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RECOVERY EFFECTS OF ZINC-OXIDE AND FRUCTOOLIGOSACCHARIDES NANO CONJUGATE ON NICOTINE-INDUCED OXIDATIVE STRESS ON CARDIAC TISSUE IN ADULT ALBINO RAT

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ABSTRACT ■ Nicotine is major toxic component of tobacco products which are capable of producing free radicals and causes oxidative stress. Zinc and Fructooligosaccharides (one type of soluble dietary fiber) both are good for cardiac health as a dietary supplement (which is digested by gut bacteria). Both have a good and significant role on cardiac health. Our main objectives of this study to prepared a new nano-conjugate and apply against nicotine induced cardiac toxicity for recovery. In this study we prepared a nano-conjugate compound with zincoxide and Fructooligosaccharides (ZnO-FNC) by chemical synthesis method and characterized by using SEM and FTIR analysis. We apply ZnO-FNC on nicotine induced albino rat and measured SOD, LPO, CAT level of heart tissue and serum LDH level. Results shows that ZnO-FNC help to reduce oxidative stress of heart tissue induced by nicotine and all the data were changed significantly ($P < 0.05$) compare to nicotine treated group and closed to the control group. ZnO-FNC has potential effects on oxidative damage and it is a new approach of nutritional therapy against cardiac stress.

Key words: Zinc-oxide, Fructooligosaccharides, Oxidative stress. Heart

INTRODUCTION

After smoking, 90% nicotine absorbed very quickly through lung, easily reach in blood and heart through diffusion process and increased heart toxicity. In a normal person (non-smoker) nicotine level in plasma is 5 to 7 ng/ml utilised for nicotinic acid, melanin synthesis and other various metabolic purposes(Russell and Feyerabend, 1975). After smoking plasma nicotine level is 10 to 50 ng/ ml(Matta et al., 2007) and causes oxidative stress, which is the main culprit for at herogenesis and thrombogenesis. Nicotine reduced catalase and superoxide dismutase enzyme activity and increased H_2O_2 level in body fluid and increased lipid peroxidase level which causes degradation of cell membrane lipid and release free radicals. Soluble dietary fibers (SDF) is a non-digestible carbohydrates induced to promote healthy bacterial growth, responsible to relief from oxidative stress. Fructooligosaccharides (FOS) is one type of SDF,more effective for cardiac health and alleviate from hyper lipidemia, cardiac fat deposition, and oxidative stressthan other SDF (Haubert et al., 2015). Normally zinc

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diminishes the percentage of free radicals in cellular physiology and it has antioxidant property which is able to protect the cell membrane from oxidative stress (Marreiro et al., 2017). Zinc oxide nano particle (ZnONP) also acts as a scavenger of oxidative stress (Jacob and Rajiv, 2019). Here we prepared a new nano-conjugate form using zinc oxide and Fructooligosaccharides (ZnO-FNC) by chemical synthesis process in the field of nano-biotechnology. The main aims of this study was to investigate the potentiality of ZnO-FNC against nicotine induced oxidative stress in rat heart tissue, specifically alteration of catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) and lactose dehydrogenase (LDH).

2. MATERIALS AND METHODS

2.1 Synthesis and characterization of ZnO-FNC:

In the first step 3.8% (w/v) Fructooligosaccharide(Sigma Aldrich Company) was added with 0.025 mol zinc acetate (Merck Pharmaceutical companies) aqueous solution and 0.1 mol NaOH (Merck Pharmaceutical companies) mixed under constant stirring drop by drop for maintain pH (11) balance and keep it at room temperature for overnight (24 hour). Then the solution centrifuged at 2,000 rpm for 10 minutes. For removed NaoH, the supernatant water was discarded and washed the pallets three times by distilled water. Pallets were dried at 80^{o}C then collect ZnO-FNC (Moharekar et al., 2014). ZnO-FNC sample was prepared in Department of Human Physiology with Community Health, Vidyasagar University, Paschim Medinipur, W.B (India). ZnO-FNC sample characterized through the FTIR (Fourier transforms infrared) spectra on Perkin Elmer Ltd software version 10.3.7 explain the chemical bonds are present in this conjugate sample. For FTIR measurement we prepared pellet of ZnO-FNC with KBr 1 wt % and take spectrum (Vidyasagar University). The surface morphology and elemental analysis of the synthesized ZnO-FNC was done by field emission scanning electron microscopy (FESEM, FEI Inspect F50, and The Netherlands) Sigma model Carlzeics (Jadavpur University, West Bengal, India). The films of the sample were prepared on a carbon coated copy grid by just dropping a very small amount of the sample on the SEM grid which was then allowed to dry by putting it under a mercury lamp for 5 minutes (Weiss and Moser, 2015). From FESEM we understand the average practical size of ZnO-FNC.

