Abstract

Objective: The objective of this study was to investigate the effect of doses of Chitocal for 4 weeks on body weight, lipid profiles, kidney function and histological structure of kidney and intestine in male rats.

Methods: Twenty adult male albino rats (200 - 215 gm) were divided into 2 groups: The first group was considered as control group. The second group was treated orally with Chitocal (50 mg/kg b.w) by use of intragastric tube. Different physiological parameters were performed including recording of the body weight and measuring lipid profiles, creatinine and urea levels.

Results: Body weight gain, total cholesterol, triglyceride, LDL cholesterol, and VLDL cholesterol levels were significantly (p< 0.05) reduced in Chitocal treated rats when compared with the control rats. HDL cholesterol and urea levels were significantly (p< 0.05) increased in Chitocal treated rats, but creatinine level was none significantly (P< 0.05) increased when compared with the control rats. Histological examination of Chitocal treated rats kidney’s showed congestion of blood vessels between renal tubules, aggregation of inflammatory cells in lumen and wall of blood vessels, enlargement of renal tubules and aggregation of inflammatory cells around glomerulus. In intestine tissue of Chitocal treated rats moderate increase of monocytes in lamina propria and hyperplasia of goblet cells was observed, aggregation of inflammatory cells in epithelium, adhesion of intestinal villi, hyperplasia in epithelium and aggregation of inflammatory cells in lamina propria.

Conclusion: From the results of this study it can be conclude that the treatment with Chitocal produced a significant reduction in body weight and lipid profiles, but it is incapable of improving the kidney functions. Also there are histopathological effects on kidney and intestine tissues in treated rats.

Effect of Weight Reduction Drug Chitocal Contained Gymnema sylvestr Extract on Body Weight, Lipid Profiles, Kidney Function and Histological Structure of Kidney and Intestine in Male Albino Rats

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Introduction

Obesity refers to an abnormally high proportion of total body fat. Obesity increases the risk of type 2 diabetes, cardiovascular disease, cancer, and premature death. The excessive storage that creates obesity eventually leads to the release of excessive fatty acids from enhanced lipolysis, which is stimulated by the enhanced sympathetic state existing in obesity [1]. The release of these excessive free fatty acids then incites lipotoxicity, as lipids and their metabolites create oxidative stress to the endoplasmic reticulum and mitochondria. This affects adipose as well as non adipose tissue [2]. Pharmacological factor involved in obesity and diabetes includes lipoprotein lipase, having a central role in the metabolism of both triglyceride-rich particles and high density lipoproteins (HDL) [3].

Approximately 80% of the world’s population currently uses herbal medicines in healing different ailments [4]. Chitocal is a highly effective weight loss formula which acts as carbohydrate blaster and fat absorption blocker. Chitocal contains high density chitosan which is a natural polysaccharide comprising copolymers of glucosamine and N-acetyl glucosamine, and can be obtained by the partial deacetylation of chitin, from crustacean shells, the second most abundant natural polymer after cellulose [5].

Chitosan is an extraordinary fat binder. Chemically speaking, chitosan is an amino polysaccharide that has the ability to “bind” lipids in the stomach before they are absorbed through the digestive system into the blood stream. Not only does chitosan attract and inhibit fats, it offers an array of other desirable physiological benefits that can foster optimal health and longevity such as hypcholesterolemic, antimicrobial, and wound healing properties. Of equal significance is the fact that when chitosan is combined with other compounds such as citric acid, ascorbic acid and phytochemicals called indoles, its action is enhanced, making it far more valuable as both a fat binder and dietary health aid. [6]

Chitocal contains Gymnema sylvestre extract. Gymnema sylvestre R. belongs to the family Asclepiadaceae. It is a woody climber found in western India. The active compound of this plant is a group of acids termed as gymnemic acids. Gymnemic acid molecules has the same arrangement of glucose molecules and has large molecular weight make it has the affinity on the glucose receptor and make competitive inhibition with glucose on the receptor in small intestine, Gymnema sylvestre is well recognized in traditional medicine as a remedy for diabetes mellitus and used in folk, ayurvedic and homeopathic systems of medicine, Gymnema leaves were also used for stomach ailments, constipation, water retention, and liver disease [7]. It brings blood glucose homeostasis through increased serum insulin levels provided by repair or regeneration of the endocrine pancreas [8].The G. sylvestre leaf and callus extracts reduced blood sugar and lipid profiles such as cholesterol, triglyceride, HDL, LDL, VLDL in alloxan-induced diabetic Wistar rats [9].

