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Research Article

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Nutritional, Biochemical and Histological Studies on the Effect of Inulin in Chicory Roots on the Immune System of Male Rats

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ABSTRACT

In this research, the powdery dried chicory roots and pure inulin are used as food additives, separately to improve the functions of the immune system disorders in forty male albino rats through the existing content of inulin. The obtained experimental results after feeding for 42 days showed that the body weight gain, feed intake and feed efficiency ratio of chicory roots (10%) group was the best in comparison to all other groups. Immunoglobulins (IgM, IgG and NO) analyses recorded high significant differences between (-ve) and (+ve) control groups and all the treated groups. The best IgM value (77.692 and 99.612 mg/dL) was recorded for chicory roots 10% and pure inulin 1.0% with significant differences in comparison with (-ve) and (+ve) control group respectively. Histopathological studies showed the liver portal triads were apparently normal with a few round cells infiltration, Kidneys were apparently normal with preserved nephrons histomorphology and apparently normal spleen with preserved lymphoid population of white pulp and normal sinusoids and lympho-reticular network of red pulp in chicory roots (10%) group. The obtained results recommended using chicory roots (10%) in the production of functional and medicinal food to enhance the nutritional quality and the physiological status of the formulated food.

Keywords: Chicory roots; Immune system; Inulin; IgM; IgG; NO; Liver; Kidney; Spleen

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INTRODUCTION

The immunity may be categorized into subsystems, collectively with the innate immunity (naturally) and the adaptive immunity (artificially) [1]. Immune system troubles can bring about maximum cancers, autoimmune ailments and infections. Immune deficiency occurs even as the immune machine is a whole lot much less energetic than conventional, or whilst the usage of the drug immunosuppressive [2]. Dexamethasone is Corticosteroids its biologically energetic synthetic derivatives, further to its use in a spread of inflammatory ailments and autoimmune ailments make it a number of the most commonplace pills [3]. Inulin is a heterogeneous aggregate of fructose polymers and determined in nature as plant repository carbohydrates [4]. Inulin is the second most crucial compound

after starch it consists of 40 devices of fructose (β-form) associated with each different via (β-2→1 linkage) and mayended with one glucose unit [5]. Inulin in flora plays an essential issue in abiotic pressure tolerance, and itsosmoregulatory characteristic can defend plant life from drought, salt and cold stresses [6,7]. Inulin turn out to be decided in masses of plant life like artichoke leaves are optionally to be had alternatives due to their full-size content material cloth [8], agave, onions, leeks, plant trovh, garlic, wheat and asparagus [9]. Usually, inulin is extracted commercially from chicory [10]. Currently, the essential approach for inulin extraction from flora is precipitation through the usage of ethanol [11]. Chicory (*Cichorium intybus*) perennial herb of the asteraceae circle of relatives is nearby to the Mediterranean region, mid Asia and northern Africa. Historically, chicory has become grown by means of manner of the historical Egyptians as a medicinal plant in the treatment of some diseases [12]. Previous studies have showed that the chicory roots have various medicinal homes. It is antioxidant [13,14], anti-maximum cancers cancers [15], antimicrobial [16], antifungal [17], anti-inflammatory [18], anti-malarial [19], anti-allergy [20], anti-hepatotoxicity [21] and antidiabetic [22,23].

This research, aimed to study the effect of inulin on the immune system and improve the body's functions, through the use of chicory roots as a rich source of inulin and pure inulin, conducting chemical analyses of this plant, fatty acids, flavonoid compounds. Male albino rats will be fed with different concentrations of these powdery dried plants and pure inulin. Also, studing the effect of dexamethasone on the immunosuppressive in male rats by measuring the levels of different immune proteins and other immunological parameters.

EXPERIMENTAL SECTION

Chemicals

The used chemicals and reagents in this study are of highest analytical grade. Pure inulin, Ethyl alcohol formalin and all other chemicals were purchased from Industrial and Pharmaceutical Chemicals Co., Sharkia, Eygpt. Dexamethasone was injected as solution (3 ampoules of 2 mL); Eash ampoule contains 8 mg and it was purchased from Amriya for Pharmaceutical Industries, Alexandria, Egypt.

Plant Material

The roots of the chicory plant were obtained from a plantation in the new Salhiya city in Al Sharkia, Eygpt. Chicory roots were well cleaned, then cut and dried under vacuum then, milled by innovative mill to give a fine powder.

HPLC Analysis of Flavonoids Compounds

This analysis was performed for the detection and quantification of particular flavonoids compounds present following a modified method [24]. HPLC analysis was carried out using an Agilent 1200 series equipped with quaternary pump, auto sampler and column comport ant set at 35°C, malti ware length detector set at 330 nm, 280 nm for detection of flavonoids compounds and phenols compounds, degaser column used for fractionation Zorbax ODS, 4.6×250 mm and the flow rate of mobile phase during run was 1 mL/min.

Detection of Inulin and Sugars

Sugars standards all with a purity exceeding 99.0% were purchased from Sigma-Aldrich Chemicals Co. (St Louis, MO, USA).

Sample Preparation

Samples were diluted 1:10 (w/v) as method described earlier [25] with deionized water and then filtered through a 0.22 μm filter membrane. An aliquot of 1.5 mL of these solutions was placed in vials for the analysis.

Equipment and Operating Conditions

The chromatographic system Agilent (series 1200) coupled to the refractive index detector was equipped with a quaternary pump, degasser and auto injector. The chromatographic data were acquired using the Agilent software. The samples obtained as described above were analyzed using an Aminex-carbohydrate HPX-87 column under isocratic condition with deionizes water. The flow rate was 0.5 mL/min. The column temperature was maintained at 85 °C and the detector at 50 °C. Sample detection was performed by comparing retention time's standards.The chromatographic conditions according to a study [26] with some modifications.

