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Inulin Improves Hepatosteatosis in Humans

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ABSTRACT

Fatty liver is a common clinical finding; it is widely spread in an epidemic form all over the globe. Its finding ushers the presence of other grave abnormalities including; obesity, type 2 diabetes mellitus, cardiovascular diseases beside the ongoing pathology of the liver itself. Hypertriglyceridemia usually accompanies the aforementioned abnormalities. Inulin type prebiotic escapes digestion in the upper part of the gut and reaches the colon, where it enhances the beneficial strains of the microbiota which suppress the pathogenic strains that encourage lipogenesis. Our aim is to study the effect of inulin fructans on patients with hepatic steatosis. Four grams of inulin fructans were given daily for each of the twenty eight obese type 2 diabetic female patients of this study for a period of three weeks. Parameters estimated: Liver Fat Index (LFI), fasting serum insulin level and fasting serum liver transferases (GGT, AST & ALT). Both liver fat index and fasting serum insulin level showed a significant decrease after inulin intake. Again, GGT showed marked numerical drop and AST dropped significantly after inulin ingestion while ALT showed no significant change. In conclusion, inulin fructans seems to be a valuable add on therapy for hepatic steatosis treatment.

Keywords: Inulin, Hepatosteatosis, Type 2 diabetes, Obesity, Liver fat index, Fasting insulin, Liver enzymes

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INTRODUCTION

Non-alcoholic Fatty Liver Disease (NAFLD) is a common clinical finding. The term NAFLD covers a spectrum of liver diseases ranging from simple fatty infiltration of the liver cells to non-alcoholic steatohepatitis (NASH) [1]. It is a disease of all ages and it has been reported in children as young as two years of age; but it is more common in adults and its prevalence increases with age. It also has been described in pregnant females [2]. According to recent studies from different countries, the prevalence of the disease has nearly doubled in the last twenty years [3-6]. This increase was more dramatic in adolescent population. The incidence reached 174% [7]. NAFLD usually accompanies certain metabolic health problems, like obesity, type 2 diabetes mellitus and the metabolic syndrome. The prevalence of these problems is tremendously rising to epidemic figures. NAFLD likewise reached these Figures [8].

These individuals usually have bulky adipose tissue rich in triglycerides and Free Fatty Acids (FFA). Besides, the faulty dietary habits of ingesting much carbohydrates and fatty food add to the problem through *de novo* synthesis of more fatty acids and triglycerides [8].

Human Adipose Tissue (AT) is not an inert one; it is in a continuous state of turnover. The ample turnover products of this bulky adipose tissue reach the liver and engorge the hepatic cells with plenty of FFAs and triglycerides [9,10].

These disordered metabolic steps lead to other metabolic disorders, like oxidative stress and systemic inflammation [11,12]. All proceed insidiously, paving their way to the clinical complications which characterize obesity, type 2 diabetes mellitus and the

metabolic syndrome. These complications usually insult the liver itself, the cardiovascular system and aggravate the diabetic state [13,14].

Observational and interventional data from animal [15,16] and human studies [17,18] could prove the existence of different strains of microorganisms in the large gut which might exert a change in the body fat stores. Some strains of this microbiota produce harmful products which drive the metabolic pathways towards increasing lipogenesis and triglycerides formation. These result in increased adiposity and accumulation of fat in the different adipose tissue compartments of the body, together with hepatic steatosis [19-21]. On the other hand, when the beneficial strains of the microbiota flourish over the harmful ones, they produce an opposite effect. Their peptide products negatively affect lipogenesis, triglycerides production, adiposity and fat accumulation in the different adipose tissue compartments and the liver [22-25].

Inulin fructans is an edible dietary fiber. It is a non digestible oligosaccharide which reaches the colon intact. There it can be consumed by certain beneficial microbiotas, which flourish on the expense of other harmful strains. These beneficial strains can promote the endogenous release of certain gut hormones for the benefit of the host [26]; and can negatively affect hepatic fat content [27-30].

The aim of the present work is to study the effect of inulin fructans in the treatment of hepatic fatty infiltration in obese type 2 diabetic females.

MATERIALS AND METHODS

Subjects

Overweight or obese type 2 diabetic females are the subject of this study. These were selected from the diabetes clinics of the governmental hospitals. These twenty eight patients aged 40-65 years were selected according to the following criteria.