2.2 Preparation of Nicotine and other applied sample solution: Nicotine (Purchase from Sigma Aldrich Company) was dissolved in 0.9% physiological solution (saline) and injected through intra-peritoneal (ip) according to effective dose of 3.5 mg/kg body weight one time daily 24 hour duration (Chattopadhyay and Chattopadhyay, 2008). On the basis of Tao et al., (2011) we prepared three doses of ZnO-FNC like 225 mg, 112.5 mg and 56.25 mg per kg body weight. In between them 112.5 mg / kg body dose of ZnO-FNC is more effective compare to other doses. Zinc oxide nanoparticle (ZnONP) and FOS (Fructooligosacarids) both are purchase from Sigma Aldrich Company and both are apply as a same dose like 112.5mg/ kg body weight. All the prepared samples like ZnONP, Fructooligosacarids and ZnO-FNC are diluted in deionised water according to dose (112.5 mg/kg body weight) and stored at 2ºC to 8ºC.

2.3 Animal Care, grouping and mode of treatment:

Experiment was carried on male Albino rats (100-120 gm body weight) were obtained from were procured from SAHA ENTERPRIZE, 386 / 2, Nilachal, Birati, Kolkatta-700051, Email: sanjoy.animal2015@gmail.com. Regn.

No. 1828 / PO / BT / S / 15 / CPCSEA and keep them CPCSEA approved animal house of Vidyasagar University (Registration No. 2013/ GO/Re/S/18/CPCSEA/2018) and ethical clearance from animal ethical committee (Ref. no. VU/ IAEC/2/4). The animals were housed in a clean polypropylene cage in a standard temperature (20±2ºC) with relative humidity (45–60%) under 12-h light and dark cycles during the whole study period. Then animal were acclimatised in animal house for 15 days. Then animals were divided into 6 groups and each group having 10 animals. Throughout the experiments animals are provided normal standard balanced diet with water *ad-libitum*.

- **Group-I:** Control group : Animals were treated with 0.9% normal saline (i.p) for 14 days and next 21 days treated with distilled water (orally administrated) then scarified.
- **Group-II**: (Nicotine treated group) Animal were treated with nicotine (3.5 mg/kg BW) for 14 days (i.p) then scarified.
- **Group-III:** Auto-recovery group : This group were treated 14 days by nicotine (i.p) and next 21 days treated with distill water (orally administrated) then scarified.
- **Group-IV:** Zinc oxide nano particle (ZnONP) treated group : nicotine treated (i.p) for 14 days, next 21 days applied ZnONP (orally administrated) then scarified.
- **Group-V**: Fructooligosaccharides applied group : First 14 days treated with nicotine (i.p), next 21 days applied Fructooligosacchari-des as a supplement (orally administrated) then scarified.
- **Group-VI**: ZnO-Fructooligosac-charides nano-conjugate (Zn-FNC) applied group : First 14 days treated with nicotine (i.p), next 21 days applied Zn-FNC as a supplement (orally administrated) then scarified.

2.7 Tissue and serum collection: After scarifying, blood sample was collected by cardiac puncture technique in EDTA container and heart tissue stored in ice container. Here we use polytron homogenizer instrument (at 40ºC) for tissue homogenate. 0.1gm heart tissue was homogenate in 1 ml phosphate buffer (0.05M, pH7) solution. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was used for the estimation of superoxide dismutase (SOD), catalase (CAT) and Lipid peroxidation (MDA) level on the other hand anticoagulant mixed blood sample was centrifuged at 3000 rpm for 10 minutes for separation of serum and it was utilized for the analysis of lactose dehydrogenase (LDH) activity.

