REVIEW ON CHITOSAN INTAKE AND ENHANCEMENT IN FECAL EXCRETION OF ENDOCRINE DISRUPTORS

ABSTRACT:

Chitin is most abundantly found non-digestible carbohydrate similar to cellulose. It has gained a lot of interest in last few years due to its wide range of applications. They are used as an alternative, dietary supplement. Chitin on deacetylation forms chitosan, an active component of chitin. The degree of deacetylation and molecular weight are the factors that elucidate varying degree of biological property of chitosan. The ability of chitosan in fat and lipid excretion has obtained keen eye among various researchers. This property of chitosan is made use in enhancing the elimination of various harmful environmental lipophilic xenobiotics. Endocrine disruptors are a group of environmental pollutants causing various hazardous effects in living system. They are highly lipophilic, persistent and can accumulate in various tissues. Thus correlating the ability of chitosan to increase excretion of fats and lipophilic xenobiotics showed a promising positive outcome.

Keywords: Endocrine disruptors, chitosan, dietary fiber, biomedical applications, endocrine disruptor's excretion.

1. INTRODUCTION:

Endocrine system is a collective word, comprising of endocrine glands, hormone producing tissues, hormones and their corresponding receptors [1]. It is a sophisticated system collaboratively working with nervous system governing the body systems [2]. It presides over an individual's growth and development, starting from childhood to adulthood and has pivotal role in the reproductive process. Endocrine system is a highly regulated system, mainly involved in maintaining the homeostasis of internal system [1]. The endocrine glands are mainly involved in the generation of hormones, or chemical messengers, that get secreted into the interstitial fluid, further diffuses into the circulatory system reaching destined organs and tissues [3]. The glands assigned under endocrine system include the pineal glands, pituitary gland, thyroid gland, parathyroid glands, thymus gland and adrenal glands [1]. Fig 1 shows the hormones released specific endocrine glands. Based on their molecular structure hormones are categorized as polypeptides, steroids, amines and eicosanoids [1]. The endocrine system is hooked up with various performances namely; guarantee homeostasis by regulation of nutrient metabolism, water and electrolyte balance, regulation of growth and cells production, control the responses of the body to external stimuli, control reproduction, control and integrate circulatory and digestive activities in collaboration with the autonomic nervous system [1].

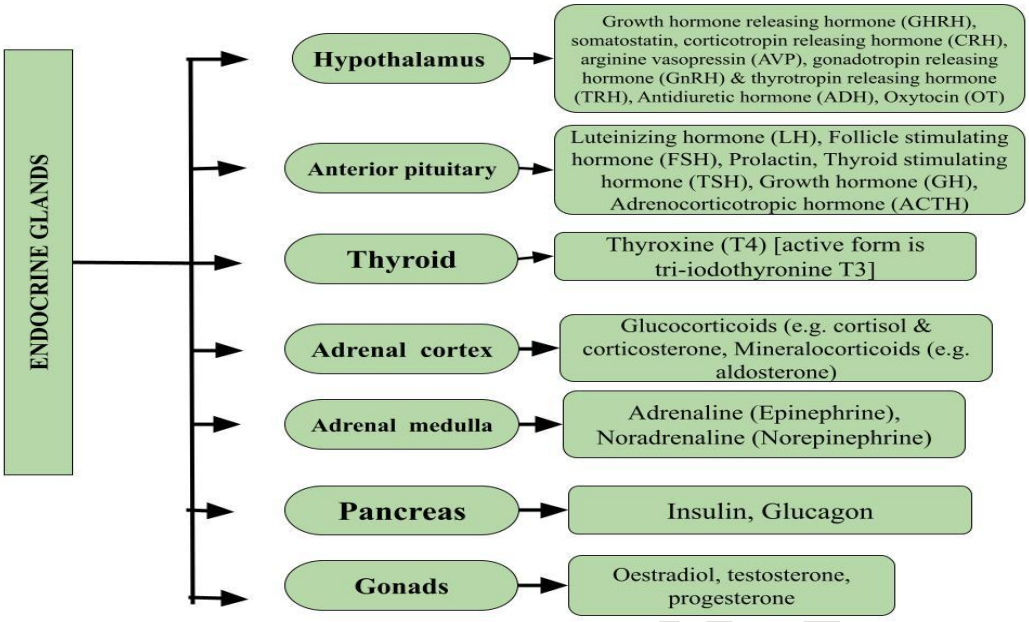

Figure 1: Endocrine glands and hormones released

On release of hormones, they are delivered to their specific target cells and bind to distinct receptors either present on the cell membrane or within the cell walls. When the hormone molecules bind to its specific receptor, they induce a variety of hormonal effects, based on the type of hormone binding and the target cell to which it binds. Components that affect the activation of the target cell are the amount of hormone in circulation, the number of receptors available on the cell surface and the sensitivity of particular receptor to particular hormone. Maintaining hormone secretion within a particular range is very much essential or the target organ becomes too active, and this achieved through negative feedback [1].

