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RESEARCH ARTICLE

Effect of Tannin and Plant Tannins on some Organs and Physic-Chemical Characters of Diabetic Wistar Rats

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Abstract

A seven weeks feeding trial was carried out to investigate the effect of 1% tannin, 5% tannin, 1.1% tannin in gum of Neem (*Azadirachta indica)* and 3.3 % tannin in pod of okra (*Abelmoschus esculentus*) on lipid profile, glucose, protein and body weight of diabetic rats, in addition the histological change in liver and kidney of experimental rats was studied. The rats were randomly assigned to five groups which are orally consumption different concentration of tannin. Group number 1, 2, 3, 4 and 5 were received 0.0 (control), 1.0, 1.1, 3.3 and 5 % tannin, respectively. Results shown significant reduction in cholesterol, HDL, LDL, triglyceride, glucose level at $p \leq 0.05$, but there was slight reduction in body weight and no significant change in protein level. Group number 5 (received 5 % tannin) in which all rats were death within the first week. This result was indicated 5 % tannin is unsafe dose for the rats, but all treated rats was continued the experiment until seven weeks without abnormal clinical signs. These findings are indicated 1, 1.1, and 3.3 % tannin were safe dose for tannin. Results in histological study showed liver and kidney tissues were not affected by different concentration of tannin except 5% tannin which affected on tissues of kidney.

Introduction

The tannin compounds are widely distributed in many species of plants, where it plays a role in protection from predation, and also as pesticides, and in plant growth regulation. It plays an important role in the ripening of fruit and the aging of wine. Tannins are incompatible with alkalis, gelatin, heavy metals, iron, limewater, metallic salts, strong oxidizing agents and zinc sulfate, since it forms complexes and precipitate in aqueous solution. Quercitannic acid is one of the two forms of tannic acid, which found in oak bark and leaves. The other form is called gallotannic acid which found in oak galls. Tannic acid is safe when it's used in the small amounts found in foods. However, tannic acid seems unsafe when applied to the skin to treat diaper rash, prickly heat, and minor burn or sunburn. The tannic acid might also be unsafe when used to treat cold sores and fever blisters on the lips. The tannic acid might be easily absorbed through the lips and cause harmful side effects. In large amounts, tannic acid can cause side effects such as stomach irritation, nausea, vomiting, and liver damage. Regular consumption of herbs with high tannin concentrations seems to be associated with an increased chance of developing nose or throat cancer throat. The high consumption of tannic acid can cause kidney, liver, skin damage, fever or infectious disease and heart failure. The appropriate dose of tannic acid depends on factors such as the user's age, health, and other conditions. Tannins can help to improve blood lipid levels [1]. Tulasi leaf powder (1 % tannin) was caused reduction in fasting blood sugar, uronic acid, total amino acids, total cholesterol, triglyceride, phospholipids and total lipids [2]. **Okra pods** regulating blood sugar is a key factor key in the prevention of diabetes and heart disease because the fiber in okra pods controls the rate at which sugar is absorbed from the small intestine into the blood stream [3] **.** Death of the animal treated with tannin was associated with a progressively developing hepatic necrosis and nephritis and a temporary acute gastroenteritis. It was accompanied by loss of weight and edema in many organs, evidence of stimulation of the

spleen, adrenal cortex and testes, and atrophy of the thymus. Recovery in survivors was associated with a temporary increase in weight of the spleen and testes and persistence of loss of weight in the adrenal, pyloric stomach, and skin [4].

Objectives of this study were investigated effect of concentration of tannin on lipid profile, glucose and body weight of diabetic rats, and assessment the histological change in liver and kidney of different experimental rats

Material and method

2.1 Experimental design:

The experimental rats were conducted according to the guidelines of the Committee for purpose of control and supervision of experiments on Animals, Adult males albino rats, weighing 180–218 g, age 12-14 week, fed with a standard pellet diet, the main component of this diet is carbohydrate (Table 1), they are kept for 15 days as adaptation period before start the experiment. All rats were put in clean metal cages, were cleaned daily and kept under conditions that prevented them from experiencing unnecessary pain and discomfort according to guidelines approved by the Ethical Committee. These males rats are divided into five groups: Group 1 were given the standard diet without tannin (Control), group 2 were given standard diet with 1% tannin , group 3 were given standard diet with1.1% tannin (Neem Gum) , group 4were given standard diet with 3,3 % tannin (Okra pods) and group 5 were given standard diet with 5% tannin.

