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Assessment The Interaction Effect Oftio_2nps And Some Short Chain Fattyacids On Immunity And Gut Microbiota Balance In Laboratory Rats

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ABSTRACT

This study was conducted in the laboratories of Food Science Department, College of Agriculture and Animal House of the College of Veterinary Medicine at Tikrit University for the period from 1stOctober-2020 to end of April-2021 to aimed of evaluate the effect of interaction between TiO2NPs synthesized by Fusarium oxysporum and Short-chain fatty acids (SCFAs)as Acetic (C2), Propionic (C3) and Butyric acid (C4), when orally dosage to laboratory rats that induced immunosuppression with Sandimmune on the level of immunoglobulins and interleukins as well as the estimation of gut microbial balance of laboratory rats.The results showed the formation of TiO2NPs through the use of biosynthesis of the filter Fusarium oxysporum to convert the primary compounds Ti (NO3)4 into their nanoparticles with the average size at 54.70 nm. The animal's group of immunosuppression induced (G2) were significantly ($p<0.05$) caused in decreased the IgG, IgA andIL-6 concentration, which at 856, 134 (mg/dL) and1.35 (Pg/ml)respectively compared to the animals control group (G1) that at 1026, 177 (mg/dL) and 2.26 (Pg/ml) respectively. the orally dosage fromTiO2NPs and each of SCFAs were significantly affects in increased the IgG, IgA and IL-6 concentration to became significantly similar of its parameters in animals control group.The results showed that the total counts of bacteria, Coliform were significantly increased, while lactic acid bacteria (LAB) were significantly decreased in the G2 groupand appeared at log 9.73, 3.49 and 8.91cfu/gof intestine compared with the its account in G1 which was at log 6.68, 4.30 and 7.04cfu/g, while the orally dosage from TiO2NPs and each of SCFAs at the G3-G9, were caused in increased the LAB and decreased the total bacteria and Coliform accounts in the animal's intestine.

Keywords: TiO_2NPs, Short chain organic acids, Sandimmune,IgG, IgA, IL-6.

Introduction

The science that is concerned of molecules within the scale of 1-100 nanometers is identified as Nano-science.The nanoparticleswereoccurred when change of the material properties in their nano dimensions less than 100 nanometers.These modify was effects on the physical and chemical properties modification such as their surface area, melting point, freezing and other properties, nanoparticles are the basic units of nanotechnology and are prepared by biological methods, their approach is bottom-up technology in preparation (Ahmed, 2020).

Titanium dioxide (TiO2) is a white, incombustible, odorless, and inexpensive powder. While the TiO2 nanoparticles have a high refractive index, are environmentally friendly and are resistant to corrosion in nature. These particles are available in nature and it has three crystal forms, namely rutile, anatase, and brookite (Chunyang et al., 2021).

Short-chain fatty acids (SCFAs) are saturated fatty acids consisting of one to six carbon atoms, including acetic (C2), propionic (C3) and butyric (C4) in the largest quantity with a generally fixed molecular ratio of 60:20:20, respectively. In the colon as well as in the feces, other SCFAs such as iso-Butyric (C4), valeric (C5 and iso-valeric (C5) are present in smaller quantities, SCFA contribute to improving the immune responses of the host in addition to its ability to maintain the level of proliferation Colon cells differentiate to protect and maintain a healthy colon by increasing mucin production and improving both oxidative stress and immune response (Dorsilla et al., 2021).

Materials and Methods

Initialization of laboratory animals

Healthy and disease-free of 54 adult male Albino rats at the age 8-9 weeks, weights range at 213-217g were obtained from the College of Veterinary Medicine, University of Tikrit, they were randomly distributed into nine groups, each one contained six animals. The animals were placed in cages made of plastic, after covering their floors with sawdust, which was replaced four times a week. The animals were fed a basil diet according to NRC, (1995), that contained (g/kg) Casein 84.95 pure protein at 158.5 g, 100 gm oil, 5 g a mixture of vitamins, 50 g mixed mineral salts, 50 g of cellulose, 100 g of glucose and 536.5 g of starch. The animals were raised under the supervision of a specialized veterinary staff, taking into account the aspect of hygiene. The experiment was design as follow:G1 control group, G2 group of animals orally dosage ofSandimmune, G3 group animals orally dosage TiO2NPs + Sandimmune, G4 group animals orally dosageof acetic acid + Sandimmune, G5 group animals orally dosage of propionic acid + Sandimmune, G6 group animals orally dosage of butyric acid + Sandimmune, G7 group animals orally dosage ofTiO2NPs + acetic acid + Sandimmune, G8 group animals orally dosage ofTiO2NPs+propionic acid+ Sandimmune, G9 group animals orally dosage ofTiO2NPs+butyric acid+ Sandimmune.