Chitin is abundantly found in nature in the form of amino-mucopolysaccharide (2-acetamido-2-deoxy-b-D-glucose), in supporting materials of various insects, crustaceans, fungi, certain invertebrates, etc. [4]. Chitin is a white, hard, inflexible, nitrogenous composite and a major byproduct of fishery department [5]. Similar to cellulose that acts as backbone of plant cell wall, chitin acts as structural polysaccharide forming the exoskeleton of various organisms. The chemical structure of chitin is similar to cellulose, which is built by linking hundreds to thousands of glucose molecules; the only difference is that in chitin the hydroxyl group present in C2 position of glucose molecule is replaced by acetamide group. Chitin can undergo degradation by the enzyme chitinase. Chitosan is a vital component of chitin derived on N-deacetylation of chitin, has a number of application. On deacetylation, the acetamide group of chitin is replaced with amine group which is the key advantage for exhibiting various biological properties [5]. Fig 2 shows the structure of chitin and chitosan. Applications of chitin and chitosan in both biomedical division and pharmaceutical division has grown rapidly and recently receiving a great attention from the researchers worldwide due to their significant properties such as antibacterial effect, biocompatibility, biodegradability, non-toxicity and high humidity absorption [6]. The physicochemical property of chitin is an important factor that aids in exhibiting various beneficiaries. These properties include molecular weight, extent of deacetylation and moisture content of chitin. Molecular mass of chitin is an important factor in exhibiting anti-microbial and antifungal properties [5]. The polycationic property of chitosan is an important consideration; this property contributes in interaction of chitosan with anionic cell membranes which in turn results in alterations of cell permeability gradually leading to cell death [7].

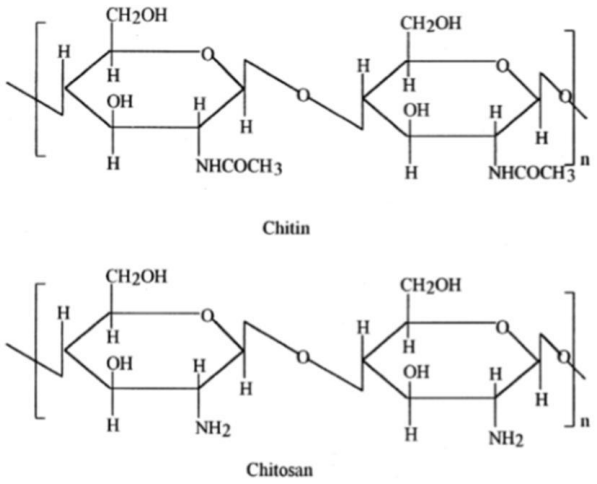

Figure 2: Chemical structure of Chitin and Chitosan

In this review, we have elaborated benefits of chitin and chitosan, major dietary fiber supplement and also its ability to decrease the accumulation of endocrine disruptors (Bisphenol-A, Phthalates, Dioxins, Polychlorinated biphenyls) in the body and enhance its excretion through feces.

2. BIOMEDICAL PROPERTIES OF CHITIN AND CHITOSAN:

Understanding the biological properties of chitin and chitosan is very much important deal for applying them in medical and pharmaceutical field. Hemostatic property was studied in invitro studies, chitosan with sulfated oligomers are proved to show anticoagulant property due to its cationic charge [8]. Analgesic effect was studied in invitro studies, chitin and chitosan shows analgesic effect on inflammatory pain due to administration of acetic acid intraperitoneal route and have proposed a possible mechanism for analgesic effect as the polycationic property of chitosan aids in protonation and the resulting reduction in pH is the key cause for analgesic effect [9]. Anti-tumor activity was studied both in invivo and invitro, chitosan and its carboxymethyl derivatives are proved to show anti-tumor property by immunostimulation property, they show increased production of lymphokines and cytolytic T-lymphocytes. Also studies by Ueno et al. suggest that activation of macrophages by chitosan mediate its antitumor effects in vivo [10] [1]. Anticholesterolemic activity, studies proved that there is decrease in fat absorption and increased excretion of fats [6]. The antimicrobial activity of chitin, chitosan, and their derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi, has received considerable attention in recent years [6]. Chitin and chitosan showed wound healing properties by activating inflammatory pathway. Minagawa et al studies showed that oligomers and monomers of chitin and chitosan showed wound healing properties [12].

