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CURATIVE EFFECTS OF ZINC OXIDE NANOPARTICLES-FOS COMPOSITE ON NICOTINE TEMPTED INTESTINAL OXIDATIVE STRESS WITH HISTOLOGICAL FEATURES AND SERUM SHORT CHAIN FATTY ACIDS MEASUREMENT.

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Abstract: Smoking and smokeless tobacco (Nicotine) intake major risk factor for gastrointestinal disorders, mucosal damage, changes in gut irrigation, and impaired mucosal immune response, inflammatory bowel diseases, and several clinical abnormalities in digestive system. Soluble dietary fibre (SDF) capable of improving the gut health by increasing anaerobic bacterial growth. Zinc oxide nanoparticles and fructooligosaccharides (FOS) are well-known antioxidants and induces the good gut bacterial growth. Present study was designed to investigate the capability of nano-composite in between Zinc oxide nanoparticles-FOS (ZnOFNC) to protect intestine from nicotine induced damages. Estimated parameters were oxidative stress measurement and histological study of large and small intestine tissues and quantitative measurement of plasma SCFA level of selective groups (Gr-I: Control, Gr.-II Nicotine treated, Gr.-III Nicotine plus FOS and Gr.-III Nicotine plus ZnOFNC). The result of this study expressed that plasma SCFA level of Gr-II were decreased and increased the ROS generation compared to Gr-I (significant level was $P<0.05$ in all cases). Whereas the Gr.-III, ZnOFNC showed its ability to expand the antioxidant level and diminished the ROS level and increased the plasma SCFA level significantly ($P<0.05$). Nicotine-induced (Gr.-II) histological changes were also recovered in Gr.-III similar to control. Throughout the several experiments like biochemical, histological and plasma SCFA level measurement it is indicated that ZnOFNC may have some potential power to diminish nicotine-induced alterations in gut health.

Keyword: **Bacterial growth, Nanoconjugate, Fructo-oligosaccharides, Oxidative stress, Nicotine, Short chain fatty acids, Zinc oxide, Intestinal tissue**

***Abbreviations*:** FOS (fructooligosaccharides), ZnOFNC (zinc oxide and fructooligosaccharides nano conjugate), ROS (reactive oxygen species), SCFA (short chain fatty acids), CFU (colony forming unit), FTIR (fourier transform infrared), UV spectrum (ultra-violate spectrum) and SEM (scanning electron microscopy).

I. INTRODUCTION:

During addiction of tobacco products (both smoking and smokeless), the principal alkaloid mainly nicotine, create a lot of harmful effects in smoker's gut, especially on the gastric mucosal layer. Which in turn causes the damages of intestinal microbes. Normally nicotine indirectly can increase the oxidative stress of intestine, alteration of intestinal mucin composition and induced damages of intestinal cellular tight junctions, intestinal inflammation along with alterations of acid-base balance. A study in 2018, established that the tobacco smoking is responsible for the development of inflammatory bowel disease leads to damaging the microbiota of intestine (Savin et al.,

2018). Another previous study reported that tobacco intake can encouraged the intestinal toxicity and it can modulate the population of gut-microbial community (Jinqiang et al., 2015). Nelson and his group (2018) observed that the female smokers' vaginal *Lactobacillus* sp percentage is going to be low than non-smokers. Benowitz (2009) also observed that internal pH of levels become altered after tobacco consumption and this situation is responsible for destruction of gut bacterial colonization. On the other hand, nicotine decreased the level of prostaglandin excretion, damages mucosal protection, and induced the inflammation small intestinal (Verschuere et al., 2012). Savin and his co-workers (2018) similarly described that the smoking can transform the composition of the intestinal microbiome and it also increased rate of proteobacteria and Bacteroidetes phyla growth which are capable for diminish the growth rate of healthy microbes of gut as well as vagina, and inner mouth cavity (Lukens et al., 2014). It is well known that gut healthy bacterium can digest the non-digestible carbohydrates through the process of fermentation and it can produce short-chain fatty acids (SCFA), lactate and ethanol etc. Gut bacterial are also pioneer microorganism, present in high population in the mammalian intestine and are responsible for maintaining gut health.

FOS is a nondigestible carbohydrate, fermented by gut bacteria. SCFA, lactate, ethanol, etc. are found after fermentation reaction in gut which are also essential elements of our metabolism (Vuyst et al., 2013). Daily FOS (10 to 30gm/day) intake significantly slower the hepatic lipogenesis, modifies the hepatic metabolism, reduced the body weight, improved bacterial growth, and increased magnesium, iron, as well as zinc absorption (Heuvel et al., 1998). Zinc (Zn^{+2}) which is the second most abundant metal ion (after Iron) of living organisms. It obligates for cell division, reduced oxidative stress, cell growth and helping for carbohydrates digestion, etc. Zinc also used for external wound healing of the body (Zalewski et al., 2005; Lu. et al., 2015). Gut bacteria have more potential capacity for zinc uptake as well as cell-bound capacity (Leonardi et al.,2013). On the other hand, the Zn-based nanoparticles (dimension ≤ 100 nm) have more physiological importance in human health. It also used for many physiological treatment purposes (Elshama et al., 2018).

With an increasing rate of tobacco product consumption and further nicotine exposure day by day, the adverse effects on body tissues and organs. As well as it is a risk factor for gastrointestinal disorders, such as peptic ulcer, Crohn's disease (CD), and several cancers become more evident and deleterious in smokers. Zinc and FOS plays a key role in scavenging reactive oxygen because of its known antioxidative properties and other beneficial effects in our body. The main objective of this observation was established a new nano-composite in between Zinc oxide nanoparticles-FOS (ZnoFNC) and analysis it's nano structure through its nano characterization like FTIR, UV, SEM, etc. At the same time for therapeutic assessment ZnOFNC applied on nicotine treated male albino rats and estimate such parameters like intestinal oxidative stress, histological study and serum SCFA quantitative measurement study to understand the intestinal health of treated animals of different groups.