The TiO_2NPs were used at 2.5 mg/mL/day which obtained from Food Science Department,College of Agriculture, that synthesized by Fusarium oxysporumsupernatantfrom Al-Sumaidai et al.,(2021) under publication. TheSandimmune (Cyclosporine-A) were used at 1 mg/kg/day, while short chain fatty acids SCFAs as Acetic, butyric and propionic acid were used at 5% of 1 ml /animal/day for each acid separately.

After the end of the specified period of the experiment, the animals were anesthetized by chloroform, then blood samples were drawn directly from the heart using the Cardiac Puncture method. A quantity between 5-8 ml of blood was withdrawn, which was placed in test tubes free of anticoagulant, then separating the serum using centrifugation at a speed of 3000 rpm for 15 minutes and keeping its at (-20)ºC until immunoglobulins IgG, IgA and Interleukin-6 are performed on them, as in (Tietz.,2005). used an ELISA System Assay.

Counting microorganisms in the small intestine: The laboratory animals were dissected and the three sections of the small intestine were taken, the duodenum, jejunum and ileum, and their contents were emptied from the remains of the digested food and washed with sterile distilled water to be ready for the process of estimating the bacterial species in them.One gram of bowel

intestine wasadded to 9 ml of physiological solution, 0.1 ml of which was drawn from the optimal dilution and then spread on De Man Regosa Sharpe MRS Agar medium and 0.1 ml of the dilution and spread on Eosin-methylene medium Blue Agar EMB and another 0.1 ml on the Nutrient Agar medium. The plates were incubated at 37 °C for 24 hours after which the developing colonies were counted on the plate for each sample (Harrigan and Maccance, 1976: Al Shammari, 2019).

Statistical analysis: The data were statistically analyzed through the experimental system within the ready-made statistical program (SAS, 2012) and using the complete random design system (CRD), as the averages were chosen according to the Duncan (Duncan, 1955) multiple range test to determine the significance of the differences between the averages of the factors affecting the studied traits at the level of 0.05.

Results and discussion

- **Effect of orally dosage from TiO_2NPs and SCFAs on induced immunosuppression rats for 28 days on some immunoglobulin parametersand interleukin-6 concentration:**The effect of interaction between TiO_2NPs and short chain fatty acids SCFAs in rats that orally dosage for 28 days on some immunoglobulin parameters was investigated in table (1).
  The results showed that the treatment of animals with asandimmune in the second group (G2) was significantly ($p<0.05$) decreased the IgG and IgA concentration, and it was becomingat 856 and 134 (mg/dL) respectively, compared with the healthy control group (G1), which was appeared at 1026 and 177 (mg/dL) respectively.The orally dosage from TiO_2NPs and each of SCFAs to laboratory rats as G3, G4, G5, G6, G7, G8 and G9, was effects on increasedsignificantly the concentration of IgG in the groups and appeared at 974, 956, 923, 902, 1008, 1022 and 1020 (mg/dL) and the IgA values were at 156, 161, 152, 167, 169, 172, and 176 (mg/dL) respectively, compared with its concentration in the group of G2.
  The results also were showed that the concentration of IL-6 was significantly decreased in the group of animals after disrupted to immunosuppression (G2), and it was appeared at 1.35 (pg/ml) compared to its concentration in the healthy control group (G1), which at 2.26 (pg/ml), while the concentrations were significantly increased in rats group immunosuppressed after treatment with TiO2NPs and SCFAs, as G3, G4, G5, G6, G7, G8 and G9 and they were became at 2.08, 2.10, 2.04, 2.07, 2.25, 2.17 and 2.20 pg/ml respectively.
  The results are in agreement with Harvey, (2009) who found that Sandimmune was suppresses cellular immunity and affects humoral immunity to a lesser extent, the Sandimmune molecules was diffuse into T cells and calcineurin binds to them to form a complex responsible for dephosphorylation of NFATc.
  The cyclosporine-induced immunosuppression observed in the group of animals (G2) resulted from the inhibition of calcineurin phosphatase activity which caused a decrease in the synthesis of several cytokines such as IL-2, IL-3, IL-4 and IL-6 over the course of the research.
  The resultswere agreed with Soliman et al., (2013) who found that treatment with TiO_2NPswas effects on reducedthe levels of IgG and IgA in male laboratory rats. Also, agreed with the Studies of (Donohoe et al., 2014; Louis et al., 2014) whom refer that some SCFAs induce the differentiation of regulatory T cells, which helps to control intestinal inflammation. And effected in preserving the intestinal wall from the risk of inflammatory bowel disease or colorectal cancer (CRC).

