant with the control group. In the same way Nicotine +ZnONPs (group IV) and Nicotine +vitamin C (group V) treated group represent similar result but it was significantly ($p < 0.05$) differed than control group but group III results were not fulfilled our expectations (Figure 2).

In CVD can occurs causes of abnormal blood clotting occurs. High platelet counts usually occur in association with diseases. Excess platelet counts induce clotting or even abnormal bleeding (Gregg and Pascal, 2003). In accordance with the present study, it was seen that nicotine treatment (group II) increased the platelet count, and that was significantly higher than the control group. In various studies, it has been shown that nicotine, directly or indirectly through the release of endogenous epinephrine, has been shown to induce platelet aggregation, and platelet aggregation is significantly associated with platelet count even within the normal range (Pfueller *et al*., 1988; Wurtz *et al*., 2012). On the other hand, ZnONPs and vitamin C-treated groups (groups VI and V) represent such efficacy against nicotine. In a similar way, it appeared that nanoconjugate (ZnOFNC: group VI) was capable of diminishing or maintaining nicotine-induced excess platelet counts, and the result was more satisfied than in groups VI and V. In previous references, there was no evidence that proved SCFA had the capability to reduce a high platelet count. But such studies have shown that zinc and vitamin C are both capable of decreasing platelet count and helping to prevent platelet aggregation (Mullan *et al*., 2002; Maia *et al*., 2015; Wilkinson *et al*.,1999). From the above monitoring, the possibility developed that zinc in ZnOFNC plays an important role against nicotine-induced abnormal platelet counts. At the same time, SCFA helps to diminish the toxic effect of nicotine.

Oxidative Stress and Antioxidant Markers

By the present observation it appears that the SOD of heart tissue was significantly ($p < 0.05$) decreased than control

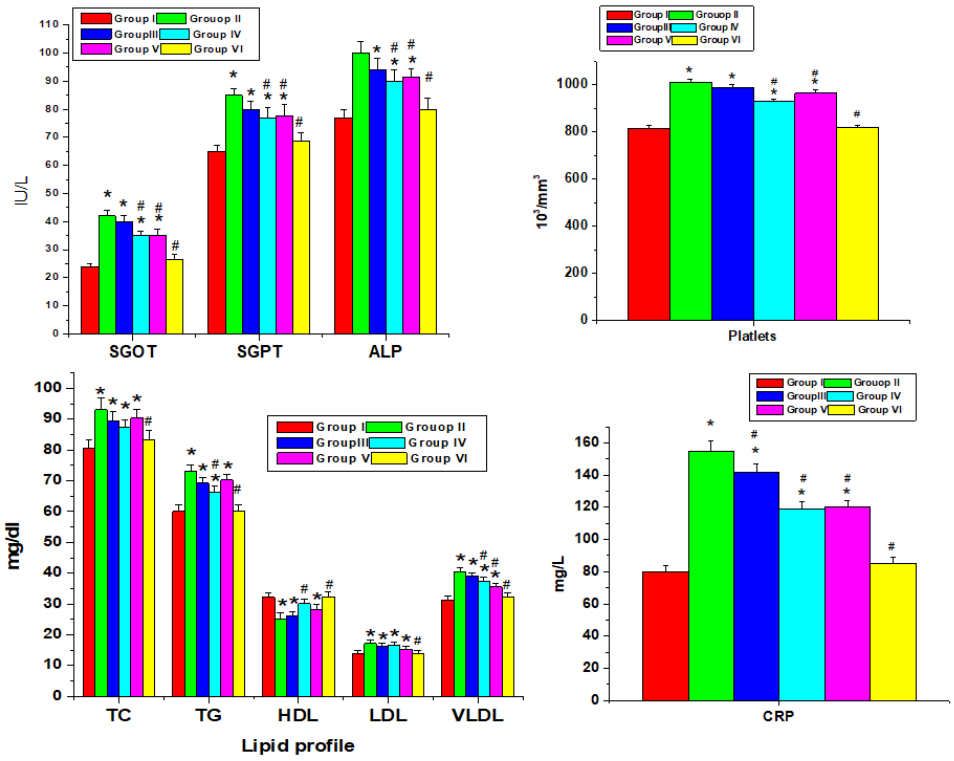

Figure 2: Histograms were show the changes of serum GOT, GPT, ALP, lipid profile, CRP level, and platelets count of different groups. Estimated values are expressed as a mean ± SEM. '*' and '# indicate the level of significance changes of group I and group II with other groups, respectively (all significant level was $p < 0.05$)