Inclusion criteria

Type 2 diabetic females, middle aged or above, overweight or obese, hypertensives or not.

Exclusion criteria

They were free from Metabolic or endocrine disorders, not under hormonal treatment or contraceptive pills, any local or systemic infection (skin, chest, urinary or intestinal), malignancy anywhere, organ failure (heart, lung, kidney or liver).

Ethical committee approval

This work has been approved by the Egyptian National Research (NRC) ethical committee. Certificate number 15011.

Consent

All patients signed their consent to be enrolled in this study.

METHODS

Each patient was given four grams of inulin daily, two grams in the morning and two grams in the evening for twenty one days.

Inulin type prebiotic was given to the patients as an add on therapy to their conventional antidiabetic treatment.

Inulin specifications

Inulin A.R. ($C_6H_{10}O_5$); N ALPHA-CHEMIKA Mumbai, 400002 (India) An ISO: 9001: 2000 Certified companies.

All patients were subjected for the following investigations before and after the period of inulin intake.

(I) Anthropometric measurements

1. Body Mass Index (BMI) weight in kilograms over height in meters squared (weight in Kg/height in m^2).
2. Waist circumference measurement to the region between the lower ribs and the iliac crest to the level of the umbilicus [31,32].

(II) Laboratory measurements

1. Fasting serum insulin level: this was quantitatively estimated using an enzyme immunoassay method according to National Committee for Clinical Laboratory Standards [33].
   Manufacturer of the kit used: Immunospect corporation 7018 Owensmouth Ave. Suit 103 Canoga Park, CA, 91303.
2. Fasting serum triglycerides: This was estimated spectrophotometrically after Fossati [34] kit used from Centronic Germany.
3. Fasting serum liver enzymes including: (a) Serum Gamma Glutanyl Transferase (GGT) was estimated quantitatively using spectrophotometerical method according to Gendler [35]. Kit used reactive GPL, Barcelona, Spain Industria 113, Nau J 08420 Canovelles-Barcelona.

b) Serum Alanine Aminotransferase (ALT) was estimated quantitatively using colorimetric method after Reitman et al. [36]. Kit used from Randox Laboratories Limited 55 Diamond Road, Crumlin, Country Antrim BT29 4QY UK.

c) Serum aspartate aminotransferase (AST) was estimated quantitatively using colorimetric method after Reitman et al. [36]. Kit used from Randox Laboratories Limited 55 Diamond Road, Crumlin, Country Antrim BT29 4QY, UK.

(III) Estimation of Liver Fat Index (LFI)

This was estimated using the equation of Bedoigni et al. [31]. The equation used four parameters: Body Mass Index (BMI), Waist Circumference (WC), serum gamma glutamyl transferase (GGT) and serum triglycerides.

STATISTICAL METHODS

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013.

Descriptive statistics were done for quantitative data as minimum& maximum of the range as well as mean ± SD (standard deviation) for quantitative parametric data. Inferential analyses were done for quantitative variables using paired t-test in cases of two dependent groups with parametric data. The level of significance was taken at P value <0.05 is significant, otherwise is non-significant.

RESULTS

Table 1 shows the results of the Liver Fat Index (LFI) and its four components: Body Mass Index (BMI), Waist Circumference (WC), fasting serum triglycerides and fasting serum Gamma Glutamyl Transferase (GGT) enzyme level; for the 28 type 2 diabetic females examined.

There was a significant decrease in the liver fat index and the fasting serum triglycerides level, together with an obvious numerical decrease in GGT while the change in BMI and WC were not appreciable.

The mean ± SD together with their P values obtained for the aforementioned parameters before and after inulin intake by the patients are recorded respectively as follow:

Liver fat index (LFI): 22.8 ± 18.2 and 21.5 ± 17.9 and P<0.001;

Body mass index (BMI): 34.8 ± 5.5 and 34.5 ± 5.4 and P=0.886;

Waist circumstance: 120.1 ± 11.3 and 120.0 ± 11.4 and P=0.811;

Fasting serum triglycerides**:** 245.6 ± 105.7 and 201.4 ± 95.5 and P<0.001;

Fasting serum gamma glutamyl transferase enzyme: 16.9 ± 7.5 and 16.3 ± 8.5 and P=0.257.