Animal Selection

Forty normal male albino rats of Spargue Dawley strain, weighting 180 ± 5 g were obtained from Faculty of Veterinary Medicine, Zagazig University, Eygpt. Each 5 rats were housed in stainless steel cage under controlled condition. Diets were fed to rats in a special non-scattering feeding cup to avoid loss of food and contamination. Tap water was provided to rats by mean of glass tubes projecting through wire cage from inverted bottles supported to one side of the cage. Experimental animals were divided into two main groups as follows:

The first main group (5 rats) was fed basal diet for 6 weeks and was used as a negative control group.

The second main groups (35 rats) were fed basal diet with immunosuppressive (by dexamethasone intrapertoneal injection. Each rat was injected by weight 7 times at 7 days; In addition, 10 mg/kg was administered to one of the groups on the 8^{th} [27]. Then the second main group was divided into seven sub groups (5 rats in cage)

Sub group (1) was fed basal diet for 6 weeks and used as a positive control group.

Sub groups (2, 3 and 4) were fed basal diet supplemented with 5, 10 and 20 g of powdery dried Chicory roots per 100 g diet for 6 weeks, respectively.

Sub groups (5, 6 and 7) were fed basal diet supplemented with 0.5, 1.0 and 2.0 g of standard pure inulin per 100 g diet for 6 weeks, respectively.

Collection of Samples

At the end of experimental period, Rats were fasted for 12 hours and sacrificed under diethyl ether anesthetized. Blood samples were collected in clean dry centrifuge tube from the hepatic portal vein. A part of blood was taken in heparinized plastic vial and analyzed immediately to have a complete blood count (CBC). After that samples were centrifuged at 4000/min, Serum separated in clean glass well-stoppered and stored at (−20°C) until analyzed.

Estimation

Blood (5 mL) was collected from the vein at the beginning and end of the experiment. Erythrocytes and plasma were separated, plasma glucose [28], serum urea nitrogen [29], serum creatinine [30,31], WBC count, Hb, RBC count, Platelet counts (PLC) were estimated the results of CBC [32], determination of IgM and IgG [33], Nitric oxide (NO) [34], Finally, determination of protein electrophoresis according to [35].

Statistical Analysis

Statistical analysis was expressed as mean ± SE. The data were statistically analyzed by completely randomized design with [36] in relation to the following model: $Y_{ij}=\mu+T_i+E_{ij}$ where, μ is the overall mean, T_i is the fixed effect of i^{th} treatments, and E_{ij} is the random error. Means were tested for significant differences using Duncan's Multiple Range test [37].

Histopathological Investigations

Small specimens of the organs liver, kidneys and spleen were taken from the experimental animals, fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70.80 and 90%), cleared in xylene and embedded in paraffin. Sections of (4-6) µm thickness were prepared and stained with hematoxylin and eosin according to the method of a study [38].

RESULTS AND DISCUSSION

Chemical Composition of Chicory Roots

Data presented in Table 1 showed the chemical composition of chicory roots, the moisture, protein, fat, ash, fiber, carbohydrate and inulin contents of chicory roots in the dry form were found as 6.51%, 5.00%, 1.00%, 9.25%, 7.28%, 33.82% respectively. These data agreed quite well with the results of Jurgonbski [39]. However, the moisture, protein, fat, ash, fiber, carbohydrate and inulin contents of chicory roots in the dry form were found as 6.61%, 5.60%, 1.73%, 8.30%, 7.82% and 69.94% respectively. The data presented in Table 1 summarize the sugars of chicory roots, sucrose 4.285%, fructose 6.050% and ribose 0.022% [40,41]. Our analyzed data of inulin in chicory roots (26.78 %) agreed with its percent (15-20%) presented by Mérillon and Ramawat [42].

Table 1. Chemical composition of chicory roots and content of sugars (g/100 g dry weight basis)

Component	Content (%)	Sugar	Content (%)
Moisture	6.51+0.32	Inulin	26.78%
Protein	5.00+0.62	Sucrose	4.29%
Fat	1.00+0.83	Fructose	6.05%
Fiber	7.28+0.92	Ribose	0.02%
Ash	9.25+0.43		
Carbohydrates	70.96		
Total	100		

Total Flavonoids Compounds

HPLC analysis indicate the presence of Apigenin 6-arabinose 8-galactose, Apigenin 6-rhamnose 8-glucose, Luteolin 7-glucose, Naringin, Rutin, Hespirdin, Apigenin 7-α neohisperoside, Quercetrin, Quercetin, Kamp.3, (2-p-coumaroyl) glucose, Acacetin-neo. Rutinoside, Naringenin, Hespirtin, Kampferol and Apegnin (Figure 1 and Table 2) that might have been responsible for their therapeutic potential. The amounts of flavonoids are showed in Table 2.