Table 1. The effect of the interaction between TiO2NPs and short chain organic acids on IgG and IgAand interleukin-6 concentration values for rats dosed orally for 28 days.

Group	IgA	IgG	IL-6
	Mg/dl		Pg/ml
G1	177a±1.2	1026a±9.64	2.26a±0.08
G2	134d±1.9	856d±7.66	1.35c±0.01
G3	156c±1.9	974b±9.88	2.08b±0.01
G4	161bc±1.2	956b±8.73	2.10b±0.01
G5	152c±2.8	923c±10.57	2.04b±0.01
G6	167b±2.5	902c±0.88	2.07b±0.01
G7	169b±3.2	1008a±12.73	2.25a±0.02
G8	172a±4.8	1022a±9.57	2.17a±0.02
G9	176a±1.57	1020a±8.88	2.20a±0.01

The different letters on the rates of the same column indicate that significant differences at 0.05 probability level.

G1: group of healthy control animals, G2: group of experimentally immunosuppressed animals, G3: group treated with TiO2NPs, G4: group treated with acetic acid, G5: group treated with propionic acid, G6: group treated with butyric acid, G7: group treated with TiO2NPs + acetic acid, G8: group treated with TiO2NPs+ propionic acid, G9: group treated with TiO2NPs + butyric acid

The IgG index is associated with many types of pathogens such as viruses and bacteria. and fungi and protects the body against them through what is called Agglutination and Immobilization or Complement Activation, Opsonization for phagocytosis and neutralization of their toxins (Mallery et al., 2010). The decrease in cytokine values coincided with the decrease in antibody secretion. Also, the interleukins were act together to trigger a chain reaction to stimulate the WBSs and various cells in the immune system against disease (Benson, 2002).

SCFAs effects on enhanced the IgA and IgG antibody responses and the interaction of GPR43 by SCFAs on dendritic cells (DCs) also regulates retinoic acid production, which in turn has been found to stimulate IgA production by B cells (Wu et al., 2017). It activates the mTOR pathway in B cells, which leads to increased glucose uptake and glycolytic activity and final differentiation into antibody-secreting plasma cells (Kim et al., 2016).

Wu et al., (2017) indicated an indirect effect of butyric acid on inflammation by maintaining the colonic epithelium barrier and showed that acid reduces inflammatory IL-6 and IL-12 production by CD11b + CD11c− intestinal macrophages. In addition, Yang et al., (2019) finding that treatment with acetic acid effected in reduced the synthesis of the inflammatory IL-6 and thus attenuated sepsis-induced inflammation in male laboratory mice and could suppress inflammation and protect the liver in infected mice. It is also beneficial in preventing metabolic syndrome in rats and humans that synthetic acetic acid and Nipa vinegar can alleviate obesity by altering inflammation, lipid metabolism and gut microbiota composition in obese rats on a high-fat diet (Beh et al., 2017).