2.2 Collection of blood sample and specific organs

Sample of eye blood was collected from each groups by heparenize capillary tube and kept in specific labeled plain container (eppendorf tube and blood container). The serum had been separated by centrifugation at 5000 rpm and stored at 5ºC until analysis. Rats were autopsied, then their liver and kidney organs were collected into labeled clean containers, and preserved in 10% formalin, then slides of liver and kidney were prepared [5].

2.3 Methods

Chemical analysis were determined according to [6].Tannin concentration was determined by [7], serum cholesterol mg/dl was measured [8], Serum HDL, LDL and triglyceride mg/dl was measured [9], glucose level (mg/dl) was measured [10] and total protein was measured [11] and Average body weight for each group was measured at 7 days interval during experiment

2.4 Preparation slides of liver and kidney of rats (treated and control)

From the autopsied rats, liver and kidney tissues were collected in clean, labeled, sterilized containers. The liver and kidney tissues are cleaned with distilled water and preserved in 10% formal saline. Sequences of steps for slides preparation were carried out [12].

2.5 Statistical analyses

Data obtained were analyzed and averaged, and assessed by using Analysis of Variance (ANOVA) [13].

Results and Discussion

3.1 Chemical analysis

Table 1 indicated the basal diet and its chemical analysis for rats as follows: 11 % moisture, 25 % protein, 3 % fat, 6 % ash and 55 % carbohydrates. These results were indicated that main nutrient in experimental rats is carbohydrates which is considered as source of glucose for experimental rats.

3.2 Lipid profile

Table 2 indicated that an average value of cholesterol level for the rats that affected by 0.0, 1.0, 1.1, 3.3 and 5 % tannin is 109.8, 35, 46, 49, and 70 mg/dl, respectively. Group that received 5% tannin was death during first week because it received more than safe dose 2.26±0.083 / kg body weight [4], but in other groups there was significant reduction on cholesterol level at $p \leq 0.05$ compared with control group (109.5%), but in 1.0 % tannin there was significant reduction at $p \leq 0.05$. These results were agreed with those values [14]. The variation on cholesterol level in four groups might be attributed to different concentration of tannin. An average value of HDL level that affected by different concentration (0.0, 1, 1.1, 3.3, and 5% tannin) was ranged from 34 – 54.5 mg/dl. It was observed that there is significant reduction on HDL level in the four groups compared with control (54.5) at $p \leq 0.05$. This results indicated that HDL level was affected by different concentration of tannin. These results are agreeing [14]. An average value of LDL level that affected by different concentration (0.0, 1, 1.1, 3.3, and 5% tannin) was ranged from 15 – 80.3 mg/d. These results indicated that LDL level during seven weeks was significantly decreased in all treated rats compared with control (80.0 mg/dl) at $p \leq 0.05$. Mean average values of triglyceride of 0.0, 1, 1.1, 3.3 and 5% tannin were 60.5, 45, 44, 40 and 53 mg/dl, respectively. These findings are indicated that there is significant reduction in triglyceride at $p \leq 0.05$. These results are agreed [2].

3.3 Glucose, Protein and Body weight

Table 3 in which it was observed that glucose level for all treated groups during seven weeks was ranged115 – 127 mg/dl compared with non treated group which is 144.5 mg/dl. These results indicated that the five groups of rats were suffering from diabetic disease because normal range for glucose level for rats was 117.0 ±1.96 mg/dl [15]. The rats that suffer from diabetic after treatment the glucose level were reduced from 144.5 to 115 mg/dl. It is also observed there was significant reduction in all treated groups at $p \leq 0.05$, but it is clearly reduction in 3.3 and 55% tannin. These results are agreed with those results [16]. Mean values of protein level of five groups were ranged 6 – 7%. These results indicated that protein intake equal protein consumption because there no reduction in protein in all treated groups compared with control group (7 %). An average value of body weight of all treated groups during seven weeks was varied 148 -201 g compared with control group (191.5 g). It was observed that there was no significant reduction in groups that treated with 1.1 and 3.3% tannin at $p \leq 0.05$. These results are with agreeing those results [17]

3.4 Histological study for liver and kidney

Slides for liver of all treated diabetics rats are normal (Figure 6- 10) while in kidney all in group(1, 1.1, and 3.3 %) is normal (Figure 1, 3, 4, and 5) except 5 % tannin concentration in which many renal tubules are dilated and show flattened lining epithelium, large area of local necrosis is demonstrated by nuclear debris and pyknotic nuclei. (Figure 2). These results indicated that 3.3 % tannin is safe dose but 5% tannin is unsafe dose. These results are agreed [4].