G. sylvestre leaf extract when orally administered to experimentally induced hyperlipidaemic rats, reduced the elevated serum triglyceride (TG), total cholesterol (TC), very low-density lipoprotein (VLDL)-and low-density lipoprotein (LDL)-cholesterol in a dose-dependent manner. Gymnema sylvestre
R. was investigated in normal and obese streptozotocin induced diabetic rats and was found to produced significant reduction in glucose level, lipid profile and body weight [10]. The aim of the present study was to investigate the effects of daily oral consumption of chitocal for 4 weeks on body weight, kidney function, lipid profiles and histological structures of kidney and intestine in rats to show the preventive and side effects of Chitocal.

Materials and Methods

Animals and experimental design

Twenty adult 12 weeks old, male albino rats (Rattus norvegicus), weighing 200-215 gm were obtained from animal’s house of the College of Science, University of Baghdad, Iraq. They were housed in standard plastic cages. The animals were kept in a well ventilated room, temperature of 24- 28°C with 12 hrs natural light and 12 hrs darkness. The rats had free access to tap water and dry rat pellets obtained from local market ad libitum. The rats were allowed to acclimatize for ten days and then divided into 2 groups; (10 per group) as follows: control group treated with normal saline, and Chitocal treated group which received 50mg/kg b.w. Chitocal intragastrically for 4 weeks. (Chitocal produced by the Arab Co. for Gelatin and Pharma. for Al-Debeiky Pharma.Egypt: (Compositions: Citosan H.D. 500mg, Ascorbic Acid 100mg, and Gymnema sylvestre Extract 50mg ).

Collection of blood samples and biochemical analysis

At the end of the experiment, blood samples were taken by cardiac puncture and blood was collected in clean EDTA tubes, then plasma was separated by centrifugation (3000 rpm for 15 min.) and stored at -20°C. Triglycerides level was estimated by use of Randox kit according to [9]. Cholesterol level was estimated by Randox kit according to Allain et al. 1974 [11]. The HDL Cholesterol (HDL-C) was estimated by BioMaghreb kit according to Demacherp 1980 [12]. VLDL composition and LDL cholesterol can be calculated with reasonable accuracy by the Friedewald formula [13]. Kits of creatinine, and urea were purchased from Spinreact, S.A. Ctra. Spain. Creatinine was determined by kinetic method described by [14], determination of urea was according to the enzymatic method of [15].

Histological examination

Animals were killed and small piece of kidney and intestine tissues taken from experimental animals were fixed in 10% neutral formalin, alcohol-dehydrated, paraffin-embedded and the section to mean thickness of 4 μm. The histological examination was evaluated by assessing the morphological changes with Hematoxylin and Eosin (H&E) stains [16].

Statistical analysis

Data are expressed as the mean ± SE. The statistical significance was carried out using one-way analysis of variance test followed by Duncan’s Multiple Range Test (SPSS statistical software package) [17]. A possibility of P value (p< 0.05) was considered as significant differences between means.

Results and Discussion

Changes in body weight

From table 1, it is shown that, there was a significant (p< 0.05) decrease in the mean body weight of the Chitocal treated rats when compared with normal control groups. The results revealed that Chitocal decreased body weight gain and had an anti-obesity potential. These findings are in agreement with other investigators [9]. Studies reveal the ability of Gymnema sylvestre to support enzyme activity for glucose utilization and to moderate glucose uptake into the intestines [9]. Gymnemic acid isolated from the leaves of Gymnema sylvestre has been reported to inhibit the intestinal absorption of
glucose and oleic acid and reported to exert beneficial effect in diabetes and obesity [18]. Gymnemate promoted weight loss in fatty rat model which exhibits progressive overweight, hyperlipidemia and hyperglycemia. [19]. The weight reducing effect may be due to inhibition of lipogenesis in rats [20]. The possible mechanism of action may be due to the hydrolysis of triglycerides and activation of insulin by the enzyme lipoprotein lipase [21]. Gymnema helps to promote weight control by its ability to reduce the cravings for sweets and control blood sugar levels [22]. A peptide isolated from Gymnema, gurmarin, has also been shown to block the sweet taste of glucose and sucrose in animal models [22]. Gurmarin temporarily binds to the sweet and bitter receptors on the tongue, thereby blocking the taste sensation and reducing sweet cravings [22]. Gurmarin, another constituent of the leaves, and gymnemic acid have been shown to block the ability to taste sweets in humans [23].