2.8 Biochemical estimation:

2.8.1 Superoxide dismutase (SOD) estimation: 1mM diethylenetriaminepenta acetic acid added with 3ml Tris buffer (50mM, pH 8.2), 45 µl pyrogallol mixture (10mM pyrogallol added in 10mM HCl) and 10 µl of tissue supernatant mixed with aliquot of assay mixture and noted optical density (OD) at 420 nm. SOD activity was expressed as units/ milligram protein (Marklund and Marklund, 1974).

2.8.2 Catalase (CAT) estimation: After homogenisation 0.1ml supernatant of tissue homogenate was added with 2.9 ml phosphate buffer (0.5M, pH 7) and then this mixture was transferred in a cuvette used as a control sample. In a cuvette 0.1ml supernatant of tissue homogenate also added with 1.9 ml phosphate buffer. Then mixed 1ml of H_2O_2 (11nm) solution with the previous aliquot assay mixture and immediately

observed the OD value at 240 nm with a one minute interval for 3 times (Beer and Sizer. 1952). Activity of catalase was calculated using following formula $\Delta A \times 10^3/40$mg protein in sample (U/mg protein).

2.8.3 Lipid Peroxidation (MDA) estimation: First of all 2 ml 0.375 % and15% thiobarbituric acid and trichloroacetic acid were added respectively. Then 200 µl tissue supernatant and 1.0 ml distilled water were added with this mixture then boiled on a water bath at 95 ^{O}C for 20 min. After that the solution get pink colour and cooled under tap water. Then mixed 3 ml n-butanol to stop the internal reaction and observed OD value at 532 nm (Wills, 1969).

2.8.4 Lactose dehydrogenase (LDH) estimation: Serum LDH activity was estimated using Kit (Coral Clinical System, A Division of Tulip Diagnostic Pvt. Ltd., Vema, Goa, India). The LDH activity was expressed as Unit/L.

2.8.5 Protein estimation: The protein was determined by Lowry *et al*., (1951) using cardiac tissue for the indirect measurement of enzyme.

2.9 Statistical analysis: All data are expressed as the Mean ± Standard Error of Mean (SEM) and One Way Anova is used for statistical analysis by Origin 6.0 professional software (details shows in table).

3. RESULT AND DISCUSSION

3.1 Characteristics of ZnO-FNP:

In general, formation of NPs and their characterization were observed by following use of instruments. In this study, Field Emission Scanning Electron Microscopy (FE-SEM) analysis was done by using sigma model Carlzeics. SEM analysis is to provide supplied particle shape and size (nm). FE-SEM images of ZnO-FNC explain the structure is uniform spherical shaped and average particle size 40 nm which have shown in figure-1.The FTIR spectroscopy is also carried out to find out the purity and nature of NPs and bio-functionalized NPs synthesized by eco-friendly chemical synthesis method. In addition, it has been also showed the chemical bonds which were present in supplied sample. 493.19 denote the Zn-O stretching. 2101.45 gave CH_2 and 2346.58 give the CH_3 stretching. 3389.76 to 3786.54 indicate the O-H stretching (Silverstein et al., 1981) shows in figure 2.

3.2 Effects of ZnO-FNC on oxidative stress:

Nicotine, the major component of tobacco product plays an important role in the development of lung, heart and liver complications. It causes oxidative damage in these organs. As we know that nicotine itis a potential oxidant, which is capable of producing free radicals and reactive oxygen

Table 1. Shows the antioxidant and oxidative stress markers in different groups (Mean ± SEM).a, b, c d, e and f different letters denote significance within control groups or treatment groups (enteritis) at $P < 0.05$. (One way ANOVA)