3. ENDOCRINE DISRUPTORS:

EPA, U.S Environmental protection act, defines endocrine disruptors as agents that interfere with the normal homeostasis of natural endogenous hormones (biosynthesis, secretion, mobilization, binding to specific receptors, elimination and negative feedback) [13]. The term “Endocrine disruptors” gains a

growing concern among mankind due to their hazardous nature. They are highly stable in environment ranging from months to years due to its persistent nature. Endocrine disruptors are classified into natural or synthetic based on the occurrence [14]. Fig 3 shows the classification of endocrine disruptors. The sources of endocrine disruptors are drinking of contaminated water, ingestion of contaminated food items, contacting with contaminated soil [15]. Commonly encountered endocrine disruptors are [16] [15].

- Pesticides – DDT, Atrazine, chlorpyrifos, 2,4-dichlorophenoxyacetic acid, glyphosate
- Plasticizers - BPA, phthalates
- Heavy metals – Lead, cadmium
- Coolants – PCB, BFR
- Personal care products – Triclosan, Parabens
- Solvents – Glycol ethers, phenols, cyclosiloxanes
- Paints – Tributylin, Nonylphenol.
- Drugs - Ethinyl estradiol

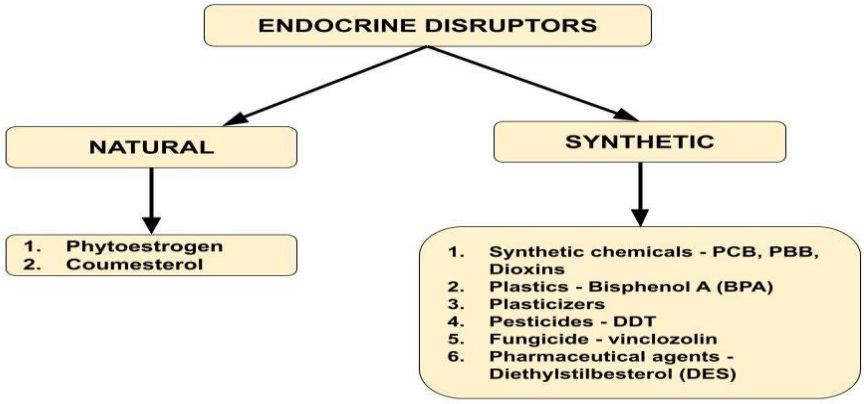

Figure 3: Classification of Endocrine Disruptors

Humans are exposed to endocrine disruptors through various routes namely oral, inhalation, dermal, biological transfer through placenta or breast milk. They exhibit their actions mainly thorough either of the following ways: mimic naturally present endogenous hormones and result in excessive stimulation or they may act as antagonist by binding to receptors and resulting in no signal transduction or they can interfere with synthesis of hormones [15]. A study on effects of Diethyl hexyl phthalate (DEHP), a plasticizer, on insulin resistance in adipose tissues showed that, DEHP increased oxidative stress in the adipose tissues which was main reason for insulin resistance which showed reduced uptake of glucose [17]. An invivo study on the effects of Bisphenol-A in insulin signal transduction and subsequent glucose oxidation in liver of albino rats showed that BPA decreased the oxidation of blood glucose which resulted in hyperinsulinemia [18]. This caused drastic decrease in glycogen level in the liver proving that BPA brings impairment to glucose oxidation. Another invivo study showed that BPA administration impairs the insulin signaling system leading to decreased glucose uptake in the skeletal (gastrocnemius) muscle [19].

4. BIOTRANSFORMATION OF ENDOCRINE DISRUPTORS:

Poly-chlorinated biphenyls are chlorine containing compounds with 209 members [20]. Biotransformation occurs mainly in liver aided by cytochrome P450 enzyme system. Biotransformation mainly depends on

the chlorination of PCB; highly chlorinated PCB are resistant to metabolism and are found to deposit in adipose tissues whereas low chlorinated PCBs are prone to biotransformation into respective metabolites [20]. In phase I they undergo hydroxylation to form monohydroxylated PCB which further forms dihydroxylated PBC. Due to unstable nature dihyhroxylated metabolites are less frequently observed. A study evidences that less chlorinated PCBs are metabolized to yield monohydroxylated derivatives [21]. Another study on rabbits states that insoluble highly chlorinated PCBs are found to yield dechlorinated metabolites [22]. In phase II they are converted into more water soluble compounds by conjugating compounds like glucuronic acid and sulfate conjugates assisting in easy excretion.