**Lipid profile:**

Table 1, shows the effect of Chitocal on the levels of plasma lipid profile. Chitocal produced a significant (p< 0.05) decrease in plasma cholesterol, triglycerides, VLDL and LDL-cholesterol levels compared with normal rats, but plasma HDL-cholesterol level slightly increase when compared with normal rats. These findings are in agreement with other studies. Preuss et al. [24] showed a significant lowering of cholesterol with Gymnema sylvestre ingestion in hypertensive rats fed a high sucrose diet, whereas the placebo group showed a significant increase in cholesterol levels. The total cholesterol was decreased, moreover LDL+VLDL (low-density and very-low-density lipoprotein) cholesterol decreased, the proportion of HDL (high-density lipoprotein) cholesterol to the total cholesterol was increased and the serum triglyceride was decreased in fatty rat exhibits hyperlipidemia after treated with Gymnema sylvestre [19]. The HDL-cholesterol is involved in transport of cholesterol from peripheral tissue to liver and thereby acts as protective factors. This indicates that Gymnema sylvestre may help to increase transport of peripheral tissue cholesterol to liver and thereby decrease blood level cholesterol. The possible mechanism of action of Gymnema sylvestre for increased HDL may be due to increase in lecithin activity [25]. The possible mechanism of action in reduction of LDL-cholesterol may be oxidation of LDL by enzymes. Gymnema sylvestre is reported to produce hypolipidemic effect by inhibition of intestinal absorption of fatty acid in rats [18].

According to animal studies, the leaves are also noted for lowering serum cholesterol and triglycerides [26]. While studies have shown that a water-soluble acidic fraction of the leaves provides hypolipidemic actions, the specific constituent in the leaves responsible for this action has not been clearly identified. Some researchers have suggested gymnemic acid as one possible candidate [27]. Antihyperlipidemic activity which may be due to presence of flavonoids, phenol, tannis (phenolic compounds) and tritterpenoids found in the preliminary phytochemical screening [28]. The possible mechanism for decreased lipid levels could be either insulin releasing effect of Gymnema sylvestre active compounds or insulin sensitizing activity, because insulin has been proved to inhibit the activity of the hormone sensitive lipase in adipose tissue and suppresses the release of lipids [21].

Chitosan able to chelate many substances like lipid and uric acid), the mechanism of conjugation mediated by high positive charge in the surface of chitosan molecule. The activity of chelation depends on the highly reactive amino group which is able to chelate lipids and many transitional ions. [29]
There are several proposed mechanisms for cholesterol reduction by chitosan. The latest findings in this field consider more than one hypothesis. The entrapment caused by a viscous polysaccharide solution is thought to reduce the absorption of fat and cholesterol in the diet. On the other hand, the presence of the amino group in its structure determines the electrostatic force between chitosan and anion substances, such as fatty acids and bile acids [30].

**Creatinine and urea:**
Chitocal produced a significant (p<0.05) increase in plasma levels of urea and none significant (P > 0.05) increase in plasma levels of creatinine, when compared with normal control groups as shown in Table 1. No study available reported the effect of Chitocal on kidney function parameters, but the result of histological examination in this study may support these results because they showed negative effects of Chitocal on kidney tissue, and this was affect kidney function. The treatment with *Gymnema sylvestre* extract found to decrease serum creatinine and urea levels, and this may be correlated with decrease in glucose level by *Gymnema sylvestre* [21], and the increases in these levels in our finding may be due to the other composition of Chitocal.