Table1: Show basal diet and its chemical analysis

Basal diet of experimental rats							
Ingredients	Sorghum	Wheat bran	Ground cake	Con.	Salt	Antitoxic	Vitamins
(%)	62.5	5.0	22.0	5.0	0.1	0.1	0.2
Chemical analysis of basal died of experimental rats							
Ingredients	Moisture		Protein	Fats	Ash		CHO
(%)	11.0		25.0	3.0	6.0		55.0

*Diet for experiment rat was analyzed (5).

Table 2: Show lipid profile affected by various concentration of tannin during seven weeks.

Various concentration	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)
0.0 % tannin (Control)	109.8	54.5	80.3	60.5
1% tannin	35.0	38.0	18.0	45.0
1.1% tannin (Neem)	46.0	43.0	16.0	44.0
3.3% tannin(Okra)	49.0	34.0	15.0	40.0
5% tannin	70.0	40.0	18.0	53.0

Table 3 : show glucose, protein and body weight affected by various concentration of tannin during seven weeks.

Various concentration	Glucose level mg/L	Protein level %	Body weight g
0.0 % tannin (Control)	144.5	7.0	191.8
1% tannin	126.0	7.0	158.0
1.1% tannin (Neem)	127.0	7.0	201.0
3.3% tannin(Okra)	115.0	7.0	184.0
5% tannin	1150	6.0	148.0

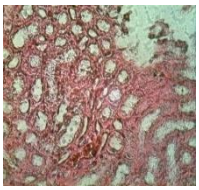

Figure (1): Control of kidney tissue

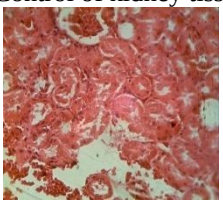

Figure (2): Tissue of kidney (5%Tannin)

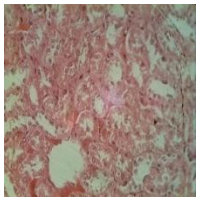

Figure (3): Tissue of kidney (1%Tannin)

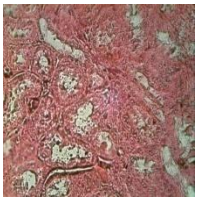

Figure (4): Tissue of kidney (3.3%Tannin).

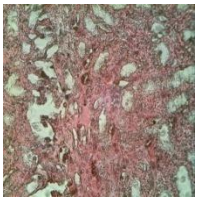

Figure (5): Tissue of kidney (1.1% Tannin)

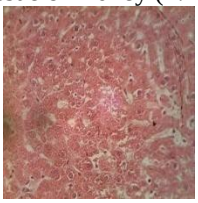

Figure (6): Control of Tissue liver

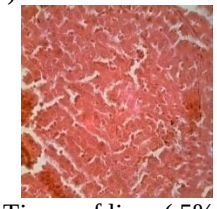

Figure (7): Tissue of liver (5% Tannin)

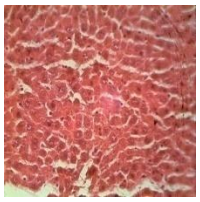

Figure (8): Tissue of liver (1% Tannin)

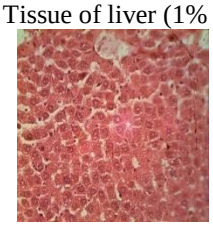

Figure (9): Tissue of liver (3.3% Tannin)

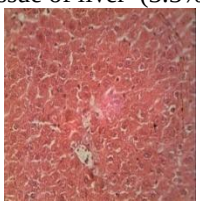

Figure (10): Tissue of liver (1.1% Tannin).

Conclusion

There are significant reduction in cholesterol, HDL, LDL, triglyceride and glucose in all treated groups. But there is slight reduction in body weighty and no change in protein level for all groups**.**

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