II. MATERIALS AND METHODS

2.1 Synthesis and characterization of ZnOFNC: 3.8% (w/v) FOS is a stabilizing agent, mixed properly with 1% (w/v) zinc acetate substrate solution and then 0.1M NaOH, the reducing agent solution was added drop by drop for maintaining the pH 11. This mixture of solution was placed on a magnetic stirrer for 24 hours. After that the solution was centrifuged at 2,000 rpm for 10 minutes. Then the supernatant was discarded and the precipitate was washed three times with distilled water for removal of extra NaOH and the ZnOFNC pallets were collected and dried at 80ºC. The synthesized ZnOFNC was characterized through the FTIR, UV spectrum and SEM.

2.2 Animal maintenance: Experimental animal were Wistar strain male albino rats (6 to7 week) body weight in between 100-120 g and ethical clearance Ref. no. VU/ IAEC/2/4 (ethical committee of Vidyasagar University). Animals were kept in clean polypropylene cage for 15 days before starting the experiment. Rats were maintained in accordance with the guidelines of the rule of Institutional Animal Ethics Committee of Vidyasagar University, Midnapore, and keep them CPCSEA approved the animal house of Vidyasagar University (Registration No. 2013/ GO/Re/S/18/CPCSEA/2018).

2.3Experimental design: Total duration of experiment was 15 days. Animals are divided in to three groups. Group-I (Control group): In this treatment protocol, animals had treated with 0.9% normal saline (orally administrated). Group II (Nicotine treated group): the animals had treated with the nicotine (dose was 3.5 mg/kg body weight dose, s.c,). Group-III (Nicotine + FOS treated group) and Group-IV (Nicotine + ZnOFNC treated group): The animals were treated with FOS and ZnOFNC (established dose was 80 mg/kg body weight, orally administrated) 6-hours later of nicotine (dose was 3.5 mg/kg body weight dose, s.c,) treatment respectively.

2.4 Tissue preparation: Intestinal tissues (Large and small) were used for the microbiological study and oxidative stress measurement. Before homogenization of intestinal tissues were washed by 0.5 M PBS and removed the internal blood clots. Then homogenate with chilled 0.05 M PBS (phosphates buffered saline, pH 7 and $9g1^{-1}$NaCl) solution and centrifuged for 1000 rpm for 5 minutes (at 4ºC) and the supernatants were used for microbiological analysis and ROS measurement (Maity et al., 2013).

2.5 Oxidative stress (ROS) measurement: 1mM of diethylene-triamine penta-acetic acid was added with 3ml tris buffer (50mM, pH 8.2) for SOD activity measurement. Then added 10 µl of tissue supernatant with the aliquot and 0.2 mM pyrogallol mixture (10mM pyrogallol added in 10mM HCl) was also added with assay mixture. The OD value was noted at 420 nm. For CAT activity determination, the total reaction volume was 3mL. Where the aliquot solution was prepared with 0.05M Tris-buffer, 5mM EDTA (pH 7.0), and 10mM H_2O_2 (in 0.1M potassium PBS, pH 7.0). After that 50 µL of tissue supernatant was added to the aliquot solution and recorded the OD value with time duration at 240 nm.

During the reduced glutathione (GSH) enzyme activity estimation the required sample was mixed with 25% of TCA and centrifuged at 2,000 rpm for 15 min to settle the precipitated protein and the supernatant was aspirated and diluted to 1mL with 0.2M sodium phosphate buffer (pH8.0) then, 2mL of 0.6mM DTNB was added with it. After 10 minutes OD value was recorded at 405 nm. A standard curve was obtained with standard reduced glutathione. In oxidized glutathione (GSSG) enzyme activity estimation at first 0.5mL supernatant sample was added with 2 µl of 2-vinylpyidine were mixed well and incubated for 1 hr at 37 ^{0}C then 4% sulfosalicylic acid was mixed with the mixture and centrifuged at 1000 rpm for 10 min to settle the precipitated proteins and take OD value at 412 nm in the spectrophotometer and calculated with a standard GSSG curve.

During the Glutathione Peroxidase (GPx) enzyme activity determination, the reaction mixture was prepared by 50mM potassium phosphate buffer (pH 7.0), 1mM EDTA, 1mM sodium azide, 0.2mM NADPH, 1U glutathione reductase, and 1mM reduced glutathione. Then the sample was incubated for 5 min at 25◦C and then added 0.1mL of 2.5 mM H_2O_2 and OD value was taken at 340nm wavelength. The activity of Glutathione Peroxidase was expressed in terms of nmol NADPH consumed/min/mg protein. For the Glutathione Reductase (GR) enzyme activity determination 2.0 mL of 9 mM GSSG, 0.02mL of 12 mM NADPH, Na4, 2.68 mL of 1/15M phosphate buffer (pH 6.6), and 0.1mL of tissue sample was mixed and incubated at 37 ^{0} C for 5 minutes. The OD value of the mixture was taken at 340 nm. Where the GR activity was stand for in terms of nmol NADPH consumed/min/mg protein. During Glutathione-S-Transferase (GST) enzyme activity study 2.85mL of 0.1M potassium phosphate (pH 6.5) containing 1mM of GSH, 0.05mL of 60mM 1-chloro-2,4-dinitrobengene and 0.1mL of tissue samples was mixed well and incubated at 25ºC for 5 minute. Then the OD value was taken at 340 nm and the GST's activity was expressed in terms of nmol NADPH consumed/min/mg protein (Mahapatra et al., 2009).