Table 1: Liver fat index and its parameters

	Before		After		Change^		#P
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	**Median (IQR)**	**Range**	
Liver Fat Index (LFI)	22.8 ± 18.2	0.6–80.3	21.5 ± 17.9	0.5–79.4	-1.4 (-1.6–0.7)	-3.5–0.5	**<0.001***
Body Mass Index:BMI (kg/m^2)	34.8 ± 5.5	25.4–47.9	34.5 ± 5.4	25.4–47.9	0.0 (-0.5–1.3)	-0.7–1.5	0.886
Waist Circumference:WC (cm)	120.1 ± 11.3	81.0–145.0	120.0 ± 11.4	81.0–145.0	0.0 (-0.9–1.1)	-0.9–1.8	0.811
Triglycerides (mg/dL)	245.6 ± 105.7	91.0–434.0	201.4 ± 95.5	80.0–392.0	-41.5 (-58.5–28.5)	-115.0–2.0	**<0.001***
Gamma glutamyl transferase:GGT (IU/L)	16.9 ± 7.5	5.3–36.9	16.3 ± 8.5	6.5–38.1	0.0 (-1.1–1.1)	-7.2–4.3	0.257

N=28, ^Negative values indicate reduction, #P-value of paired t-test, *Significant

The succeeding figures illustrate the degree of changes in the mean fat liver index (LFI) and its four components (BMI, WC, fasting serum triglycerides and fasting serum GGT enzyme levels) before and after the period of inulin ingestion by the patients.

Table 2 shows the mean ± SD values of the following other indices for hepatosteatosis before and after inulin intake in the 28 patients enrolled in the study together with their P values.

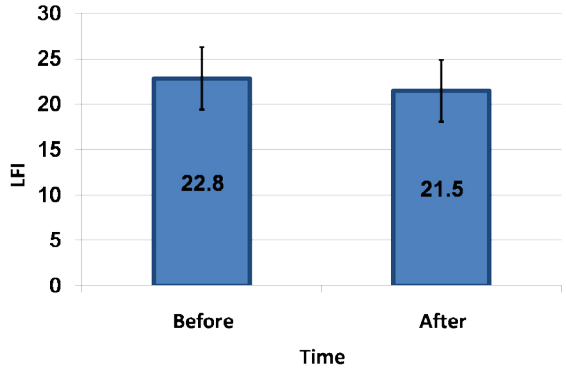

Figure 1: Mean liver fat index (LFI) before and after inulin intake period. Figure shows a significant reduction in liver fat index after inulin intake from 22.8 ± 18.2 to 21.5 ± 17.9

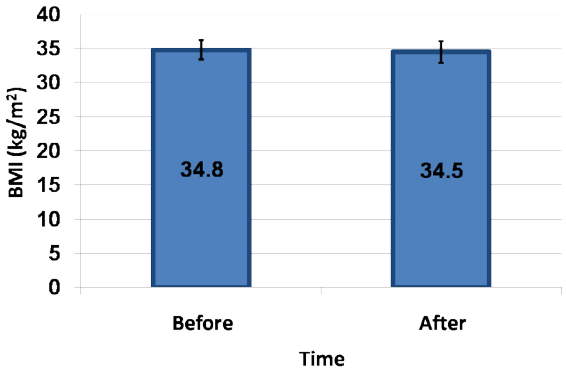

Figure 2: Mean body mass index (BMI) before and after inulin intake. The decrease in body mass index was not significant after inulin intake 34.8 ± 5.5 to 34.5 ± 5.4

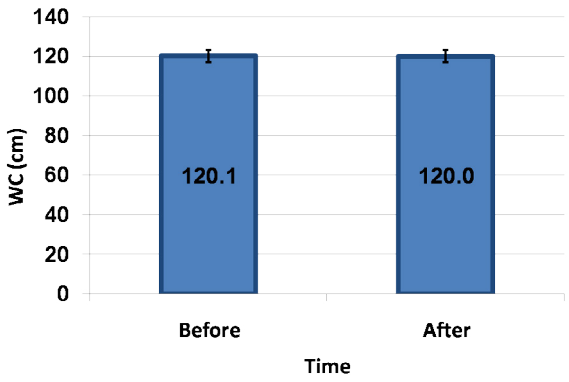

Figure 3: Mean waist circumference (WC) in centimeters before and after inulin intake. The decrease in waist circumference was not significant after inulin intake 120.1 ± 11.3 to 120.0 ± 11.4