level. But the CAT level of vitamin C treated group (group V) result was significantly ($p < 0.05$) differed than group II, III and IV. On the other hand, the MDA level results of the FOS, ZnONPs, vitamin C, and ZnOFNC treated groups were significantly ($p < 0.05$) low than the nicotine treated group. In similar way LDH level results of the FOS, ZnONPs, vitamin C, and ZnOFNC treated groups were also significantly ($p < 0.05$) low than the nicotine treated group. In all cases of ZnOFNC treated group results shown that the SOD, CAT, MDA and LDH all were insignificant with normal level (Figure 3).

Antioxidation events catalase (CAT) and superoxide dismutases (SOD) are very important antioxidants that are defence against oxidative stress in the body. Causes of polyunsaturated fatty acid peroxidation include an include an increase in free radicals and increased MDA (malondialdehyde level) production in the cells. Oxidative stress leads to the accumulation of DNA damage, genomic instability, and hypomethylation of DNA. Similarly, the other most important enzyme is lactate dehydrogenase (LDH), whose extracellular activity increases under the condition of oxidative stress and is an is an indicator of tissue damage found in almost all the body's tissues, including those in the blood, heart, kidneys, brain, and lungs (Farhana and Lappin. 2020). Our observations have indicated that intracellular SOD, CAT, and both antioxidants were decreased and MDA and LDH were both increased in the nicotine-treated group. Such labs reported that nicotine induces ROS production that induces DNA damage, genomic instability, hypomethylation of DNA, and cell lysis (Wang *et al.,* 2000). On the other hand, the present study revealed that ZnOFNC is able to normalise nicotine-induced SOD, CAT, MDA, and LDH levels in heart tissue. Studies have demonstrated that SCFAs can inhibit oxidative stress. Bosch *et al.* (2021) reported that butyrate prevents ROS generation (increased SOD and GSH levels), cell apoptosis, and induces epigenetic regulation. Such neurological studies have shown that SCFAs can reduce brain and intestinal tissue injury induced by oxidative stress (Gao *et al.,* 2018; McLoughlin *et al.,* 2017). In addition, Marreiro and his group (2017) showed that zinc protects cells against oxidative damage, acts in the stabilisation of membranes, and inhibits the enzyme nicotinamide adenine dinucleotide phosphate oxidase. On the basis of the above references in our study, such possibilities arise that zinc and gut fermented SCFA may both be found in ZnOFNC. For that reason, ZnOFNC gives the best result against nicotine-induced oxidative stress compared to ZnONPs.

Histological Analysis

Shrinkage fibrous tissue, karyopyknosis nuclei, necrotic-cardiac muscle, etc. were found in the histological heart tissue section of nicotine-treated group. FOS, ZnONPs, and vitamin C unable to change nicotine tempted heart tissue damages. But we recognised that the histological section of the nicotine + ZnOFNC-treated group was in normal shape.