Effect of orally dosage from TiO_2NPs and SCFAs on induced immunosuppression rats for 28 days on the intestinal microbiota balance:

The effect of orally dosage each of TiO_2NPs and SCFAsalone or mixed to laboratory rats for 28 days on the balance of the intestinal microbiota was illustrated in Table (2).

The results showed that the total counts of microorganisms and coliform were significantly ($p<0.05$) increased and the LAB was decreased in the intestines of animals that treated with Sandimmune G2 and appeared at log 9.73, 3.49 and 8.91cfu/g respectively comparing with the same counts of the control group G1 which appeared at 6.86, 4.30 and 7.04 log cfu/g respectively. The treatment of laboratory rats with each of TiO_2NPs and SCFAs as G3, G4, G5, G6 and G7. And G8 and G9 were effects on total counts of bacteria and appeared at 5.78, 7.69, 7.31, 7.37, 6.03, 6.14 and 6.07 log cfu/g respectively.while the counts of coliform bacteria were becoming at 4.56, 6.36 and 6. 67, 6.59, 5.42, 5.51 and 5.48 log cfu/grespectively. Also, the LAB was appeared at 3.79, 4.32, 4.46, 4.40, 4.82 and 4. .94 and 4.89 log cfu/g respectively.

Table 2.Effect of orally dosage from TiO_2NPs and SCFAs on induced immunosuppression rats for 28 days on the intestinal microbiota balance.

Group	Total Count	lactic acid bacteria	Coliform
	Log cfu/g		
G1	6.86c ±0.03	4.30b ±0.07	7.04b ±0.06
G2	9.73a ±0.13	3.49c ±0.1	8.91a ±0.1
G3	5.78e ±0.1	4.19b ±0.08	4.56e ±0.16
G4	7.69b ±0.12	4.32b ±0.17	6.36c ±0.03
G5	7.31b ±0.06	4.46b ±0.21	6.67c ±0.1
G6	7.37b ±0.19	4.40b ±0.24	6.59c ±0.08
G7	6.03d ±0.52	4.82a ±0.17	5.42d ±0.3
G8	6.14d ±0.13	4.94a ±0.21	5.51d ±0.5
G9	6.07d ±0.13	4.89a ±0.24	5.48d ±0.7

The different letters on the rates of the same column indicate that significant differences at 0.05 probability level.

G1: group of healthy control animals, G2: group of experimentally immunosuppressed animals, G3: group treated with TiO2NPs, G4: group treated with acetic acid, G5: group treated with propionic acid, G6: group treated with butyric acid, G7: group treated with TiO2NPs + acetic acid, G8: group treated with TiO2NPs+ propionic acid, G9: group treated with TiO2NPs + butyric acid

These results were agreement with Mahdy et al., (2017) whom conducted that TiO_2NPs has a significant antibacterial effect on Staphylococcus aureus and Escherichia coli. The mechanisms of microbial inhibition of TiO_2NPs involving the release of positively charged ions into the reaction medium bound (negatively charged) with a thiol group (-SH) for proteins located on the cytoplasmic membrane and this reaction leads to their capture by the cell wall and increased permeability, causing deformation of the structure of cellular components such as DNA, ribosomes, cellular enzymes and finally microbial cell death (Khashan et al., 2021).

These results agreed with the findings of Farup and Hestad, (2016), who indicated that there is a change in the production of SCFAs when some human diseases occur, such as irritable bowel syndrome, and these diseases can even be a guide for measuring gut health.

Deleu et al., (2021) indicated that acetic acid is an important regulator of gut acidity and helps maintain a stable environment as it helps maintain the acidity of the gut at a level that can survive beneficial microbes and prevent harmful microbes from surviving. It also binds to receptors in the lining of the gut controlling in appetite and regulation of fat storage, these receptors play important roles in promoting the secretion of gut hormones that regulate appetite.

High SCFA leads to lower pH in colon, which sequentially affects the microbial composition. In addition, most SCFA is absorbed in colon. This phenomenon can be explained by the lower ability of Bacteroides compared to Firmicutes species to tolerate SCFA at low pH at 5, which leads to a shift in microbial composition (Den Besten et al., 2013). The low pH prevents the overgrowth of pathogenic bacteria sensitive to acidic pH (Kettle et al., 2015).

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