Dioxin is a general term given for group of congeners like polychlorinated dibenzodioxin (PCDD), polychlorinated dibenzofurans (PCDF). PCDD and PCDF are released into environment naturally on forest fire, waste incineration and are released anthropogenic way during industrial processes [23]. Among dioxins tetra chloro dibenzodioxins (TCDD) are considered to be the most toxic and hazardous compound. Metabolism of dioxins mainly occurs in liver with the assistance of cytochrome P450 enzyme systems. On phase I, they are majorly converted into hydroxylated metabolites. Metabolism of PCDD taken place by initially adding a single oxygen molecule which forms epoxides in the presence of cytochrome monoxygenase enzyme system [23]. Then in phase II, they undergo conjugation with glucuronic acid and sulfate conjugates in the presence of enzymes namely: UDP-glucuronosyl transferase and sulfotransferases [23].

Bisphenol-A (BPA) is a chemical compound which is white crystalline solids, used in production of various synthetic polymers [24] It is termed as xenoestrogen as it is proven to be similar to estrogen 17-β-estradiol [25]. They are rapidly biotransformed and excreted in urine and feces. Metabolism is taken place in liver assisted by cytochrome enzymes. Majorly noted metabolites of bisphenol- A are 2,2 bis (4-hydroxyphenyl)propanol and 3-hydroxy bisphenol-A; other metabolites include 4-isopropylhydroxy phenol and BPA dimers [24]. Further conjugation with glucuronic acid and sulfate conjugates helps in easy elimination of BPA metabolites.

Phthalates, diesters of 1,2-benzenedicarboxylic acid (Phthalic acid) are man-made chemicals with wide range of industrial applications [26]. Phthalates with low molecular weight are employed in the manufacture of solvents, varnish, plasticizers, personal care products etc., whereas those with higher molecular weight are used in production of highly flexible vinyl plastics which are used for medical devices, food contact application, flooring and other consumer products [26]. They undergo rapid metabolism in liver. The phase I of detoxification process yields metabolites based on their molecular weight. Polar and low molecular compounds are metabolized into hydrolytic monoesters whereas high molecular weight compounds are initially forms hydrolytic monoesters and then form oxidative metabolites on enzymatic oxidation. After which they are conjugated with glucuronic acid for easy removal.

5. DIETARY FIBRE:

According to American Association of Cereal Chemists (AACC), dietary fibres are edible parts of plant or analogous carbohydrates that are rebellious in digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine [27]. The functions of dietary fiber are based on its physicochemical properties. They are particle size, bulk formation, surface area, hydration, solubility, viscosity and ability to adsorb metal ions or organic molecules [27]. Dietary fibers are ubiquitously found in various food items in the form of cellulose, hemicellulose, lignins, pectins, gums, mucilages. Supplementation of normal human diet with un-digestible carbohydrates found to bring alterations in population and metabolic characteristic of gut microbiota [28]. This property helps in fermentation of certain fibers by the gut microbes in the colon resulting in benefits like increased absorption by small intestine, absorption of water soluble nutrients. Dietary fibers shows a variety of therapeutic beneficiaries

like increasing the volume of fecal bulk, decreasing the time of intestinal transit, decrease cholesterol and glycaemic levels, trapping substances that can be dangerous for the human organism (mutagenic and carcinogenic agents), stimulating the proliferation of the intestinal flora etc. [29]. Low dietary intake is associated with complications like irregular bowel moments, constipation, fluctuations in blood glucose levels, rise in cholesterol level and disturbance in normal gut microbe's level.

Among these rise in cholesterol level can lead to obese condition which further brings a number of complications like coronary heart disease, type 2 diabetes, hypertension, stroke, dyslipidemia, insulin-resistance, glucose intolerance, metabolic syndrome [30] [31]. Chitin, a major component involved in building exoskeleton of insects, fungi and marine organisms like crustaceans, shrimps etc., is a major dietary fiber similar to cellulose. The deacetylated form of chitin, chitosan is widely used as dietary supplement. A substance with natural origin and non-toxic in nature that helps in anti-obesity treatments that has been highly recommended to control obesity is chitosan [30]. The mechanism by which chitin and chitosan involved in fat excretion was studied by L-K Han et al. They suggested that decrease in intestinal fat absorption is the possible way for increased excretion of dietary fats from the body. Impairment in intestinal absorption of fats was due to decrease in pancreatic lipase activity. In order to support this statement they performed an invitro pancreatic lipase activity study [32]. The test results were positively evidencing that administration of chitin and chitosan showed significant dose dependent decrease in lipase activity of pancreatic juice. Administration of 7% -15% of chitin and chitosan to high fat-diet ICR mice, showed increased excretion of fat in feces. The same study showed that high fat-diet ICR mice, serum samples showed increased level of hyperlipidaemic condition, with increased serum cholesterol and triglycerides levels, and precipitation of fatty liver with triglycerides and cholesterol building up in liver. But this condition was managed by administering chitin and chitosan in a dose dependent manner [32].