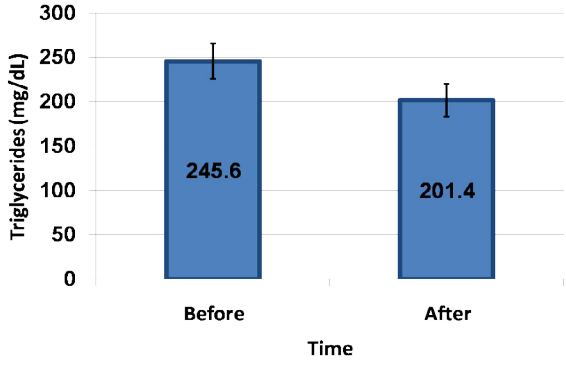

Figure 4: Mean fasting serum triglycerides (TG) in mg/dl before and after inulin intak. Figure shows a significant reduction of triglycerides after inulin intake period from 245.6 ± 105.7 to 201.4 ± 95.5

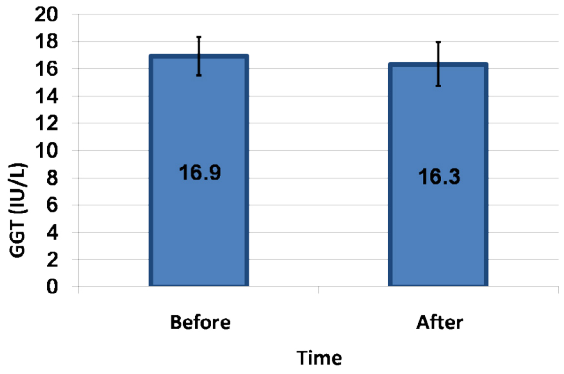

Figure 5: Mean fasting serum gamma glutamyl transferase (GGT) level in IU/L before and after inulin intake. There was a non significant decrease in GGT after inulin intake, it decreased from 16.9 ± 7.5 to 16.3 ± 8.5

Fasting serum insulin in mIU/L**:** 15.3 ± 8.9 and 12.3 ± 7.6 and P<0.001;

Fasting serum aspartate aminotransferase (AST) enzyme level in IU/L**:** 18.1 ± 4.9 and 16.5 ± 5.7 and P=0.04.

Fasting serum alanine aminotransferase (ALT) enzyme level in IU/L**:** 8.1 ± 2.5 and 8.3 ± 3.1 and P=0.684.

Table 2: Other indices for hepatic steatosis

	Before		After		^Change		#P
	Mean ± SD	Range	Mean ± SD	Range	Median (IQR)	Range	
Insulin (mIU/L)	15.3 ± 8.9	4.0-29.4	12.3 ± 7.6	2.9–26.1	-2.8 (-5.2-1.4)	-9.9–5.0	**<0.001***
Aspartate aminotransferase:AST (IU/L)	18.1 ± 4.9	10.0-26.0	16.5 ± 5.7	7.0–23.0	-2.0 (-4.0–1.0)	-10.0–6.0	**0.044***
Alanine aminotransferase:ALT (IU/L)	8.1 ± 2.5	3.0–12.0	8.3 ± 3.1	3.0–14.0	0.0 (-2.0–3.0)	-3.0–3.0	0.684

N=28, ^Negative values indicate reduction, #P-value of paired t-test, *Significant

The following three Figures 6-8 illustrate the changes in the mean parameters of the other indices used to evaluate hepatosteatosis after inulin ingestion period.

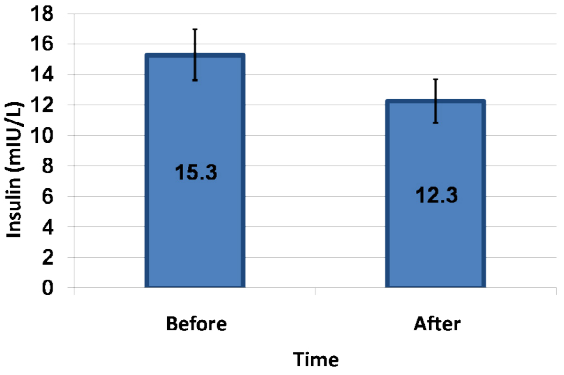

Figure 6: Mean fasting serum insulin level in mIU/L before and after inulin intake. Fasting insulin level decreased significantly after inulin intake from 15.3 ± 8.9 to 12.3 ± 7.6