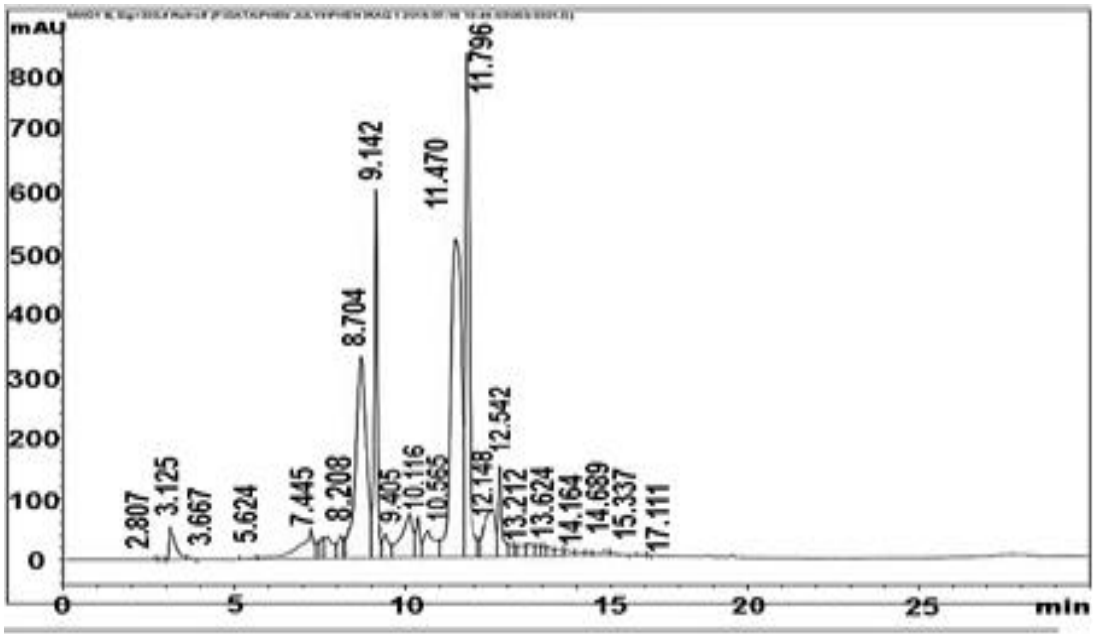

Figure 1. HPLC chromatogram total flavonoids in chicory roots

Table 2. Total flavonoid compounds of chicory roots

Flavonoids	Chicory roots conc. (ppm)
Apigenin-6-arabinose-8-galactose	37.96
Apigenin 6-rhamnose-8-glucose	11.59
Luteolin-7-glucose	74.85
Naringin	259.33
Rutin	20.37
Hespirdin	1008.88
Apigenin-7-O-neohisperoside	3.61
Quercetrin	21.77
Quercetin	0.67
Kamp.-3, (2-p-coumaroyl)glucose	56.1
Acacetin-neo.rutinoside	10.44
Naringenin	0.17
Hespirtin	3.43
Kaempferol	2.07
Apegnin	2.94
Total	3530.81

Effect of Chicory Roots and Pure Inulin on Body Weight

The mean values of body weight gain percent, feed intake and feed efficiency ratio of rats fed diet supplemented with different levels of chicory roots, (5.0, 10.0 and 20.0 g/100 g deit), and pure inulin (0.5, 1.0 and 2.0 g/100 g deit) for 6 weeks are summarized in Table 3. After feeding, the body weight gain percent recorded the highest values (47.34 ± 1.98%) by (-ve) control group, while the value significantly decreased to reach (17.37 ± 1.34%) for (+ve) control group and the experimntal results showed that a high significant difference ($p \leq 0.05$) by all groups. In

comparison with the (-ve) and (+ve) control groups, the body weight gain percent of chicory roots group (10%) was the best result (25.96 ± 1.31%) in the three groups of chicory (5.0%, 10.0%, 20.0%) and pure inulin group (1.0%) recorded the best result (13.76 ± 0.70%) in the three groups of pure inulin (0.5, 1.0 and 2.0 g/100 g diet). As for feed intake revealed that negative control (764.45 ± 7.39 g) is higher than ($p \leq 0.05$) positive control (633.31 ± 17.54 g). When added chicory roots and pure inulin separately, the feed intake increase by the six levels of these groups, the feed intake (711.43 ± 16.99 g) of male rats were fed diet contains chicory roots (10%) had the best efficiency in feed utilization and the highest weight gain during the experimental. Finally, we could conclude that the feed efficiency ratio of chicory roots (10%) group was the best results in comparison with the feed efficiency ratio of positive control and other group of rats except negative control group which recorded the highest value of feed efficiency ratio. This results agreement with [43] the weights of the animals were shown to be reduced in comparison with the negative and positive control. This might be attributed to the effect of dexamethasone used which is in agreement with the studies reported earlier.

Table 3. Body weight gain percent, total feed intake and feed efficiency of rats fed on diets supplemented with different levels of chicory roots and pure inulin

Treatments	body weight gain %	Total feed intake (g)	Feed efficiency ratio
(-ve) control	47.34 ± 1.98^{a}	764.45 ± 7.39^{a}	0.112 ± 0.002^{a}
(+ve) control	17.37 ± 1.34^{d}	633.31 ± 17.54^{b}	0.045 ± 0.006^{d}
Chicory roots 5%	16.12 ± 1.26^{d}	681.27 ± 17.48^{ab}	0.046 ± 0.001^{d}
Chicory roots 10%	25.96 ± 1.31^{bc}	711.43 ± 16.99^{ab}	0.071 ± 0.002^{bc}
Chicory roots 20%	9.61 ± 1.75^{f}	623.44 ± 36.79^{b}	0.016 ± 0.003^{f}
Inulin 0.5%	9.36 ± 0.55^{f}	620.34 ± 35.74^{b}	0.028 ± 0.001^{e}
Inulin 1.0%	13.76 ± 0.70^{de}	673.60 ± 22.86^{b}	0.040 ± 0.002^{d}
Inulin 2.0%	10.22 ± 1.45^{ef}	672.48 ± 41.94^{b}	0.028 ± 0.003^{e}

Mean values of five rats ± Standard Errors.

a, b, c and d of small letters in the same column are significantly differences ($P \leq 0.05$)

Effect of Chicory Roots and Pure Inulin on Fasting Serum-Glucose

The data obtained are present in Figure 2, there was a high significant difference between (-ve) control and all groups. And showed that the highest fasting serum-glucose value was (295.68 ± 12.78mg/dL) of treated groups recorded for pure inulin (2.0%), while the lowest value (125.504 ± 3.44mg/dL) was recorded for chicory roots 10% in comparison with (-ve) and (+ve) control groups. These results are in accordance with [44] when treated rats with *Cichorium intybus* (chicory), the level of sugar decreased to normal value. This effect might be due to the active inulin in this plant. This is due to hormonal level of glucagons and insulin, that way regulating lipid and carbohydrate metabolism by lowering serum glucose level.