Endocrine disruptors like (Polychlorinated biphenyls, Dioxins, Bisphenol-A, Phthalates) in environment are grouped as Persistent Lipophilic Organic Pollutants (PLOPs) [33]. Exposure to these chemicals is mainly due to accidental intake of PLOPs which contributes to 90% of total PLOPs uptake with no specific exposure [33]. Due to their lipophilic nature they are bound to dietary lipids, hence they are embodied along with dietary lipids and undergoes metabolism similar to fats/ lipids. Studies suggest that intake of dietary supplements with either soluble or insoluble fiber can result in decreased accumulation of environmental lipophilic toxins and chemicals in the body tissues and on other hand enhance the excretion of these toxins [34]. Considering these evidences, studies on chitosan, a dietary fiber mainly of marine origin, also provided positive correlation in fecal excretion of fats and environmental toxins like Bisphenol-A [35], Dioxins [36],[37],[38], Phthalates [35] and PCB [38].

6. EFFECT OF CHITOSAN INTAKE AND FECAL EXCRETION OF COMMON ENDOCRINE DISRUPTORS:

6.1 Bisphenol A:

A study was designed by Noriyuki Kohda [35] on intake of chitosan and fecal excretion of Bisphenol A (BPA) in rats. The study was employed on twenty four weeks old male Sprague Dawley rats. The rats were quarantined for 7 days, room temperature and humidity were maintained accordingly and the rats were fed with normal diet. The rats were divided into two groups: control group and test group. For 7 days, the control group was fed with 100 g/Kg body weight of BPA and the test group (Chitosan group) was fed with 100 g/Kg body weight of BPA along with 5% chitosan. The degree of deacetylation and viscosity of chitosan was determined. Body weight and diet consumption were routinely measured. On termination of the study, the rats were euthanized using ether anesthesia and the feces were collected in sanitized aluminium foil bags. The wet weight of the feces were noted and they were freeze dried for 3

days and used for further gas chromatographic analysis to determine the presence of BPA in collected feces.

The results suggested that there were no significant loss or gain in body weight in both the control group and chitosan treated group. The fecal dry and wet weight of chitosan treated group were found to be significantly higher that the control group. Also fat excretion was increased in chitosan treated group and decrease in fat breakdown in the body. They confirmed that chitosan showed effective decrease in fat metabolism. There was increased excretion of BPA in first two days, and a significant decrease in day 3 but again showed increased excretion in day 4-7. The chitosan treated group show a correlation between the BPA excretion and fat indigestibility.

6.2 Dioxins:

A study was designed by Noriyuki Kohuda [36-38] on intake of chitosan and fecal excretion of Dioxins in rats. The studied was done using eighteen 4-weeks old Sprague dawley rats for twenty days. Before the experiment the rats were quarantined for 7 days and maintained with appropriate humidity, temperature, dark and light cycle and fed with normal purified diet. After quarantine the rats were grouped two groups, three rats per group, based on its body weight. For the first 14 days control group was fed with normal diet and the test group was fed with normal diet along with 5% chitosan. Then on the 15^{th} day the rats were transferred into individual metabolic cages and the test group was orally administered with a single dose of dioxin solution containing 40ng of each congeners (1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD), 2,3,4,7,8- pentachlorodibenzofuran (PeCDF) and 3,3',4,4',5-pentachlorobiphenyl (PeCB)) along with 5% chitosan, while the control group were given only dioxin. Body weight and feed consumption was routinely measured and noted. The fecal dry and wet weights were noted on days 15-20. The wet feces collected were freeze dried for three days and dried weight was measures and further the dried feces were ground to analyse the fat content and dioxin content in the feces. The feces samples were analyzed using gas chromatography for determining the concentration of dioxin present in it. Considering the last 5 days when the dioxins were administered, they calculated the amount of dioxin excreted by multiplying fecal concentration of dioxin (ng/g) by fecal weight in 5 days (g/day).

The results suggest that there was no significant change in body weight or diet intake. Measuring the weight of the feces, the weight of feces in 5% chitosan treated group was notably high when compared to the control group. The PeCDD, PeCDF, PeCB and the total dioxins excretion was high in chitosan treated group that the control group. Considering the fact that dietary fiber chitosan increase the excretion of fats, the analyses showed increased excretion of fat in the chitosan treated group. Correlating the fecal fat excretion and lipophilic xenobiotic excretion on intake of dietary fibers, the chitosan treated group showed a positive high excretion of dioxins through feces in chitosan treated group than in control.