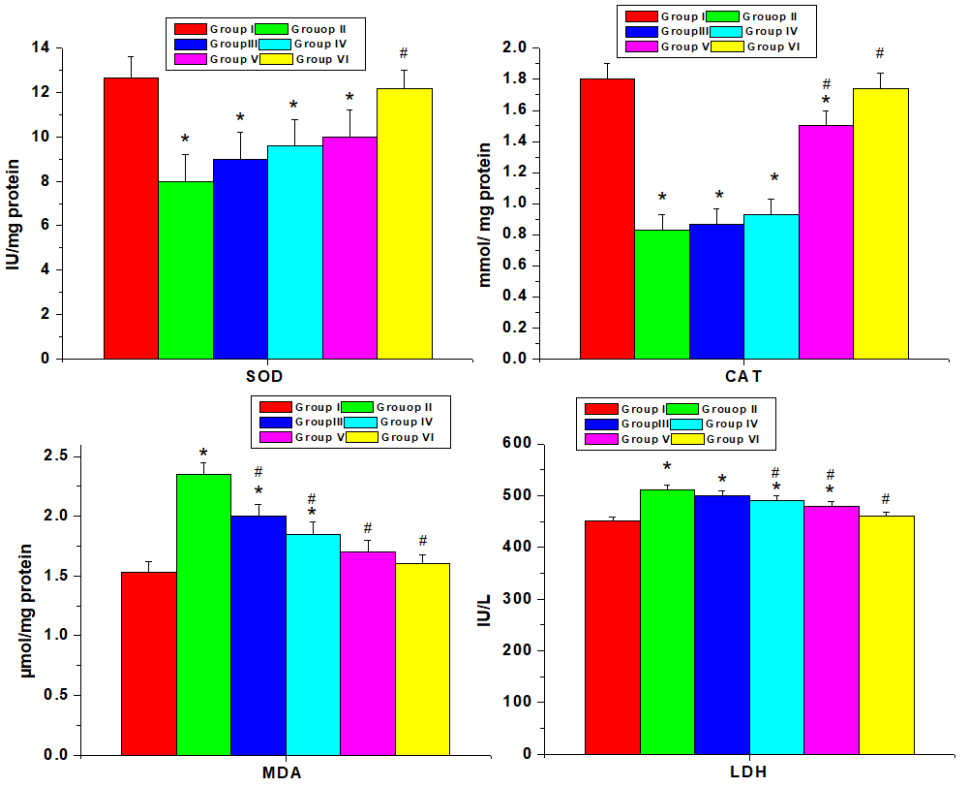

Figure 3: Histograms were show the changes of SOD, CAT, MDA and LDH level. Estimated values are expressed as a mean ± SEM. Here * and # indicate the level of significance changes of group I and group II with other groups respectively (all significant level was $p < 0.05$)

No karyopyknosis nuclei or necrotic-cardiac muscle were found, which was similar to the control group (Figure 4).

The histological analysis of a tissue specimen allows them to diagnose and determine the severity of disease. In the present study heart tissue section of nicotine treated shown several damages that were shrinkage fibrous tissue, karyopyknosis nuclei, necrotic-cardiac muscle, etc. Nicotine significantly enhanced the expression of transforming growth factor-β1, recruitment of inflammatory cells, production of reactive oxygen species (ROS), stimulates fibrogenesis and activates the collagen-producing cells. (Han *et al.,* 2021; Jensen *et al.,* 2012; Kisseleva and Brenner,2008;). All above factors are induced tissue damage. In our study it was observed that ZnOFNC (group VI) could prevent or helped to recovered the nicotine induced damages of heart tissue. Because the histological images of heart tissues of this group looked normal as like as control group. No such damages like shrinkage fibrous tissue, karyopyknosis nuclei, necrotic-cardiac muscle, etc were not recognised. Here such possibilities occur that SCFAs are synthesised from ZnOFNC through fermentation. Most of studies recognised that anti-inflammatory effect of SCFAs able to inhibit cardiac fibrosis and help to prevent heart failure (Chen *et al.,* 2020; Durholz *et al.,* 2020; Lewandowski *et al.,* 2002). Zhang and his group (2019) established that zinc also help to prevent ROS generation, spleen fibrosis (Lin *et al.,* 2018, Zhang *et al.,* 2019). Previous references help to established such possibilities arise that zinc and SCFA may be synthesised from ZnOFNC and they were help to diminished tissue damaging factors like oxidative stress, tissue fibrosis, represent anti-inflammatory effect, etc.

ECG Analysis

ECG records the electrical signals in the heart and it able to quickly detect the heart problems and monitor the heart's health (Figure 5).

P and T wave intervals and amplitude analysis

In this experiment P and T waves were flattened as well as showing prolonged time interval in case of nicotine induced group which was significant (at $p < 0.05$) in compared to control group. Other groups like FOS and vitamin C treated group were unable to represent any effective changes (abnormal P and T wave interval and amplitude) against nicotine treated groups. Whereas P and T wave intervals and only P wave amplitudes of ZnONPs treated group were significantly ($p < 0.05$) differ than group II. ZnOFNC treated group result significantly ($p < 0.05$) normalised P and T wave intervals and amplitudes pattern of ECG compare than group II and insignificant with control group (Figures 5, 6).