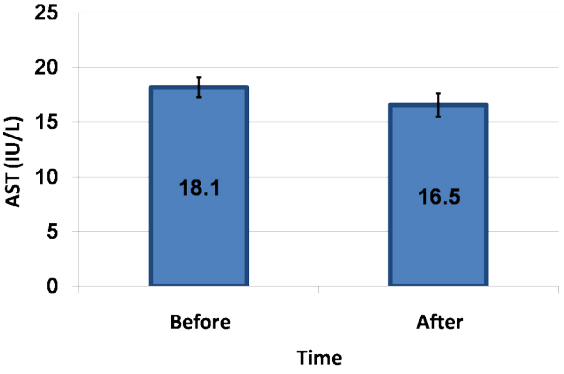

Figure 7: Mean fasting serum aspartate aminotransferase (AST) enzyme level in IU/L before and after inulin intake.Serum level of AST decreased significantly after inulin intake from 18.1 ± 4.9 to 16.5 ± 5.7

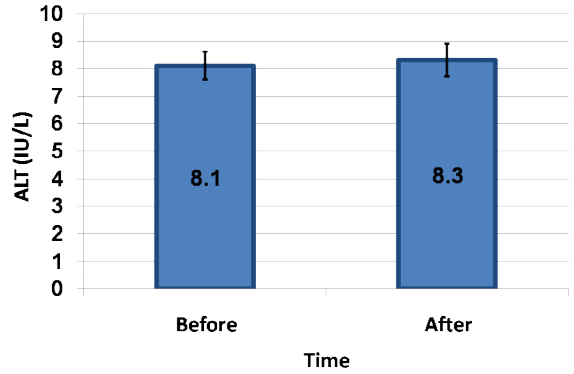

Figure 8: Mean fasting serum level of alanine aminotransferase (ALT) enzyme level in IU/L before and after inulin intake.There was a non significant change in serum level of ALT after inulin intake from 8.1 ± 2.5 and 8.3 ± 2.1

DISCUSSION

Non-alcoholic Fatty Liver Disease (NAFLD) is not just a simple process of fat accumulation in the liver cells; it is a more serious and concernable pathological health problem. It heralds three dreadful clinical entities.

The first: belongs to the liver itself; NAFLD encompasses a wide range of hepatic fatty infiltration extending from simple hepatic steatosis to an inflammatory condition termed non-alcoholic steatohepatitis (NASh), up to hepatic steatofibrosis or cirrhosis [1]. NAFLD is a common disease and represents 75% of chronic liver diseases. It is also one of the most common indications for liver transplantation in the United States and worldwide [3].

The Second: the presence of intrahepatic fat means the presence of other metabolic disorders; and contrary to previous belief, simple hepatic steatosis is not a benign condition. All grades of fatty liver are often accompanied by different degrees of each of the following; hypertriglyceridemia, insulin resistance; with their concomitant varying grades of blood sugar abnormalities. The latter, varying from impaired glucose metabolism (impaired fasting glucose and impaired glucose tolerance) up to frank type 2 diabetes mellitus [1].

Thirdly: cardiovascular diseases are commonly seen in liver disease patients including NAFLD; and contribute significantly in the morbidity and mortality of liver disease patients [37-40]. As in the general population the leading cause of death in NAFLD patients is cardiovascular disease but the risk hazard is nine folds the general population [41].

In view of the aforementioned facts, it is plausible to treat fatty liver rigorously to avoid hepatic pathological evolution leading to cirrhosis; and to improve hepatic metabolic performance [42], and to decrease the morbidity and mortality of its consequent cardiovascular complications.

At present, treatment of hepatic steatosis is not a simple task. All available pharmaceutical preparations stop short from achieving complete cure [43].

Inulin is a natural edible and safe compound which is known to have a prebiotic effect. It is a non digestible, fermentable oligosaccharide. It escapes digestion in the upper gastrointestinal tract and reaches the colon where it is consumed by certain microbes there. These latter flourish and exert a beneficial effect on the gut endocrine epithelial cells which secrete hormone like peptides. These regulate important biological functions for the benefit of the host [44-45].

In few experimental studies on mice and rats, inulin-fructose, showed improvement of hepatic steatosis [46].

Such studies are not recorded in humans so far. The present work studies the effect of inulin-fructose intake on human hepatic steatosis in type 2 diabetic female patients.

In order to assess the effect of inulin intake on fatty liver; we have to estimate the amount of liver fat before and after inulin ingestion.