For lipid peroxidation (MDA) measurement 2 ml 0.375 % and15% thiobarbituric acid and trichloroacetic acid were added and mixed well. After that 200 µl tissue supernatant and 1.0 ml distilled water. Supernatant mixed aliquot solution was boiled on a water bath at 95 ^{0} C for 20 min (the solution get pink colour) then cooled it under tap water. 3 ml of n-butanol added with it to stop the reaction and OD value was taken at 532 nm (Wills, 1966).

2.6 Histological observation: The intestinal tissues (large and small) were collected from different groups and analysis was done by Haematoxylin and Eosin (H/E) staining method. Formaldehyde (10%) was used for 24 hours (for tissue fixation) and then dehydrate them ascending gradation of alcohol. After impregnation, intestinal tissues were transferred to melted paraffin wax at ≤ 68 ^{0}C in an embedding bath for the elimination of air bauble from tissue. Then all the tissues paraffin block were used for tissue section was cutting (microtome at 0.5 mm thickness) and then stained by haematoxylin-eosin followed by mounting with coverslip using neutral DPX medium (Titford, 2009).

2.7Quantitative analysis of SCFA in plasma through GC-MS:

2.7.1 Sample preparation: Blood samples were collected in tubes containing heparin and heparin mixed blood samples were centrifuged and collect the plasma samples. For SCFAs analysis the plasma samples were stored in a −80 °C until analysis for the analysis the samples were thawed at 4°C. During application them in GC column 100 µL of 1.0 M formic acid were added to 100 µL of plasma. Then samples were vortexed (for 10 min) and then centrifuged (13,000 g for 10 min at 4 °C) and 100 µL clear supernatant was transferred into GC vials for analysis. The internal standard solutions (acetic acid-d4, propionic acid-d6 and butyric acid-d7) and SCFA standard mixture (acetic acid, propionic acid and butyric acid) were prepared according to 50:5:5 ratio and 2-ethylbutyric acid used for prepared of internal standard solution (IS) and HPLC grade water used for those solution preparation. On the

other hand, the blank sample was prepared to correct the base line of SCFA peak area (Kim et al., 2022 and Wang et al., 2020).

2.7.2 Instrumentation and analytical conditions: Shimadzu GC-2010 (Shimadzu Corp) with capillary column (BP21 FFAP 30 m × 0.53 mm i.d., 0.50 µm film thickness) was performed for GC-ms. Nitrogen was used as a carrier gas which flow rate was 30 mL/min. Similarly, the auxiliary gases (Hydrogen and dry air) for the flame ionization detector flow rate were 30 mL/min and 300 mL/min. At that time the detector and injector temperatures were 240 °C and 220 °C respectively and the total run time was 12 min. The method was validated according to the guidelines ICH (International Conference on Harmonization); 2005. The concentrations of the plasma SCFAs (acetic acid, propionic acid and butyric acid) from the spiked and non-spiked sample were used to calculate the recovery percentage of each analyte.

LOD (limit of direction) and LOQ (limit of quantification) were calculated established on the basis of ICH (2005) method. Here equations of LOQ = 10 SD/slope and LOD = 3.3 SD/slope where SD (standard deviation) was the estimated uncertainty of the responses (slopes of regression lines) and the slope was obtained from the calibration curve. In full scan mode m/z range 40-150. Each SCFA determined by calibration curve was the calculated through correlation coefficient (R^2) value over 0.99 (n = 5 in each group).

2.8 Statistical analysis: All the data are expressed as the Mean ± Standard Error of Mean (SEM), One Way ANOVA and using Origin 6.0 professional software.

III. RESULT AND DISCUSSION:

3.1 Characteristics of ZnOFNC: UV-vis spectrum analysis we got one important pick of ZnOFNC at 358nm shows in Figure 1A. FTIR spectroscopy help to investigate various biochemical bonds from FTIR spectrum pecks (cm^{-1}) (Mark et al., 1987). The ZnOFNC sample's FTIR spectrum pecks shown in Figure 1B. In ZnOFNC spectrum the hydroxyl group, carboxyl group, aldehyde group, and alkyne group were also present. Similarly, we got such pecks in ZnOFNC which were 743, 595 and 489 picks representing Zn-O stretching bonds. But new strong O=C=O was stretching found in only the ZnOFNC sample at 2346.58 which may help to bind FOS and ZnO both particles and help to formed of ZnOFNC sample shown in Figure 1B. On the other hand, the Figure- 1 C represents the SEM image of ZnOFNC. Which were spherical shaped and the average size was 45nm.

Before discussing the entire result of several experiments, we would like to discuss the characterisation of ZnOFNCs nano-conjugate. FTIR spectrum pecks (cm^{-1}) of ZnOFNC samples were represent various biochemical structural bonds (Mark et al., 1987). Where we got several functional groups like hydroxyl, carboxyl, aldehyde, and alkyne groups that were present in both samples of FOS (Lambertz et al., 2017) and ZnOFNC. Similarly, we got Zn-O stretching bonds that were present in the sample of ZnOFNC. But here a strong O=C=O bond was found that may be expressed by a that could be helpful for binding of FOS and ZnO compounds and that was helped to form ZnOFNC (Figure- 1B). On the other hand, we received one important pick of ZnOFNC at 358nm in UV spectrum. Very recent study reported that 200–600 nm UV picks are indicating better Zn-O stretching (Aldalbahi et al.,2020). At same time SEM images shown that the ZnOFNC particles was spherical shaped and the average size of ZnOFNC was 45nm (Figure- 1C). Above characteristics of ZnOFNC were represent that FOS and Zinc acetate capable to form a nano conjugate form.