QRS, RR, QT and PR intervals analysis

The QRS, RR, QT and PR intervals in nicotine tempted group significantly ($P < 0.05$) prolonged compared to control group.

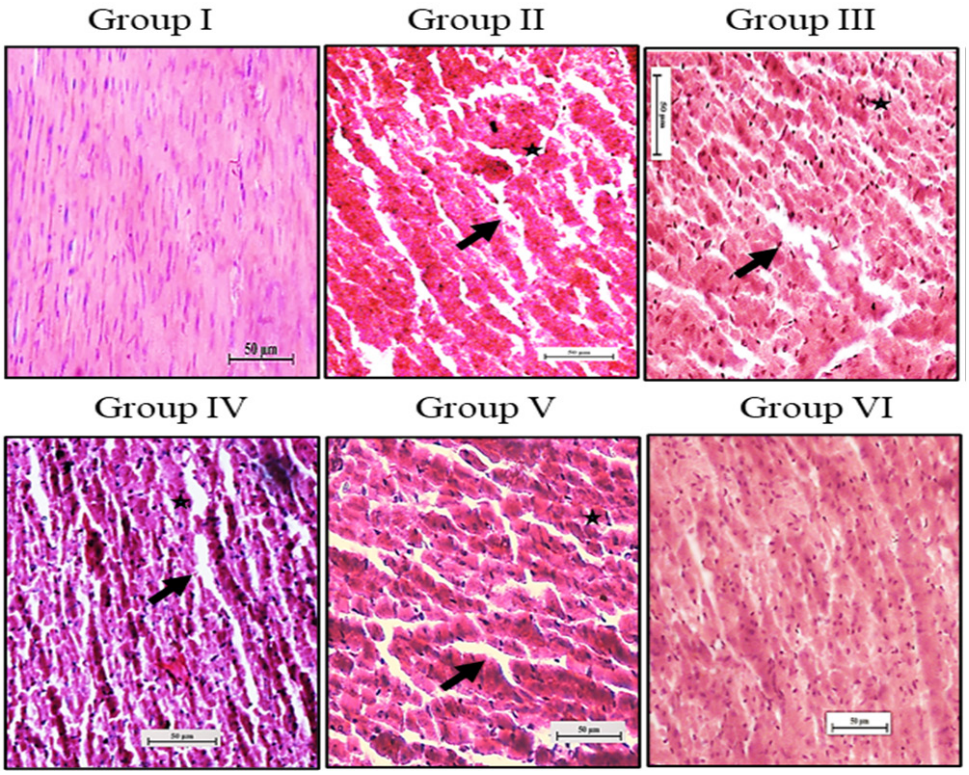

Figure 4: These pictures show the longitudinal section of the heart tissue of different groups. The original magnification of heart tissue was X500. Single arrows of heart tissue are showing the necrotic-cardiac muscle, arrows heads are indicating the shrinkage of fibrous tissue and '*' symbols are indicated karyopyknotic nuclei

On the other hand, ZnOFNC has the capability to normalised those intervals. But other groups like only vitamin C and FOS unable to changes the same intervals in nicotine induced animals. Somehow only ZnONPs have some efficacy only on QT interval which was significantly ($P < 0.05$) reduced than nicotine treated group (Figures 5, 6).

Heart rate analysis

The nicotine treated group heart rate was significantly ($p < 0.05$) reduced compared to control group. Other groups like FOS and vitamin C treated group the activity was powerless on nicotine tempted abnormal heart rates. But the heart rate of ZnONPs treated group was significantly altered with nicotine treated and control group. That means ZnONPs have some ability to improve the nicotine tempted abnormal heart rate. But ZnOFNC ameliorate the conditions of heart rate significantly ($p < 0.05$) in nicotine pretreated animals and insignificant with control group (Figures 5, 6).