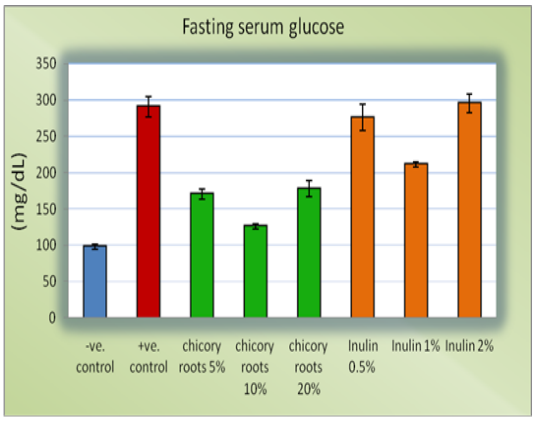

Figure 2. Fasting serum glucose of rats fed on diets supplemented with different levels of chicory roots and pure inulin

Effect of Chicory Roots and Pure Inulin on Kidneys Functions

The data are summarized in Table 4, showed a high significant difference ($p \leq 0.05$) between (-ve) control and other groups' values except groups which fed on chicory roots 10% (0.825 ± 0.052 and 32.432 ± 2.382 mg/dL) and pure inulin 1.0% (0.980 ± 0.056 and 54.98 ± 2.637 mg/dL) recorded the best results in comparison with (-ve) and (+ve) control groups for creatinine and urea, respectively.

Table 4. Kidney functions of rats fed diet supplemented with different levels of chicory roots and pure inulin

Treatments	Creatinine (mg/dL)	Urea (mg/dL)
(-ve) control	0.814 ± 0.075^{de}	31.212 ± 2.725^{d}
(+ve) control	2.014 ± 0.099^{a}	68.392 ± 2.825^{a}
Chicory roots 5%	0.996 ± 0.066^{de}	40.502 ± 3.987^{c}
Chicory roots 10%	0.825 ± 0.052^{e}	32.432 ± 2.382^{d}
Chicory roots 20%	1.264 ± 0.039^{c}	37.146 ± 2.742^{cd}
Inulin 0.5%	1.442 ± 0.065^{c}	60.382 ± 3.998^{ab}
Inulin 1.0%	0.980 ± 0.056^{d}	54.980 ± 2.637^{b}
Inulin 2.0%	1.366 ± 0.056^{c}	69.132 ± 4.066^{a}

Mean values of five rats ± Standard Errors.
a, b, c, d and e of small letters in the same column are significantly differences ($p \leq 0.05$)

Effect of Chicory Roots and Pure Inulin on HB, RBCs, Platelets and WBCs

The data tabulated in Table 5, showed that the lowest value (10.384 ± 0.839 g/dL) of HB for the all groups recorded for (-ve) control group, while all groups recorded an increase and a clear improvement in the concentration of HB for the treated groups and the best result (15.958 ± 0.728 g/dL) was recorded by chicory roots (10%) in comparison with the (+ve) and (-ve) control groups. On the other hand, the highest RBCs value (4.506 ± 0.306 µL) was recorded by (+ve) control group, while a high significant difference ($p \leq 0.05$) between (-ve) and other groups' values except groups which fed on chicory roots10% (4.034 ± 0.335 µL) and pure inulin 1.0% (4.294 ± 0.357 µL) recorded the best results in comparison with (-ve) and (+ve) control groups, respectively. In case of Platelets, the highest levels (475.4 ± 26.464µL) was recorded by (+ve) control group, while other groups were decreased in comparison with (+ve) control group except chicory roots 10 % was recorded the best results (208.6 ± 24.115µL) in comparison to (-ve) and (+ve) control groups, and the highest WBCs value (13.316 ± 0.908µL) recorded by (+ve) control group,

while the best result (6.086 ± 0.608 µL) recorded by chicory roots 10% with significant differences in comparison with (-ve) and (+ve) control groups. Finally, we could conclude that the hemoglobin (HB), red blood cells count (RBCs), Platelets and white blood cells count (WBCs) of chicory roots (10%) was the best result in comparison with the other groups, the significant reduction of WBC and lymphocytes counts observed in the study [45]. These results were a good indicator of the improvement of the immune system in food supported chicory roots at a concentration of 10%.