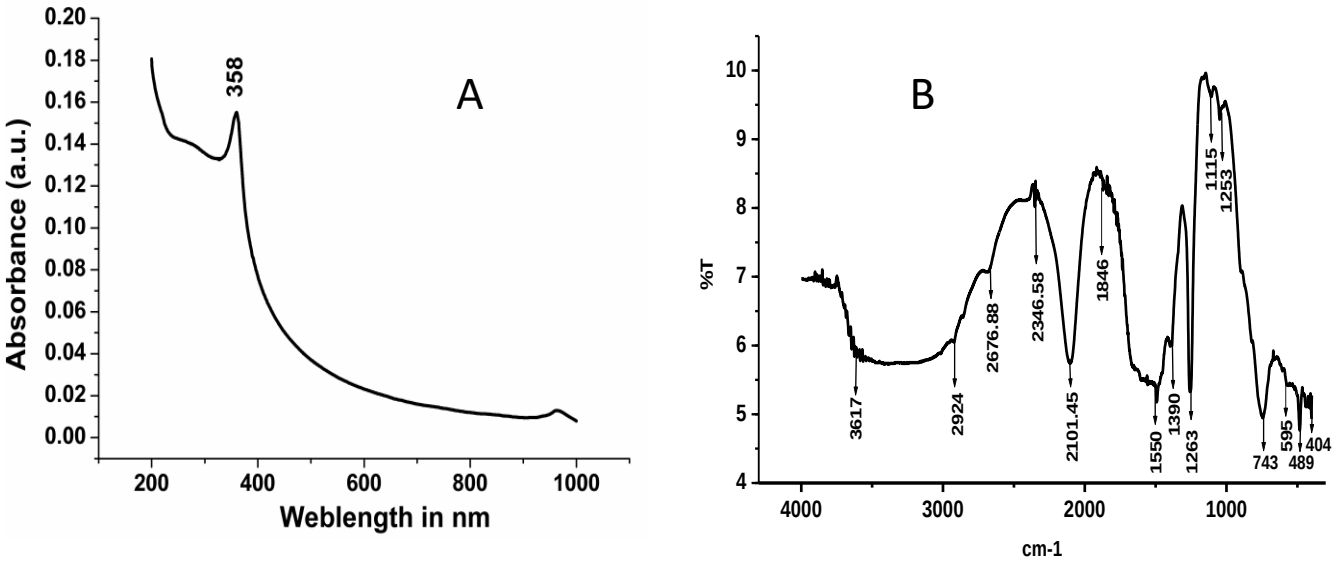

Figure 1: A' image represents UV spectrum given one important pick at 358nm (absorption value). Image 'B' shows FTIR spectrum, and image 'C' represent SEM image

3.2 Oxidative stress study: In present study we observed that the antioxidant enzyme activity and toxic enzyme activity changes due to nicotinic toxic effects and observed the therapeutic as well any recovery role of ZnOFNC (Figure-2). The results of the antioxidant elements study have been appropriately summarized, tabulated, and subjected to statistical analysis before completion and graphic illustration and ANOVA test was used in this study to compared between means of different groups (group I, II, III and IV). First of all, the SOD, CAT, GSH, GSSG, GR, and GPx level of nicotine treated group was significantly decreased comparison to the control group. The result of Group IV (Nicotine with ZnOFNC) we observed that SOD, CAT, GSH, GSSG, GR, and GPx levels of the large and small intestine were significantly ($P<0.05$) raised in relation to nicotine treated group and insignificant with the control group. On the other hand, the MDA level was significantly depleted in the large and small intestine of group IV (21.15±1.40 and 25.45±1.70 µmol/mg protein) compared to the group II (39.26±1.30 and 39.56±1.60 µmol/mg protein) but insignificant with the control group (19.63±1.60 and 23.03±1.12 µmol/mg protein). FOS treated group (group III) represent similar result as like as group II (Figure-2).

Micro-ecology helps to builds with microorganisms, that is a very important part of gastrointestinal system and controlled the mental and physiological health of human (Guarner et al. 2006). Micro-ecology imbalances are promoting illness and induced the development of various diseases (Guarner and Malagelada. 2003). The beneficial micro-biota is found in a high population in our digestive tract. Savin and his group (2018) reported that nicotine toxicity influenced on oxidative stress as well as oxidative stress-related elements (like MMP-9 release) are other factors responsible for modification of intestinal tight junction's strength, changes in mucin composition, and acid–base balance of intestine. Present observation, it was revealed that nicotine toxicity increased the toxic substrates like MDA level significantly increased and on the other hand the antioxidant enzyme (SOD, CAT, GSH, GR, GSSG, GST & GPx) activity decreased significantly ($P<0.05$) (Figure-3). Tissue damaging factors such as MMP-9 highly activated causes of nicotine induced toxicity (Seow et al., 1994) and it also effects on the rectal mucosal thickness along with induced inflammatory bowel disease (Zijlstra et al., 1994). Nicotine