ECG that can help diagnose certain heart conditions, including abnormal heart rhythms and CVD. The nicotine-treated QRS, RR, QT, and PR intervals were significantly (at $p < 0.05$) longer than the control group. The QRS and QT interval indicates ventricular depolarization and ventricular systole respectively. A prolonged QRS and QT interval increased the risk of sudden heart death, myoclonic jerking and cardiovascular collapse respectively (Chen *et al.,* 2015). PR interval arises for AV nodal conduction. The delayed PR interval increased the risk of first-degree AV block (Alemzadeh-Ansari, 2018). The RR interval represents the heart rate, and a longer RR interval indicates that the heart rate is decreasing (Goldberger *et al.,* 2018). In cardiac muscle action potential, the repolarizing action depends on the K^+ efflux, but nicotine directly inhibits cardiac-A-type potassium (K+) channels. So, the rate of action potential becomes slower, leading to various changes in the ECG pattern (Alemzadeh-Ansari, 2018). In the next observations, the nicotine-prompted periods of P and T wave intervals were significantly (at $p < 0.05$) longer where the P and T wave amplitudes had flattened than in the control group. The flattened T wave is the cause of hypokalaemia, which means a lack of K+ ions, which increases the risk of myocardial ischemia (Hanna *et al.,* 2011). But a flattened and prolonged P wave indicates left atrial abnormality and increases the risk of sudden cardiac death (Issa *et al.,* 2023).

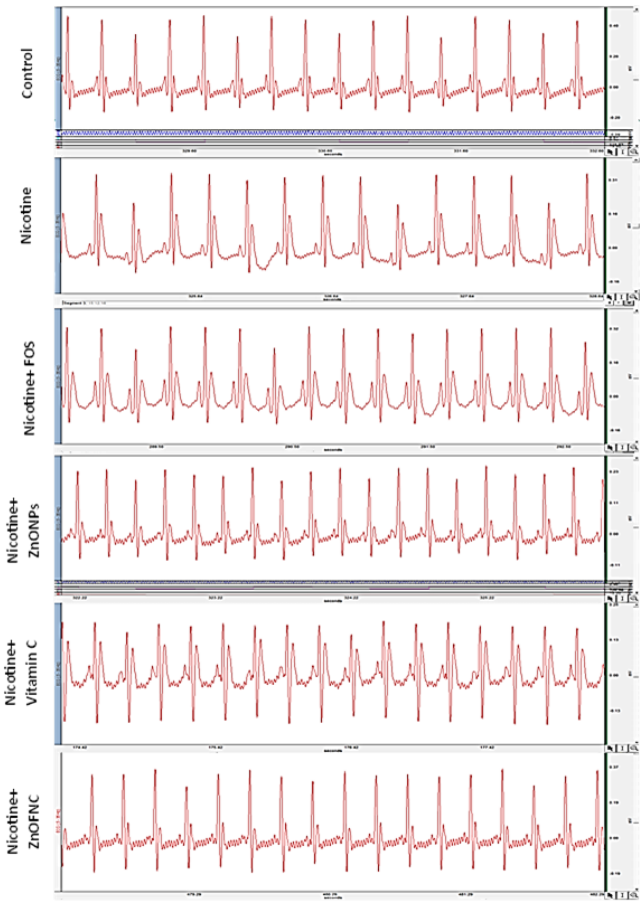

Figure 5: Electrocardiography pattern image sample of all groups established on rat model. Here QRS, RR, QT, PR intervals and P and T wave intervals and amplitudes with heart rate are estimated from above ECG pattern

In our treatment protocol, it was noted that ZnOFNC was able to recover the nicotine-tempted heart damage. Because in the ZnOFNC-applied group, all ECG patterns were close to the normal structure as in the control group. Here, such possibilities arise that ZnOFNC may be able to release a zinc supplement. In various lab reports, it was established that zinc has the greatest role in the electrophysiology of cardiac muscle. It can increase the K+ level in cells of cardiac muscle, and resting potential is elevated (Ciofalo and Thomas, 1965). It can also regulate the calcium movement in our heart through the monitoring type-2 ryanodine receptors (RyR2), which are responsible for the release and storage of calcium in the sarcoplasmic membrane (Qian *et al.,* 2016). With the zinc supplement, ZnOFNC may be able to release SCFA as well. Various reports proved that SCFA have such activities as anti-inflammatory, prevent ROS generation, induce fatty acid oxidation, etc. (Chen *et al.,* 2020; Durholz *et al.,* 2020; Lewandowski *et al*., 2002). Palm and his group (2022), in their review article, provide a probable mechanism. According to their thought, the AMP-activated protein kinase may be heavily linked with the SCFA activation pathway, which works through the proliferator-activated receptor gamma coactivator (PGC). It is well known that PGC has a key regulatory pathway for fatty acid oxidation (FAO). For the activation of the PGC pathway, SCFAs might be able to restore the FAO capacities of heart tissue and increase its total ATP levels. Also, such indirect evidence suggests the SCFAs activate the peroxisome proliferato-ractivated receptor plus PGC pathway in the myocardium. And help improve cardiac health. Tang *et al*. (2019) reported that low concentrations of gut microbiota were unable to improve