Table 5. HB, RBCs, platelets and WBCs of rats fed on diet supplemented with different levels of chicory roots and pure inulin

Treatments	HB (g/dL)	RBCs (×10^6/µL)	Platelets (×103/µl)	WBCs (×103/µL)
-ve control	18.008 ± 0.936^{a}	4.506 ± 0.306^{ab}	184.4 ± 27.520^{c}	7.142 ± 0.630^{b}
+ve control	10.384 ± 0.839^{b}	2.896 ± 0.130^{ab}	475.4 ± 26.464^{a}	13.316 ± 0.908^{a}
Chicory roots 5%	14.728 ± 1.103^{ab}	5.201 ± 0.236^{a}	312.8 ± 19.127^{b}	4.894 ± 0.339^{cd}
Chicory roots 10%	15.958 ± 0.728^{ab}	4.034 ± 0.335^{ab}	208.6 ± 24.115^{bc}	6.086 ± 0.608^{bcd}
Chicory roots 20%	14.732 ± 1.007^{ab}	6.098 ± 0.311^{a}	352.8 ± 16.259^{b}	5.172 ± 0.357^{cd}
Inulin 0.5%	13.116 ± 1.041^{ab}	5.594 ± 0.415^{a}	369.8 ± 34.292^{ab}	4.696 ± 0.443^{cd}
Inulin 1%	14.234 ± 1.019^{ab}	4.294 ± 0.357^{ab}	259.2 ± 28.815^{bc}	6.644 ± 0.801^{bc}
Inulin 2%	13.08 ± 1.254^{ab}	3.514 ± 0.231^{ab}	360.6 ± 36.586^{ab}	5.36 ± 0.474^{d}

Mean values of five rats ± Standard Errors.

a, b, c and d of small letters in the same column are significantly differences ($p \leq 0.05$)

Effect of Chicory Roots and Pure Inulin on IgM, IgG and Nitric oxide (NO)

The data results Table 6, indicated that the highest IgM value was (260.004 ± 9.702 mg/dL) recorded for (+ve) control group, While the other groups recorded decrease a significant compared to the (+ve) control group except chicory roots 10% was recorded the best results (97.692 ± 7.907 mg/dL)compared to negative control group, As for IgG value was (860 ± 27.613 mg/dL) for group 4 which fed on 10% chicory roots, then decreased significantly followed by group 3 (970.4 ± 29.459 mg/dL) which fed on 5% chicory roots and group 7 (983.8 ± 83.104 mg/dL) which fed on 1.0% pure inulin. Note that the lowest value recorded by the negative control group. The results showed a significant improvement in the NO level of the groups fed on chicory roots and pure inulin, compared with the positive group which recorded (0.431 ± 0.028 ug) the highest value, while the negative group recoded (0.113 ± 0.008 ug) the lowest value of NO levels but the best result compared all groups recorded by group 7 which fed 1.0% pure inulin (0.174 ± 0.025 ug) in comparison with the three groups of pure inulin. Also the best result compared all groups recorded by group 4 which fed 10% chicory roots (0.211 ± 0.010 ug) in comparison with the three groups of chicory roots.

Table 6. Immunoglobulins (IgM, IgG and NO) of rats fed on different levels of chicory roots and pure inulin

Treatments	IgM (mg/dL)	IgG (mg/dL)	NO (ug)
(-ve) control	83.082 ± 6.276^{f}	850.2 ± 36.483^{de}	0.113 ± 0.008^{f}
(+ve) control	260.004 ± 9.702^{a}	1413.4 ± 67.527^{a}	0.431 ± 0.028^{a}
Chicory roots 5%	132.648 ± 12.387^{cd}	970.4 ± 29.459^{e}	0.241 ± 0.017^{c}
Chicory roots 10%	97.692 ± 7.907^{f}	820 ± 27.613^{de}	0.211 ± 0.010^{cd}
Chicory roots 20%	127.326 ± 11.243^{cde}	995.8 ± 62.577^{e}	0.224 ± 0.014^{cd}
Inulin 0.5%	151.892 ± 8.655^{bc}	1225.4 ± 85.604^{ab}	0.188 ± 0.013^{cde}

Inulin 1.0%	100.612 ± 9.864^{def}	983.8 ± 83.104^{cd}	0.174 ± 0.025^{de}
Inulin 2.0%	146.668 ± 13.233^{c}	1141.6 ± 57.066^{bc}	0.251 ± 0.030^{bc}

Mean values of five rats ± Standard Errors.

a, b, c and d of small letters in the same column are significantly differences ($p \leq 0.05$).

Effect of Chicory Roots and Pure Inulin on Protein Electrophoresis

The data results in Table 7, showed the highest Alpha-1-Globulin value (0.518 ± 0.019 g/dL) of for the groups recorded for (+ve) control group, while the all groups were recorded decrease a significant compared to the (+ve) control group except chicory roots 10% was recorded the best results (0.134 ± 0.009g/dL) compared to (-ve) control group. The highest Alpha-2-Globulin value (0.972 ± 0.052 g/dL) for the groups recorded for (+ve) control group, while the all groups were recorded decrease a significant compared to the (+ve) control group except chicory roots 10% was recorded the best results (0.414 ± 0.039 g/dL) compared to (-ve) control group. The highest Beta Globulin value (2.606 ± 0.059 g/dL) of the groups recorded for (+ve) control group, while the lowest value (0.458 ± 0.070 g/dL) recorded for chicory (20%) group, but the best results (1.080 ± 0.063 g/dL) was recorded by chicory roots 10% group compared to (-ve) control group. The highest Gamma Globulin value (1.576 ± 0.068g/dL) was recorded for (+ve) control group while the best results (0.958 ± 0.092 g/dL) recorded for chicory roots 10% with significant differences in comparison with (-ve) control group.