able to change the gut microbial ecology and increased unhealthy bacterial growth (Jinqiang et al., 2015). Hopkins and Macfarlane (2002) observed that gastrointestinal environmental factors (like changes in pH level, oxidative stress, deficiency of ions, etc.) are able to modulate the colonization of microbiota. High population of *Prevotella* sp (found in the oral cavity, gut, and vagina, responsible for bites, paronychia, urinary tract infection, brain abscesses, osteomyelitis, and bacteraemia) (*Lukens et al., 2014*). In a toxicity study it was noted that nitric oxide (NO) is a well-known gastric mucosa protection factor. ADMA (Asymmetric dimethylarginine) responsible to prevent nitric oxide synthesis and help to development of gastric mucosa injury. ADMA level was simultaneously increased presence of nicotine in a high amount in serum (Zhang et al., 2009). The cellular mechanism like increased ADMA formation, elevated ROS generation, modulated pH level of GI tract, diminished NO formation, increased number of toxic bacterial growth rate, etc. In the present study, we observed that after application of ZnOFNC with nicotine can able to abridge or prevent the nicotine-induced toxic effects. ZnOFNC Administration (group IV) significantly enhanced the antioxidant status in the large and small intestines than nicotine-treated rats (group II) and protected cells against the damaging effects. The reduction of antioxidant level (SOD, CAT, GR, GSH, GPx, GSSG & GST) in the large and small intestines of nicotine-treated rats may be enhanced or protected after application of ZnOFNC decreases. Similarly, lipid peroxidation or MDA level in the large and small intestine of nicotine treated rats may be diminished after application ZnOFNC. That may prove it has a detoxification capacity. It is known that Zn directly acts on SOD activity and increased its level and modulates oxidative stress (Bharti et al., 2014). It is proved that causes of nicotine ingestion excess H_2O_2 and lipid peroxides are generated which in turn diminish the scavenging activity of GPx and the depression of this enzyme (GPx) activity reflects perturbations in normal oxidative mechanisms during nicotine ingestion. On the other hand, the GR activity is promoting the formation of GSH from GSSG where the GSH in the cellular metabolism independent of its antioxidant properties and G detoxification of xenobiotics as a substrate for the enzyme glutathione-S-transferase with that nicotine increased the activity of marker enzyme LDH and increased the level of MDA, causes elevation of lipid peroxidation (Ray et al., 2002) shown in Figure- 2.

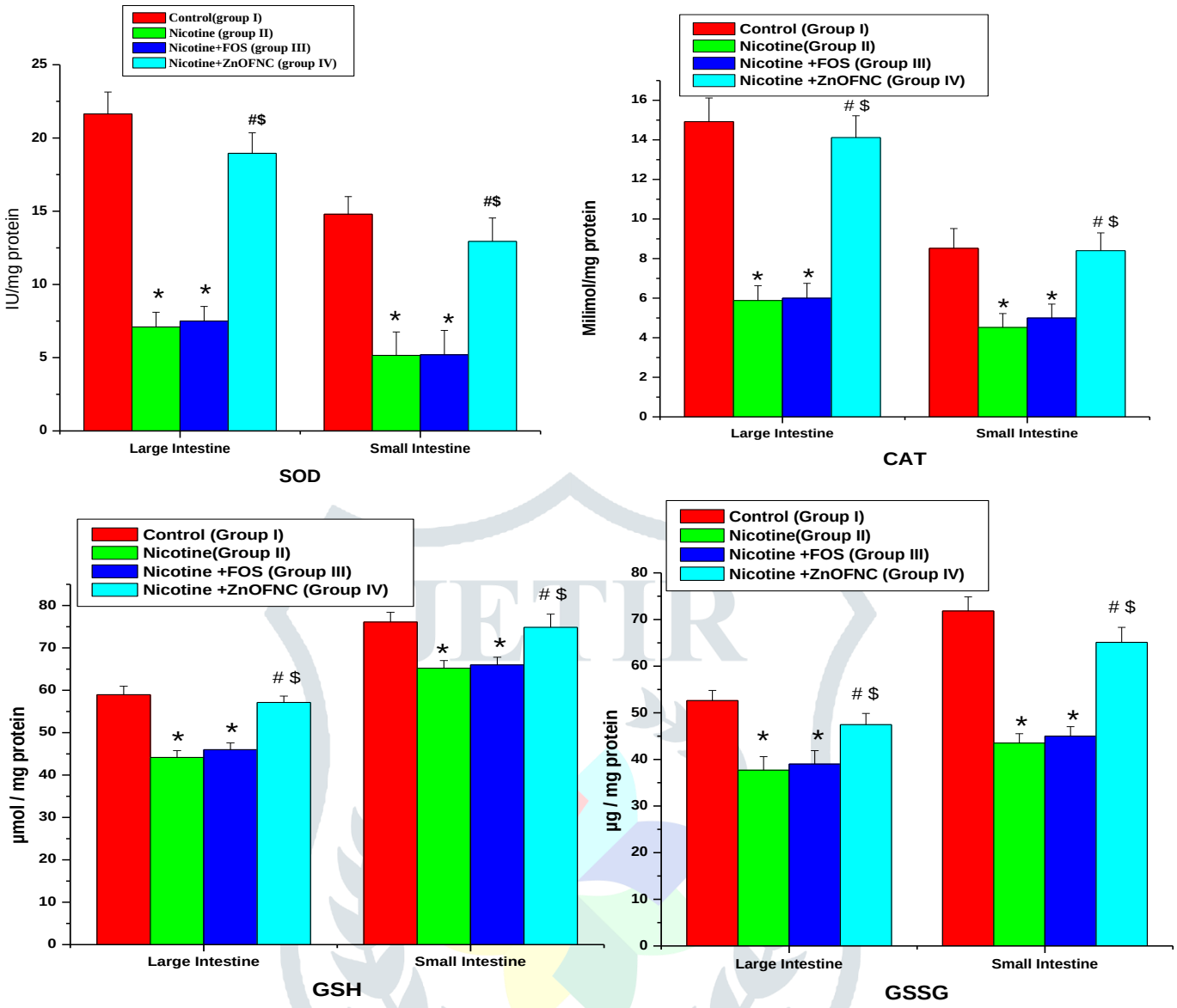

Figure 2a: The Figure represents the in vivo changes of antioxidant parameters (SOD, CAT, GSH and GSSG) of large and small intestine of different groups. Here * indicate the level of significance at $P < 0.05$ by One-way ANOVA in compare to control group. # Indicate significant changes of group II with group III and VI ($P<0.05$). Similarly, $ Indicate significant changes of group III with group VI.