6.3 Phthalates:

A study was designed by Noriyuki Kohda [35] on intake of chitosan and fecal excretion of Diethyl-phthalates (DEHP) in rats. Similar to Noriyuki et al study on bisphenol-A, the study was employed on twenty four weeks old male Sprague Dawley rats. The rats were quarantined for 7 days, room temperature and humidity were maintained accordingly and the rats were fed with normal diet. The rats were divided into two groups: control group and test group. For 7 days, the control group was fed with 100 g/Kg body weight of DEHP and the test group (Chitosan group) was fed with 100 g/Kg body weight of DEHP along with 5% chitosan. The degree of deacetylation and viscosity of chitosan was determined. Body weight and diet consumption were routinely measured. On termination of the study, the rats were euthanized using ether anesthesia and the feces were collected in sanitized aluminium foil bags. The wet

weight of the feces were noted and they were freeze dried for 3 days and used for further gas chromatographic analysis to determine the presence of DEHP in collected feces.

The results suggested that there were no significant loss or gain in body weight in both the control group and chitosan treated group. The fecal dry and wet weight of chitosan treated group were found to be significantly higher that the control group. Also fat excretion was increased in chitosan treated group and decrease in fat breakdown in the body. They confirmed that chitosan showed effective decrease in fat metabolism. There was increased excretion of DEHP in first two days, and a significant decrease in day 3 but again showed increased excretion in day 4-7. The chitosan treated group show a correlation between the DEHP excretion and fat indigestibility.

6.4 Polychlorinated Biphenyls:

A study was designed by Noriyuki Kohda [38] on intake of chitosan and fecal excretion of polychlorinated biphenyls in healthy human models. The study human subjects were selected based on the blood results and medical interview conducted by a medical doctor. Thus 6 healthy Japanese men were judged to be appropriate models for the study progress. The study period was planned to be 4 weeks. The study was designed as, on the first week there was no chitosan intake, on the second week 1g of chitosan was taken 5 minutes before breakfast each day. Then on the third week following the same manner 3g of chitosan was administered to the subjects. And the fourth week there was no intake of chitosan by any of the subjects. The study was started after clear explanation of the study to the subjects and after obtaining an informed consent form. The subjects were asked to follow the given instructions before beginning the study: not to smoke, avoid intake of un-prescribed diet, refrain excess eating, drinking and exercising, notify if they encounter any kind of illness (cold, abdominal pain etc.) and not to consume alcohol. The subjects were provided with prescribed diet for breakfast, lunch and dinner for the first 6 days of the week. Then on the second week they were provided with 20g of homogenized dried fish which contained high amount of PCBs. The subjects were asked to defecate in a sterile plastic container and cover it with aluminium foil. The samples were freeze dried for 7 days and then stored in -28^0 for analysis of fat and PCB content in it. All the congeners of PCBs namely mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona- and deca – PCB. PCB content in the prescribed diet (ng/period) was calculated by multiplying total concentration of congeners (ng/g) by the meal weight in the study period (g/period). Fecal excretion and diet content of PCBs were analyzed using gas chromatography. The fecal excretion of PCBs (ng/period) was calculated by multiplying fecal concentration of each PCB congeners (ng/g) by fecal weight (g/period).

The results showed that PCB content in the prescribed diet was found to be 10,253 ng/ period (first week). And the excretion of PCB in the second and third week showed significant increase when compared to the first week. There were no significant alterations in the fecal wet weight, fecal dry weight and fecal fat concentration during the study period. A positive correlation between the fat and PCB excretion is observed, but the correlation between fat excretion and fecal dry weight showed increased PCB excretion when compared to wet weight. During the study period, adverse reactions were monitored and the only adverse event noted was headache and fatigue. But these adverse events were not due to intake of chitosan as they were reported during the first week.

7. Conclusion:

Chitosan is considered as a valuable dietary supplement, due to its unique physical, chemical and biological properties. Being ubiquitous in origin, they are highly biodegradable, biocompatible and non-poisonous in nature. Chitosan is a de-acetylated byproduct of chitin, which is found in the exoskeleton of

insects, marine organisms (crustaceans, shrimps, etc.), fungi and many more. Chitosan possess a number of biomedical and therapeutic applications. Chitosan being a dietary fiber enhances fats and lipids excretion by inhibiting the pancreatic lipase activity, essential for dietary fat breakdown. It is a valuable source in treating obese people. This evidence paved a path to experiment on the elimination of lipophilic xenobiotic entering the biological system. This intention positively proved that chitosan can aid in the excretion of harmful environmental lipophilic compounds from the body. The endocrine disruptors (Bisphenol-A, Phthalates, Dioxins, and Polychlorinated biphenyls) are persistent, highly lipophilic in nature and can bio-accumulate in tissues. Dietary lipids contaminated with endocrine disruptors undergo the same metabolic pathway as the lipids to which they are bound. But administration of chitosan showed enhanced fecal excretion of these endocrine disruptors. On conclusion, we have reviewed that intake of chitosan can decrease the accumulation of endocrine disruptors in body tissues and enhance its excretion.