Liver biopsy is a precise method for quantitative determination of liver fat, but the technique is invasive, unacceptable by the patients and is also unethical [47]. Magnetic resonance spectroscopy is also a precise method but is expensive and is not available at hand for the present study [48]. Computed Tomography (CT), although accurate and non-invasive, it is expensive and exposes the patient to much irradiation [49,50]. Again, contrast-enhanced ultrasonography is a recently introduced modality of investigastion. Although, it equals CT in its accuracy, yet it is not commercially available and needs further clinical experience [51]. Conventional ultrasonography on the other hand does not serve accurate quantitative determination of liver fat especially in mild cases of hepatosteatosis and in obese patients with fatty liver [52].

In the present work, we resorted to three sets of parameters for the evaluation of the effect of inulin intake on hepatic fat accumulation; First: liver fat index (LFI), Second: fasting serum insulin level and third: fasting serum level of liver transferase enzymes alanine aminotransferase enzyme (ALT) and aspartate aminotransferase enzyme (AST).

Liver fat index is a simple method for hepatic fat estimation after Bedogni et al. [31]. Those workers chosed four independent variables to formulate their equation. These four variables are: Body Mass Index (BMI), waist circumference, serum triglycerides

level and serum gamma glutamyl transferase enzyme level. Applying Bedogni's formula of liver fat index before and after inulin treatment in our study, there was a significant decrease of the Liver Fat Index (LFI) after inulin ingestion compared with its level before inulin intake.

Concerning the four variables used in the equation of liver fat index and owing to the short period of inulin intake, we could not record an appreciable decline in both the Body Mass Index (BMI) and the waist circumference after inulin intake; meanwhile fasting serum Gamma Glutamyl Transferase enzyme (GGT) level decreased, albeit insignificantly, the decrease was numerically appreciable. Triglycerides, on the other hand, recorded a significant drop after inulin intake. However, both serum GGT and triglycerides are independent predictors for liver fat evaluation. In fact triglycerides are independent predictors of liver fat content as proved in all the models studied in Bedogni's work [31] and this had been confirmed by other workers [53,54].

Apart from Liver Fat Index (LFI), and in the opinion of several workers, fasting serum insulin is an independent factor in prediction of fatty liver in the general population [31,55-57]. Bedogni et al. also approved this opinion and added that, in the general population serum insulin was proved to be an independent risk factor in detection of fatty liver [31]; And it ranks second to body mass index (BMI) in its importance and strength in their model one. They also noticed that, waist circumference, despite of being the strongest independent factor in the final model of their work yet, it did not show an additional predictive value of hepatic steatosis beside insulin when this latter was in their model. They stated that they could not explain this finding [31].

In our work, besides LFI, fasting insulin is another important indicator for the effect of inulin on fatty liver. Fasting insulin recorded a significant decrease after inulin intake. Inulin decreased serum triglycerides together with intrahepatic fat as indicated by the significant drop in liver fat index. The decrease in hepatic fat resulted in improved insulin sensitivity and hence decreased fasting serum insulin level. The scenario between serum triglycerides, intrahepatic fat, insulin sensitivity, insulin resistance and fasting serum insulin level was discussed in a previous study by the authors of this work [58].

Again, we studied the amino transferase liver enzymes Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) and we should refer to their importance in evaluation of the issue of hepatic steatosis.

Bedogni et al., consider GGT as an independent detector of liver fat while ALT is not a surrogate predictor of hepatic steatosis and AST is not an indicator of fatty liver in the models they studied [59].

On the other hand, several workers from different countries reported that, despite the variable degree of severity of the different individuals of the spectrum of hepatic steatosis; elevated hepatic transferase enzymes (ALT & GGT) even in the normal range may precede or usher the onset or accompany type 2 diabetes mellitus. This happens even in the absence of obesity, metabolic syndrome or c-reactive protein elevation. Thus, they postulated that elevated intrahepatic fat (1HF) content played a central role in liver damage and glucose intolerance [60-62].

The results of our present study recorded decreased readings in two transferase enzymes (GGT & AST); the latter recorded a significant decrease after inulin intake.

CONCLUSION

Inulin seems to be a valuable compound which draws the attention for the possibility to be of appreciable benefit in the treatment of both hepatic steatosis and the accompanying metabolic disorders. Besides, being an edible (dietary) fiber, it can be safely given to children and pregnant women with hepatosteatosis.

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