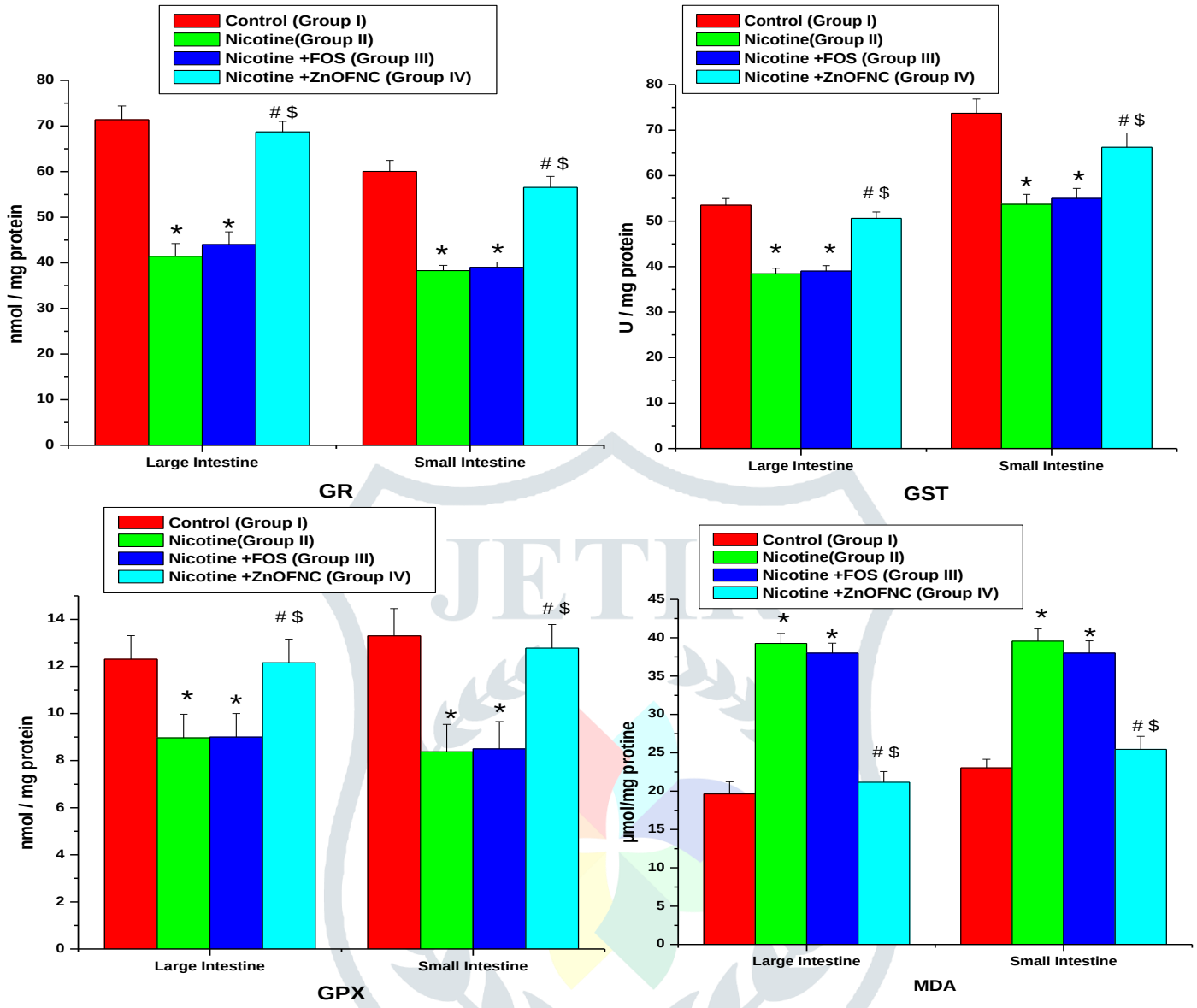

Figure 2b: The Figure represents the in vivo changes of antioxidant parameters (GR, GST, GPx) and MDA of large and small intestine of different groups. Here * indicate the level of significance at P < 0.05 by One-way ANOVA in compare to control group. # Indicate significant changes of group II with group III and VI (P<0.05). Similarly, $ Indicate significant changes of group III with group VI.

3.3 Histological study of Intestine: The cellular structural changes of large and small intestine tissues were shown in histological images of different groups (Figure-3). For nicotine treatment (group II) the histological structure of intestinal (small intestine) villus structure was altered, decreased villus number, the mucous layer of villous was thinner than normal, the epithelial cell layer of villus was damaged, reduced the density of epithelial cell layers and inflammation occurred in muscularis areas of the intestine. The group IV images were represented, the histological structure of intestine tissues there no such changes occur than control. Intestinal (small intestine) villus structures were as like as control groups. In the mucous layer no damages and inflammation were occurred in the muscularis areas of group IV, the mucous layer density is thicker than the group II and III. No such damages found in the epithelial cell layer.

In the histological section of large intestine of group II and III represented that, the villus was shrinking, the mucous layer was damaged, and sub-mucosa layer was narrow. Some area of the muscular layer was ruptured and so many gaps were formed in between the submucosal layer and muscular layer. The group IV images were represented, the histological structure of large intestine tissues there no such changes occur than control (Figure -3).