Reference:

1. Johnstone C, Hendry C, Farley A, McLafferty E. Endocrine system: part 1. Nursing Standard. 2014;28(38):42-49. doi:10.7748/ns.28.38.42.e7471
2. Cohen B, Hull K, Memmler R. Study Guide For Memmler's The Human Body In Health And Disease, 12Th Edition. Philadelphia, Pa.: Lippincott Williams & Wilkins; 2013.
3. Tortora G, Derrickson B. Principles Of Anatomy And Physiology, 13Th Edition, 2-Volume Set, Internati. John Wiley & Sons; 2011.
4. Ravi Kumar M. A review of chitin and chitosan applications. React Funct Polym. 2000;46(1):1-27. doi:10.1016/s1381-5148(00)00038-9
5. Islam S, Bhuiyan M, Islam M. Chitin and Chitosan: Structure, Properties and Applications in Biomedical Engineering. J Polym Environ. 2016;25(3):854-866. doi:10.1007/s10924-016-0865-5
6. Aranaz I, Mengibar M, Harris R et al. Functional Characterization of Chitin and Chitosan. Curr Chem Biol. 2009;3(2):203-230. doi:10.2174/187231309788166415
7. Lim S, Hudson S. Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group. Carbohydr Res. 2004;339(2):313-319. doi:10.1016/j.carres.2003.10.024
8. Park P, Je J, Jung W, Ahn C, Kim S. Anticoagulant activity of heterochitosans and their oligosaccharide sulfates. Eur. Food Res. Technol. 2004;219(5):529-533. doi:10.1007/s00217-004-0977-3
9. Okamoto Y. Analgesic effects of chitin and chitosan. Carbohydr Polym. 2002;49(3):249-252. doi:10.1016/s0144-8617(01)00316-2
10. Jeon YJ, Kim Sun. Antitumor activity of chitosan oligosaccharides produced in ultrafiltration membrane reactor system. J. Microbiol. Biotechnol. 2002 12. 503-507.
11. Ueno H, Mori T, Fujinaga T. Topical formulations and wound healing applications of chitosan. Adv Drug Deliv Rev. 2001;52(2):105-115. doi:10.1016/s0169-409x(01)00189-2
12. Minagawa T, Okamura Y, Shigemasa Y, Minami S, Okamoto Y. Effects of molecular weight and deacetylation degree of chitin/chitosan on wound healing. Carbohydr Polym. 2007;67(4):640-644. doi:10.1016/j.carbpol.2006.07.007
13. Kavlock R, Daston G, DeRosa C et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. Environ Health Perspect. 1996;104(suppl 4):715-740. doi:10.1289/ehp.96104s4715
14. Diamanti-Kandarakis E, Bourguignon J, Giudice L et al. Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. Endocr Rev. 2009;30(4):293-342. doi:10.1210/er.2009-0002