Table 7. Protein electrophoresis (Alpha-1-Globulin, Alpha-2-Globulin, Beta Globulin and Gamma Globulin) of rats fed on different levels of chicory roots and pure inulin

Treatments	Alpha-1-Globulin (g/dL)	Alpha-2-Globulin (g/dL)	Beta Globulin (g/dL)	Gamma Globulin (g/dL)
(-ve) control	0.114 ± 0.017^{abc}	0.720 ± 0.037^{b}	1.108 ± 0.035^{ab}	1.105 ± 0.031^{b}
(+ve) control	0.518 ± 0.019^{bcd}	0.972 ± 0.052^{a}	2.606 ± 0.059^{a}	1.576 ± 0.068^{a}
Chicory roots 5%	0.170 ± 0.013^{cde}	0.414 ± 0.039^{c}	0.662 ± 0.048^{ef}	0.764 ± 0.068^{de}
Chicory roots 10%	0.134 ± 0.009^{ef}	0.690 ± 0.038^{b}	0.730 ± 0.055^{de}	0.958 ± 0.092^{cd}
Chicory roots 20%	0.198 ± 0.007^{bcd}	0.488 ± 0.041^{c}	0.458 ± 0.070^{g}	0.656 ± 0.070^{e}
Inulin 0.5%	0.212 ± 0.019^{ab}	0.476 ± 0.046^{c}	0.616 ± 0.047^{efg}	0.827 ± 0.081^{de}
Inulin 1.0%	0.146 ± 0.009^{def}	0.688 ± 0.070^{b}	1.080 ± 0.076^{b}	0.928 ± 0.101^{cd}
Inulin 2.0%	0.172 ± 0.009^{bcde}	0.752 ± 0.027^{ab}	1.000 ± 0.063^{bc}	1.276 ± 0.091^{b}

Mean values of five rats ± Standard Errors.

a, b, c, d, e, f and g of small letters in the same column are significantly differences ($p \leq 0.05$)

The data results in Table 8, stated that the highest T.P value (9.210 ± 0.369 g/dL) of the groups recorded for (+ve) control group, while the best results (7.002 ± 0.127g/dL) recorded for pure inulin 1.0% with significant differences in comparison with (-ve) and (+ve) control groups. The highest ALB value (5.484 ± 0.188 g/dL) for the treated groups recorded for (+ve) control group, while the best value (3.504 ± 0.088g/dL) recorded for chicory roots 10% with significant differences in comparison with (-ve) and (+ve) control groups. The highest Globulin value (4.816 ± 0.116 g/dL) of the groups recorded for (+ve) control group, while the best results (1.734 ± 0.107 g/dL) recorded for chicory roots 10% group with significant differences in comparison with (-ve) and (+ve) control groups. On the other hand, the highest A/G Ratio value (3.194 ± 0.217 ratio) for the groups recorded for chicory roots 10%, while

the lowest value (1.139 ± 0.045 ratio) recorded for (+ve) control group with significant differences in comparison with (-ve) control group.

Table 8. Protein electrophoresis (T.P, ALB, Globulin and A/G Ratio) of rats fed on different levels of chicory roots and pure inulin

Treatments	T.P (g/dL)	ALB (g/dL)	Globulin (g/dL)	A/G Ratio
(-ve) control	7.034 ± 0.096^{bc}	3.718 ± 0.128^{cde}	1.384 ± 0.049^{b}	1.242 ± 0.047^{d}
(+ve) control	9.210 ± 0.369^{a}	5.484 ± 0.188^{a}	4.816 ± 0.116^{a}	1.139 ± 0.045^{d}
Chicory roots 5%	7.522 ± 0.232^{b}	4.486 ± 0.302^{b}	2.010 ± 0.142^{fg}	2.208 ± 0.143^{c}
Chicory roots 10%	6.686 ± 0.164^{cde}	3.504 ± 0.088^{e}	1.734 ± 0.107^{g}	3.194 ± 0.217^{b}
Chicory roots 20%	6.026 ± 0.103^{e}	5.256 ± 0.138^{a}	2.616 ± 0.130^{cde}	1.406 ± 0.104^{d}
Inulin 0.5%	6.170 ± 0.121^{de}	4.030 ± 0.224^{bcde}	2.042 ± 0.129^{fg}	1.684 ± 0.136^{cd}
Inulin 1.0%	7.002 ± 0.127^{bc}	4.268 ± 0.224^{bc}	1.828 ± 0.239^{cd}	1.798 ± 0.218^{cd}
Inulin 2.0%	6.962 ± 0.304^{bcd}	4.524 ± 0.127^{c}	3.006 ± 0.223^{bc}	1.350 ± 0.128^{d}

Mean values of five rats ± Standard Errors.

a, b, c, d, e, f and g of small letters in the same column are significantly differences ($p \leq 0.05$).

Histopathological Examination

The liver of rats for (-ve) group showed the normal hepatic morphological structures (Photo A&A1), Examined sections revealed normal hepatic morphological structure with preserved lobular, portal and interlobular components and preserved vascular and biliary structures. (Photo B) liver of rats for (+ve) group showed congestion of portal blood vessels (star) and (Photo B1) macrosteatosis (star) and dilated sinusoids (arrow). (Photo C&C1) Liver of rats for chicory roots (5%) group showed hepatic parenchyma with macrosteatosis (arrow head) and portal triads round cells infiltrations (arrow). (Photo D&D1) Liver of rats for chicory roots (10%) group showed micro and macrosteatosis in some hepatic parenchyma (arrow) with a few round cells infiltration in portal area (star). (Photo E&E1) Liver of rats for chicory roots (20%) group showed micro and macrosteatosis (arrow head).The portal triads showed mild infiltration of round cells (arrow). Portal blood vessels are moderately dilated (star).The fatty change seen mostly periphero-lobular (arrow) and periportal (circle). (Photo F&F1) Liver of rats for pure inulin (0.5%) group showed congested hepatic blood vessels (stars), macrosteatosis (open arrow), and vacuolar degeneration (closed arrow) and hypertrophied kuffer cells (arrow head). (Photo G) Liver of rats for pure inulin (1.0%) group showed absolutely healthy liver (normal liver). (Photo H) Liver of rats for pure inulin (2.0%) group showed periportal (circle), (Photo H1) normal hepatic parenchyma with some degenerative changes as fatty change (open arrow), and (Photo H2) showed hydropic degeneration (open arrows) (Figure 3).