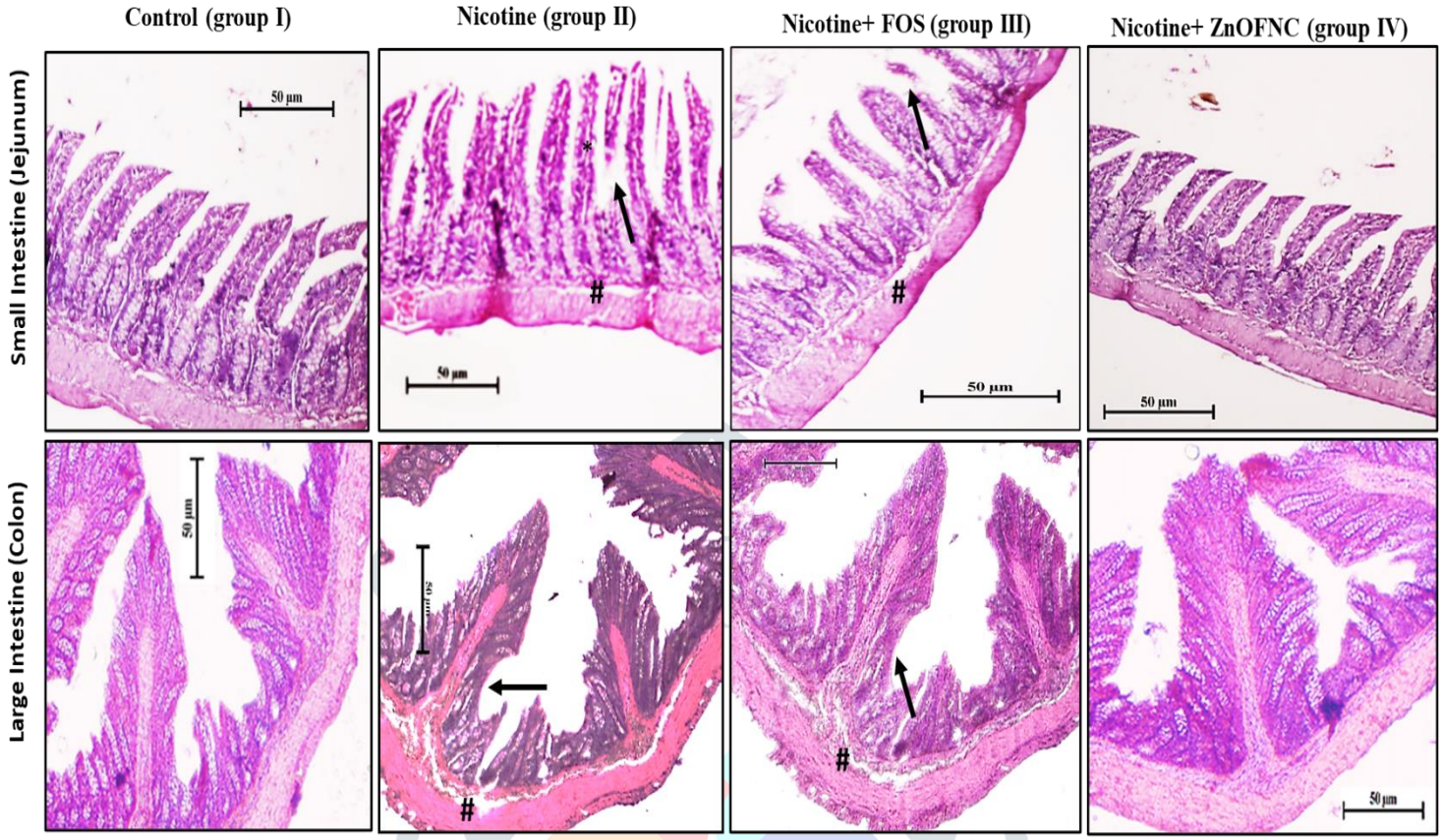

Figure 3: These pictures show the longitudinal section of the small and large intestine of groups I, II, III and IV. Original magnification was X200. Arrows of large intestine tissue are showing the necrotic muscular layer and the shrinkage of fibrous tissue. and '*' symbols are indicated shrinkonised villus and thinner mucous layer. The original magnification of small intestine tissue was X200. Single arrows of small intestine tissue are indicated the ruptured villi occurred in several areas and '#' symbols are indicated the ruptured epithelial cell layer.

3.4 GC-ms spectrum analysis: Through GC technique, the biological samples were vaporized into gaseous compounds and separate them based on boiling point of the compound. Then they separated according to their differential absorption on a porous solid or liquid support with their molecular weight and volatile capacity. The result developed on the basis of retention time. Those compounds variations depend on the partition coefficients of the compounds of biological sample (Plasma samples). The standard calibration range was in-between 12.67–28.06 which used for LOQ detection (Zhang et al., 2019). For GC-ms spectrum analysis the intensity and m/z (mass to charge) ratios are used for both qualitative and quantitative analysis. In present study the full scan mode of m/z range was 30-150. Here we got such absorption picks their m/z values were 55, 57, 60 and 74 on the based on retain time. Which was proved that in biological sample (Plasma samples) SCFAs were present (acetic acid, propionic acid and butyric acid). At that same time, compare with standard sample of SCFAs (acetic acid-d4, propionic acid-d6 and butyric acid-d7) picks that were also proved that the SCFA (acetic acid, propionic acid and butyric acid) were also present in biological samples.

Recovery percentage of SCFA of plasma: In this study the recovery percentage of SCFA (Acetic acid, Propionic acid and Butyric acid) of group I was significantly ($P<0.05$) differ than group II and III. Similarly, the recovery percentage of Propionic acid of group IV result was also significantly ($P < 0.05$) higher than group I shown in Figure 4.