	Group I	Group II	Group III	Group IV	Group V	Group VI
SOD (U/mg protein)	$4.32^{a} \pm 0.37$	$1.56^{bce} \pm 0.26$	$1.46^{bce} \pm 0.26$	$1.97^{ce} \pm 0.29$	$1.68^{d} \pm 0.26$	$3.92^{a} \pm 0.56$
MDA (nmol/ gm tissue)	$12.84^{a} \pm 1.81$	$15.97^{bc} \pm 1.84$	$15.60^{bc} \pm 1.25$	$14.72^{b} \pm 1.98$	$15.63^{c} \pm 1.30$	$13.29^{a} \pm 1.93$
CAT (U/mg protein)	$42.20^{a} \pm 1.45$	$19.44^{bc} \pm 1.66$	$22.37^{bdc} \pm 1.43$	$24.68^{dc} \pm 1.64$	$21.84^{c} \pm 1.44$	$35.17^{e} \pm 2.61$
LDH(U/L)	$436.27^{a} \pm 2.82$	$1130.86^{b} \pm 3$	$913.54^{c} \pm 2.69$	$486.57^{d} \pm 2.5$	$673.42^{e} \pm 3.2$	$365.81^{f} \pm 2.60$

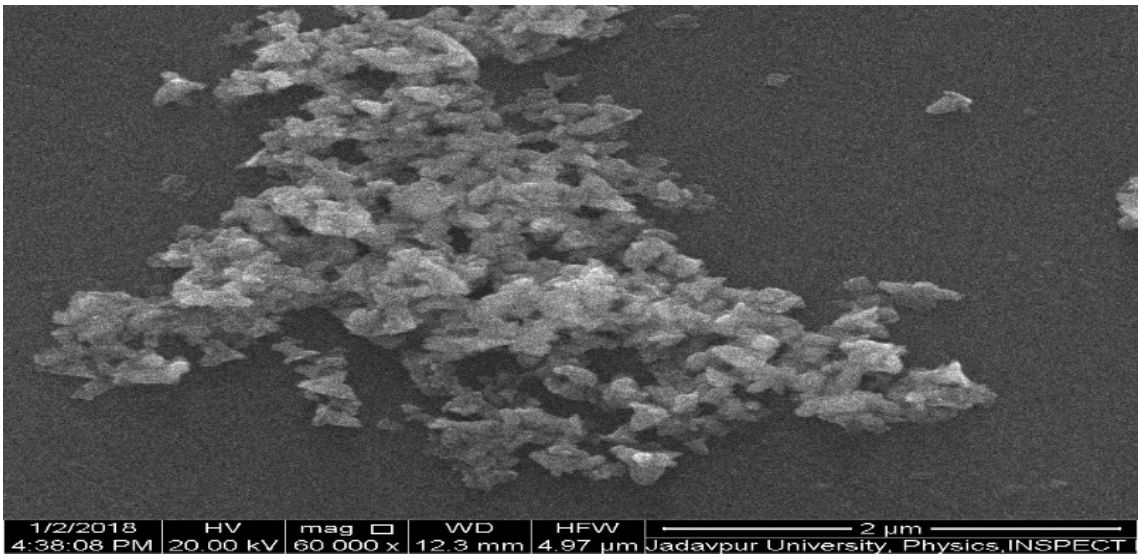

Fig. 1: Field Emission Scanning Electron Microscope image of synthesized Zn-FNC

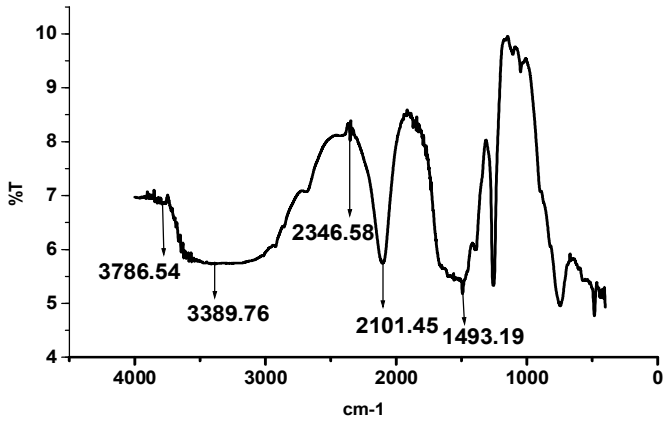

Fig. 2: Fourier transform infrared spectrum of Zn-FNP.