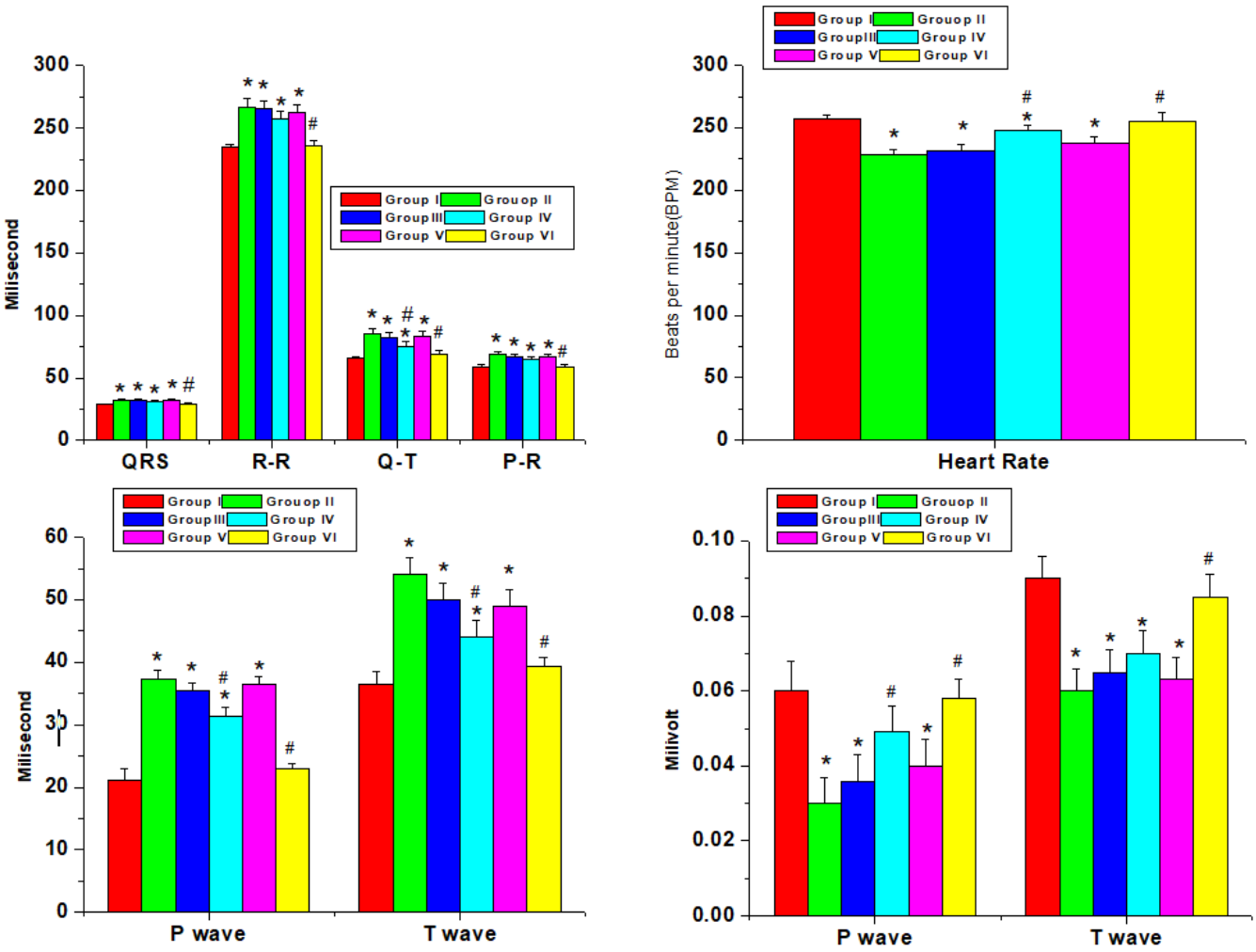

Figure 6: Histograms were show the changes of ECG wave and heart rate. Estimated values are expressed as a mean ± SEM. Here * and # indicate the level of significance changes of group I and group II with other groups, respectively (all significant level was $p < 0.05$)