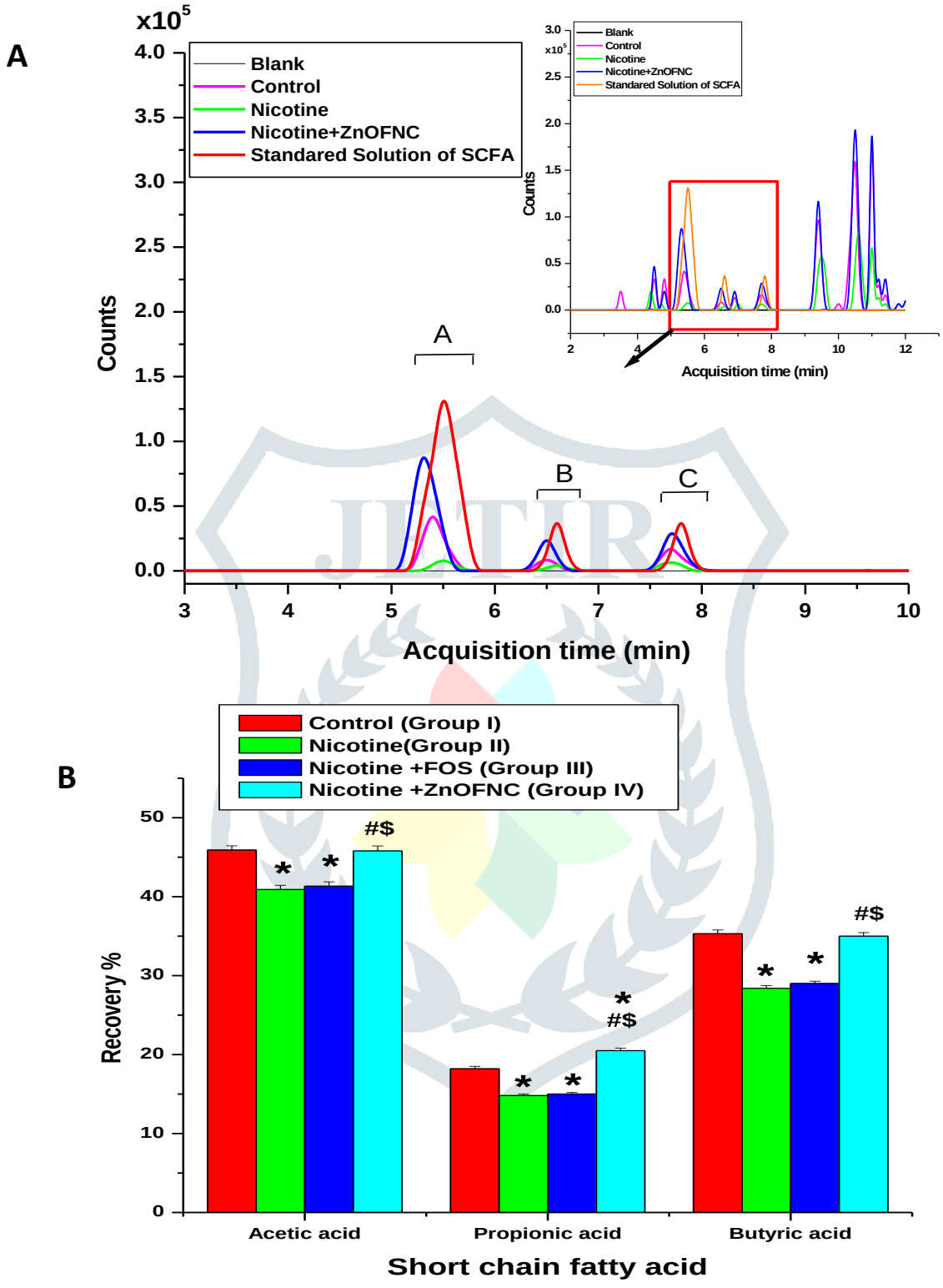

Figure 4: Image A represent the line diagram of EIC (Extracted ion chromatogram) of the quantitation ions. Here "A, B and C" represent the acetic acid, propionic acid and butyric acid level of different solution respectively. Pink, green and blue line diagram are representing the control, nicotine and nicotine +ZnOFNC group's plasma SCFA level respectively. Black and red line diagram represent the blank and standard SCFA solution.

Image B represent the graphical presentation of Recovery % (n=5) of plasma SCFA. Here * indicate the level of significance at $P < 0.05$ by One-way ANOVA in compare to control group. # Indicate significant changes of group II with group III and VI ($P<0.05$). Similarly, $ Indicate significant changes of group III with group VI.

Use of tobacco products through smoking or smokeless causes huge adverse effects on human health. All the effects are negative. Any tobacco-related products encompass more than 70 chemicals that cause several diseases primarily different types of cancers. It was also proved that tobacco contains nicotine, a highly addictive and toxic drug that induced various damages to internal organs and cellular levels. In this investigation, we try to build up a nutritional approach to treatment against nicotine toxicity at various tissue levels. Here we mainly used ZnOFNC (fructooligosaccharides and zinc oxide nano conjugate form) against nicotine toxicity in the intestinal organs. From this observation, it may be suggested that ZnOFNC activity was more prominent against nicotine-induced cellular dysfunctions at various levels and all above-mentioned studies may be going on through the increased synthesis of short-chain fatty acids (Butyrate, Propionate, Acetate) at the GI tract. Throughout study the FOS treated group (group III) represent similar result with group II (nicotine treated group). So, it can say that FOS itself unable to improve the damages causes of nicotine toxicity when it gates a composite form (ZnOFNC) it represents more beneficial result against tobacco-related damages which is a positive treatment protocol in future aspects.

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