species. So inside the cell, nicotine-induced free radicals react with bio-membranes causing oxidative destruction of polyunsaturated fatty acids and forming cytotoxic (Chiagoziem et al., 2014) components. An in-vitro cytotoxicity study on malaria parasites suggested that infected HeLa cellshows the application of chitosan–tripolyphosphate (CS–TPP) nanoparticles (NPs) conjugated with chloroquine help to reduced oxidative stress (SOD, CAT, LDH and MDA level). Chitosan is a type of dietary fiber (Tripathyet al., 2012) responsible for controlling various types of physiological functions. CAT is an antioxidant as well as dismutase enzyme found in living aerobic organism such as peroxisomes and mitochondria, specially splitting hydrogen peroxide into water and oxygen. On the other hand SOD transfer the electron from one molecule of the superoxide anion to another and form hydrogen peroxide from free radical $2O_2^{\bullet-}+2H^+\rightarrow H_2O_2+O_2$. (Ma et al., 2012). Table-1 shows the CAT and SOD level of

nicotine treated group which are significantly ($p<0.05$) decrease than the control groups. In an auto-recovery phage body able to recover the oxidative stress but very slowly. Whereas ZnO-FNC shows very quick recovery response for the level of CAT and SOD in heart tissue ($p<0.05$) which are approximately similar to control groups. Various findings reported that specially free radicals (allylic hydrogen atom) initiated lipid peroxidation (LPO) and breakdown lipids of cell membranes (Snezana et al., 2014). Lipid peroxidation has been introduced in pathogenesis of a number of diseases (Chiagoziem et al., 2014). Nicotine increased lipid peroxidation and MDA production (MDA an end product of lipid peroxidation). Wide range of MDA production may causes ather osclerosis, stroke, diabetes, and aging (Landau et al., 2013). After 15 days of nicotine treatment, MDA level was 15.974 ± 1.8416 (nmol/ gm tissue, $p<0.05$) whereas in control groups $12.48\ \pm1.8148$ (nmol/ gm tissue, $p<0.05$). In case of ZnO-FNC group (group-VI) after the end 20 days of treatment, MDA level of heart tissue reduce significantly ($p<0.05$) at 13.29 ± 1.93497 (nmol/ gm tissue). Form these result it is very clear that ZnO-FNC skilfully reduce the free radicals and it may have some antioxidant activity. Lactate dehydrogenase (LDH) is a another type of glycolytic enzyme found in various tissues like skeletal muscle, heart, liver, kidneys, brain, lungs, and red blood cells. The intensity of oxidative stress induces LDH release from the tissues and raises its level in the blood causes of stroke, cancer, heart attack, blood flow deficiency, haemolytic anaemia, hepatitis, muscle injury and tissue death (Berridge et al., 2013). Iso-enzymes of LDH like LDH-1 & LDH-2 are responsible for myocardial infection and LDH-5 responsible for liver disease (Usher et al.,1974). For nicotine toxicity LDH-1 type is exposed (Gamieldien et al., 2008). At the same time both zinc and SDF are responsible for the development of intestinal lactic acidand acetic acid producing bacterial growths which are capable to reduced LDH level (Aldunate et al., 2015 and Leonardi et al., 2013).Hamza et al., (2018) pointed out that green tea leaves extract and zinc oxide nano practical complex form potentially reduced the LDH level of liver tissue. In case of our study we found that nano-conjugate supplement (ZnOFNC) is capable for recovery of oxidative damage by lowering LDH and MDA level in 20 days (group-VI) treatment towards the control group. Therefore we may be suggested that nano conjugate dietary supplement (ZnOFNC) have a significant beneficial effect on nicotine induced oxidative damage in biological system, which may be important for the prevention of smoking (nicotine) induced cardiovascular damage for smokers.

4. CONCLUSION:

So in this present studies we try to assess the toxic effect of nicotine on cardiac tissue through the measurement of CAT, SOD, LPO and LDH level. Zinc oxide and fructooligo-saccharides nano-conjugate (ZnOFNC) efficiently diminished the oxidative stress, free radical scavenging activity and antioxidant properties within few days. We are also trying to investigate the other biomarkers related to nicotine induced cardiovascular damage in future.

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