cardiac tissue necrosis. Through the above mechanism, ZnOFNC may be able to normalise heart waves, which in turn causes the development of ECG patterns and leads to better heart health (Figure 6).

Recovery Percentage of SCFA of Plasma

The recovery percentage of plasma SCFA (Acetic acid, Propionic acid and Butyric acid) of this study was significantly (P<0.05) depleted in nicotine treated group (group II) than control group (group I). On the other hand, it appears that, the recovery percentage of plasma acetic acid and butyric acid of group VI and V were significantly ($p < 0.05$) increased than group II.

But they were also significantly ($p < 0.05$) low with the group I. Similarly, Nicotine + FOS (group III) treated group represent similar result as like as group II. Although nicotine + FOS (group VI) represent excellent result in case of the recovery percentage of plasma acetic acid and butyric acid and which was try to coverup the result of control group. But in case of the recovery percentage of plasma propionic acid group IV, V, VI represent similar result and that were significantly ($p < 0.05$) differ with group I and II (Figure 7).

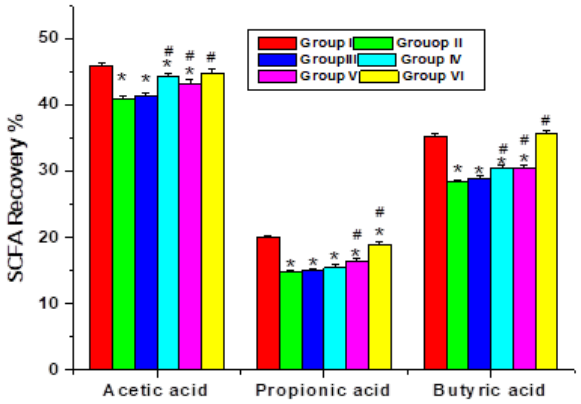

Figure 7: Histograms were show the changes of SCFA level (acetate, propionate, butyrate). Estimated values are expressed as a mean ± SEM. Here * and # indicate the level of significance changes of group I and group II with other groups respectively (all significant level was $P < 0.05$)

Such a lab report established that cigarette smoking, especially nicotine, significantly altered the gut bacterial community and decreased the diversity of gut microbiota, which are responsible for SCFA synthesis (Biedermann *et al.,* 2013; Savin *et al.,* 2018). Iris *et al*. (2019) reported that cigarette smoke extract may also suppress SCFA production. Current monitoring showed that the plasma acetate, propionate, and butyrate levels of the ZnOFNC therapeutic group significantly normalised compared to another group. Holtug and his group (1992) observed that 1 g of FOS can produce 61 mmol of SCFA per litter of blood, which is higher than other soluble dietary fibres (Besten *et al.,* 2013). Similarly, Zn supplementation plays a positive role in bacterial communities' populations and indirectly helps to increase SCFA levels in the blood (Barra *et al.,* 2021; Popovic *et al.,* 2021). On the basis of the above references, it may be assumed that zinc ions might be supportive of FOS-SCFA production.

Conclusion

The nanoparticles are moved very rapidly towards their particular sites, which are highly individual organs. Because their molecular arrangement is very similar to those of biological molecules. As for result, the body can permit them, and the side effects of nanoparticles are also significantly low. Therefore, from the overall information, it may be suggested that the ZnOFNC conjugate form has more potential effects or efficacy in maintaining cardiac health than nicotine. In the future, for more justification, we will also try to investigate molecular-level studies on nicotine-tempted hearts and ZnOFNC potential function.

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Author Contribution

All authors made substantial contributions to conception and design, acquisition of data or analysis and interpretation of data: took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to the current journal' gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

Ethical Approval

Ethical Ref. no. was VU/ IAEC/2/4 of the animal ethical committee of Vidyasagar University.

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