15. Kabir E, Rahman M, Rahman I. A review on endocrine disruptors and their possible impacts on human health. Environ Toxicol Pharmacol. 2015;40(1):241-258. doi:10.1016/j.etap.2015.06.009
16. Dodson R, Nishioka M, Standley L, Perovich L, Brody J, Rudel R. Endocrine Disruptors and Asthma-Associated Chemicals in Consumer Products. Environ Health Perspect. 2012;120(7):935-943. doi:10.1289/ehp.1104052
17. Rajesh P, Sathish S, Srinivasan C, Selvaraj J, Balasubramanian K. Diethyl Hexyl Phthalate (DEHP) is associated with insulin resistance in adipose tissue of male rat: Protective role of antioxidant vitamins (C & E). J Cell Biochem. 2013;114(3):558-569. doi:10.1002/jcb.24399
18. Jayashree S, Indumathi D, Akilavalli N, Sathish S, Selvaraj J, Balasubramanian K. Effect of Bisphenol-A on insulin signal transduction and glucose oxidation in liver of adult male albino rat. Environ Toxicol Pharmacol. 2013;35(2):300-310. doi:10.1016/j.etap.2012.12.016
19. Indumathi D, Jayashree S, Selvaraj J et al. Effect of bisphenol-A on insulin signal transduction and glucose oxidation in skeletal muscle of adult male albino rat. Hum Exp Toxicol. 2013;32(9):960-971. doi:10.1177/0960327112470273
20. Liu J, Tan Y, Song E, Song Y. A Critical Review of Polychlorinated Biphenyls Metabolism, Metabolites, and Their Correlation with Oxidative Stress. Chem Res Toxicol. 2020;33(8):2022-2042. doi:10.1021/acs.chemrestox.0c00078
21. Hutzinger O, Nash D, Safe S et al. Polychlorinated Biphenyls: Metabolic Behavior of Pure Isomers in Pigeons, Rats, and Brook Trout. Science (1979). 1972;178(4058):312-314. doi:10.1126/science.178.4058.312
22. HUTZINGER O, JAMIESON W, SAFE S, PAULMANN L, AMMON R. Identification of metabolic dechlorination of highly chlorinated biphenyl in rabbit. Nature. 1974;252(5485):698-699. doi:10.1038/252698a0
23. Inui H, Itoh T, Yamamoto K, Ikushiro S, Sakaki T. Mammalian Cytochrome P450-Dependent Metabolism of Polychlorinated Dibenzo-p-dioxins and Coplanar Polychlorinated Biphenyls. Int J Mol Sci. 2014;15(8):14044-14057. doi:10.3390/ijms150814044
24. Michałowicz J. Bisphenol A – Sources, toxicity and biotransformation. Environ Toxicol Pharmacol. 2014;37(2):738-758. doi:10.1016/j.etap.2014.02.003
25. Vandenberg L, Hauser R, Marcus M, Olea N, Welshons W. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 2007;24(2):139-177. doi:10.1016/j.reprotox.2007.07.010
26. Hauser R. PHTHALATES AND HUMAN HEALTH. Occup Environ Med. 2005;62(11):806-818. doi:10.1136/oem.2004.017590
27. Dhingra D, Michael M, Rajput H, Patil R. Dietary fibre in foods: a review. J Food Sci Technol. 2011;49(3):255-266. doi:10.1007/s13197-011-0365-5
28. Kimura Y, Nagata Y, Buddington R. Some Dietary Fibers Increase Elimination of Orally Administered Polychlorinated Biphenyls but Not That of Retinol in Mice. J Nutr. 2004;134(1):135-142. doi:10.1093/jn/134.1.135
29. Beecher G. Phytonutrients' Role in Metabolism: Effects on Resistance to Degenerative Processes. Nutr Rev. 2009;57(9):3-6. doi:10.1111/j.1753-4887.1999.tb01800.x
30. Regina M, e Silva A, Vieira F. The Chitosan as Dietary Fiber: An in vitro Comparative Study of Interactions with Drug and Nutritional Substances. 2021 .Retrieved 30 November 2021, from.
31. Aslander-van Vliet E, Smart C, Waldron S. Nutritional management in childhood and adolescent diabetes. Pediatr Diabetes. 2007;8(5):323-339. doi:10.1111/j.1399-5448.2007.00317.x
32. Han L, Kimura Y, Okuda H. Reduction in fat storage during chitin-chitosan treatment in mice fed a high-fat diet. Int J Obes. 1999;23(2):174-179. doi:10.1038/sj.ijo.0800806
33. Andreas Moser G, McLachlan M. The influence of dietary concentration on the absorption and excretion of persistent lipophilic organic pollutants in the human intestinal tract. Chemosphere. 2001;45(2):201-211. doi:10.1016/s0045-6535(00)00551-8

34. Kimura Y, Nagata Y, Bryant C, Buddington R. Nondigestible Oligosaccharides Do Not Increase Accumulation of Lipid Soluble Environmental Contaminants by Mice. J Nutr. 2002;132(1):80-87. doi:10.1093/jn/132.1.80
35. KOHDA N, INOUE S, NODA T, SAITO T. Effects of Chitosan Intake on Fecal Excretion of Bisphenol A and Di(2-ethyl)phthalate in Rats. Biosci Biotechnol Biochem. 2012;76(4):732-736. doi:10.1271/bbb.110856
36. KOHDA N, INOUE S, NODA T, SAITO T. Effects of a Chitosan Intake on the Fecal Excretion of Dioxins and Fat in Rats. Biosci Biotechnol Biochem. 2012;76(8):1544-1548. doi:10.1271/bbb.120300
37. Kitamura K, Nagao M, Hayatsu H, Morita M. Effect of Chlorophyllin-Chitosan on Excretion of Dioxins in a Healthy Man. Environ Sci Technol. 2005;39(4):1084-1091. doi:10.1021/es048577u
38. KOHDA N, INOUE S, NODA T, SAITO T. Effect of Chitosan Intake on Fecal Excretion of Dioxins and Polychlorinated Biphenyls in Healthy Men. Biosci Biotechnol Biochem. 2012;76(6):1195-1200. doi:10.1271/bbb.120067