**Table 1** Body weight and levels of plasma lipid, creatinine, and urea in rats treated with 50 mg/kg b.w. Chitocal.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control group (Mean ±S.E)</th>
<th>Chitocal treated group (treated with Chitocal 50 mg/kg b.w.) (Mean ±S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight. g</td>
<td>205.8 ± 6.57</td>
<td>204.4 ± 8.26</td>
</tr>
<tr>
<td>Final body weight. g</td>
<td>274 ± 7.77</td>
<td>226 ± 6.55*</td>
</tr>
<tr>
<td>Cholesterol mg /dl</td>
<td>91 ± 2.64</td>
<td>75.8 ± 0.97*</td>
</tr>
<tr>
<td>Triglyceride mg /dl</td>
<td>78 ± 7.51</td>
<td>58 ± 2.54*</td>
</tr>
<tr>
<td>HDL cholesterol mg /dl</td>
<td>41.62 ± 1.33</td>
<td>48.02 ± 1.37*</td>
</tr>
<tr>
<td>LDL cholesterol mg /dl</td>
<td>33.92 ± 1.53</td>
<td>16.18 ± 2.44*</td>
</tr>
<tr>
<td>VLDL cholesterol mg /dl</td>
<td>15.6 ± 1.50</td>
<td>11.6 ± 0.50*</td>
</tr>
<tr>
<td>Creatinine mg /dl</td>
<td>0.476 ± 0.076</td>
<td>0.64 ± 0.074</td>
</tr>
<tr>
<td>Urea mg /dl</td>
<td>28.8 ± 3.96</td>
<td>40.6 ± 2.96*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E of 10 animals.
* Values are statistically significant P <0.05 when compared with normal control.

**Histological examination**
The present study showed some histopathological effects in kidney and intestine tissues, histological examination of the normal control kidney, tissues showed normal histology (Fig. 1). Kidney of Chitocal treated rats showed congestion of blood vessels between renal tubules, aggregation of inflammatory cells (especially monocytes) in lumen and wall of blood vessels (Fig. 2), enlargement of renal tubules and aggregation of inflammatory cells around glomerulus (Fig. 3). Also the results showed normal histology of the normal control intestine tissue.
In intestine tissue belongs to Chitocal treated rats moderate increase of monocytes in lamina propria and hyperplasia of goblet cells was observed (Fig.5), aggregation of inflammatory cells in epithelium, adhesion of intestinal villi, hyperplasia in epithelium and aggregation of inflammatory cells in lamina propria (Fig.6).

These results may be due to the effect of secondary metabolites result from biodegradation of Chitocal components which may have side effects on kidney and intestinal tissues. The major role of kidney is exertion of drug metabolites and this may be the cause of these negative effects. Muzzarelli et al. propose a spontaneous formation of insoluble chitosan salts from bile acids whose hydrophobic nature should permit the collection of cholesterol and lipids via hydrophobic interaction [30], and this may be affect the surface epithelium of intestine and cause the adhesion of villi and hyperplasia of goblet cells. From these results it can be conclude there are negative side effects of Chitocal on histology of kidney and intestine, and further studies are needed in this respect.

**Figure 1** Section of kidney tissue belongs to normal control rat showing normal histology of the kidney (H&E) 400X.

**Figure 2** Section of kidney tissue belongs to rat treated with Chitocal showing congestion of blood vessels between renal tubules ( ), aggregation of inflammatory cells (especially monocytes) in lumen and wall of blood vessels ( ) (H&E) 400X.
Figure 3 Section of kidney tissue belongs to rat treated with Chitocal showing enlargement of renal tubules ( ) aggregation of inflammatory cells around glomerulus ( ) (H&E) 400X.

Figure 4 Section of intestine tissue belongs to normal control rat showing normal histology of the intestine (H&E) 400X.

Figure 5 Section of intestine tissue belongs to rat treated with Chitocal showing moderate increase of monocytes in lamina propria ( ) hyperplasia of goblet cells ( ) (H&E) 400X.
Figure 6 Section of intestine tissue belongs to rat treated with Chitocal showing aggregation of inflammatory cells in epithelium (→) adhesion of intestinal villi and hyperplasia in epithelium (←) aggregation of inflammatory cells in lamina propria (↓) (H&E) 400X.

References