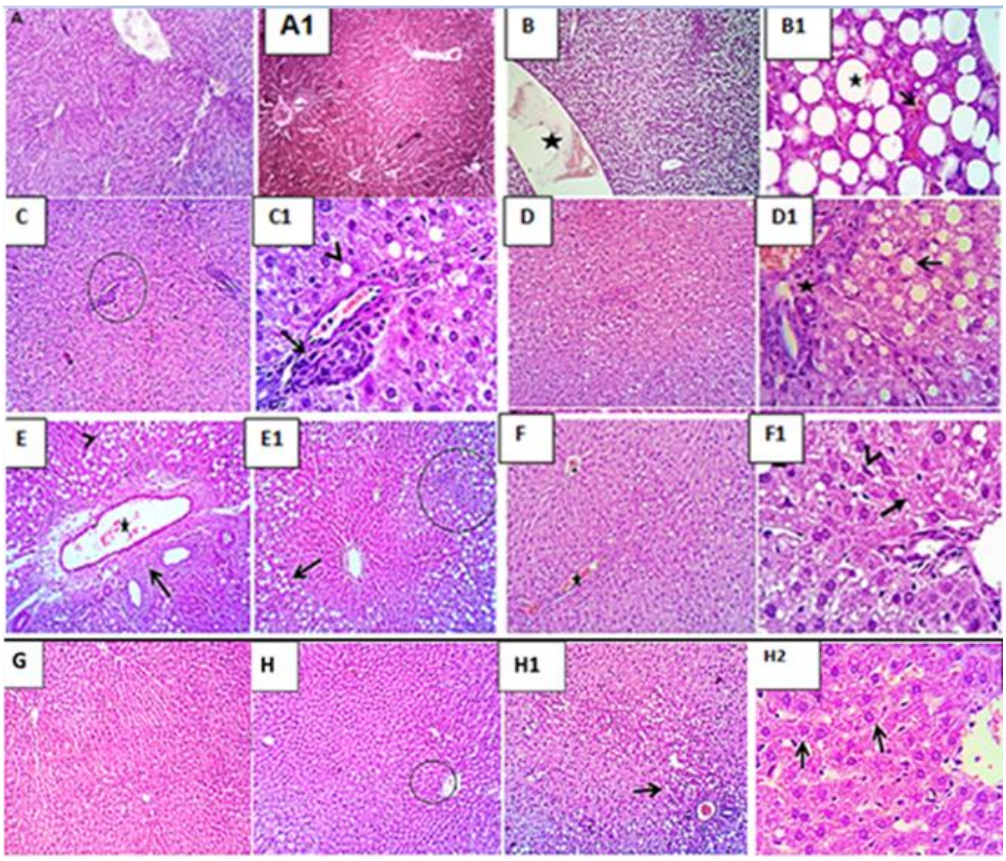

Figure 3. Liver photo micrograph of male rats: (Photo A&A1) of -ve group, (Photo B&B1) +ve group, (Photo C&C1) chicory roots (5%) group, (Photo D&D1) chicory roots (10%) group, (Photo E&E1) chicory roots (20%) group, (Photo F&F1) pure inulin (0.5%) group, (Photo G) pure inulin (1.0%) group and (Photo H,H1&H2) pure inulin (2.0%) group.

The kidneys of rats for (-ve) group showed the normal renal epithelium (arrow head) and glomeruli (star) (Photo A&A1). (Photo B) Kidneys of rats for (+ve) group showed mild congestion of renal blood vessels and intertubular capillaries (arrow). Perivascular edema (star), (Photo B1) degenerative changes in some tubular epithelium (arrow), with hyper trophied mesangial cells (star). (Photo C) Kidneys of rats for chicory roots (5%) group showed congestion of renal blood vessels (arrow) with peri vascular aggregation of lymphocytes and neutrophils (star) and degenerative changes (arrow heads) in some renal tubular epithelium, (Photo C1) Acute pyelitis with dilated blood vessels (star) and moderate infiltration of lymphocytes and neutrophils (arrows). (Photo D) Kidneys of rats for chicory roots (10%) group showed normal renal parenchyma (star). (Photo E&E1) Kidneys of rats for chicory roots (20%) group showed cystic dilatation of a few cortical tubules (star) with cloudy swelling of some renal epithelial cell (arrow) and mild congestion of intertubular capillaries (arrow heads). (Photo F) of rats for pure inulin (0.5%) group showed large number of renal tubular epithelium in cortex and medulla with degenerative and necrotic changes (arrow heads), some blood vessels shows perivascular edema (stars). (Photo F1) Moderate number of the collecting tubules arecystically dilated (stars). shrinked glomeruli are seen (open arrow). (Photo G&G1) Kidneys of rats for pure inulin (1.0%) group showed some of the renal blood vessels vacuolated tunica media (open arrow) with perivascular mononuclear cells infiltration (star), (Photo G2) Showed mild degenerative changes in the cortical and

medullary tubular epithelium (arrow heads) with hyaline cast (open arrow). (Photo H) Kidneys of rats for pure inulin (2.0%) group showing hydropic degeneration (open arrows), Degenerative changes in the cortical tubular epithelium (curved arrow), necrotic some tubular epithelium (open arrow), congested renal blood vessels (star) (Photo H1) (Figure 4).

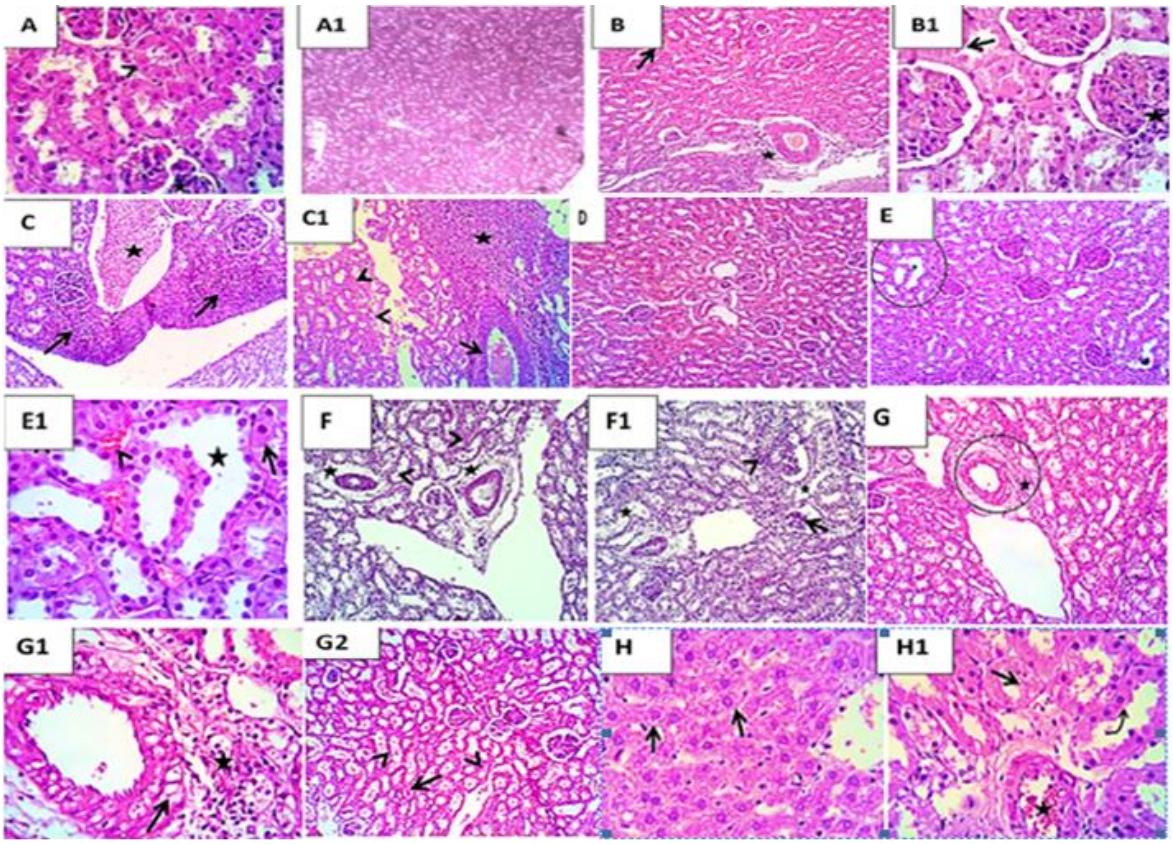

Figure 4. Kidneys photo micrograph of male rats: (Photo A&A1) of -ve group, (Photo B&B1) +ve group, (Photo C&C1) chicory roots (5%) group, (Photo D) chicory roots (10%) group, (Photo E&E1) chicory roots (20%) group, (Photo F&F1) pure inulin (0.5%) group, (Photo G,G1&G2) pure inulin (1.0%) group and (Photo H&H1) pure inulin (2.0%) group

The spleen of rats for (-ve) group showed normally arranged lymphoid cells of white pulp (circle), central aretiole (arrow head) and splenic sinusodes (arrow) (Photo A&A1). (Photo B) Spleen of rats for (+ve) group showed lymphoid hyperplasia of the white pulp with prominent germinal centers (star), mantle (arrow head) and marginal zones (arrow). (Photo B1) showed some cells mitotic divisions (arrows). (Photo B2) showed the red pulp moderately congested sinusoids (arrow heads) and (Photo B3) massive infiltration of immature lymphocytes (arrows). (Photo C) Spleen of rats for chicory roots (5%) group showed white pulp lymphoid hyperplasia with prominent germinal center (star). (Photo C1) showed the red pulp moderate congestion (arrow) and infiltration of large number of mature and immature lymphocytes (stars). (Photo D) Spleen of rats for chicory roots (10%) group showed normal preserved lymphoid population of white pulp, normal sinusoids and lympho-reticular network of red pulp. (Photo E) Spleen of rats for chicory roots (20%) group showing moderately proliferated lymphoid population of the white pulp (stars). (Photo E1) showed the red pulp congested sinusoids (star) normal population of megakaryocytes (arrow). (Photo F) Spleen of rats for pure inulin (0.5%) group showed normal lymphoid white pulp population (stars), (Photo

F1) showed the red pulp moderate infiltration of mature (open arrow) and immature lymphocytes (arrow head). (Photo G) Spleen of rats for pure inulin (1.0%) group showed mild to moderate proliferation of the lymphoid population of the white pulp (star). (Photo G1) the red pulp is massively replaced by infiltration of large number of mature (open arrow) and immature lymphocytes (arrow head). (Photo H) Spleen of rats for pure inulin (2.0%) group Showed mild pyelitis (open arrow) and mild to moderate proliferation of the white pulp lymphoid population (star). (Photo H1) the red pulp is massively and nearly totally replaced by infiltrated mature and immature lymphocytes (open arrows) (Figure 5).

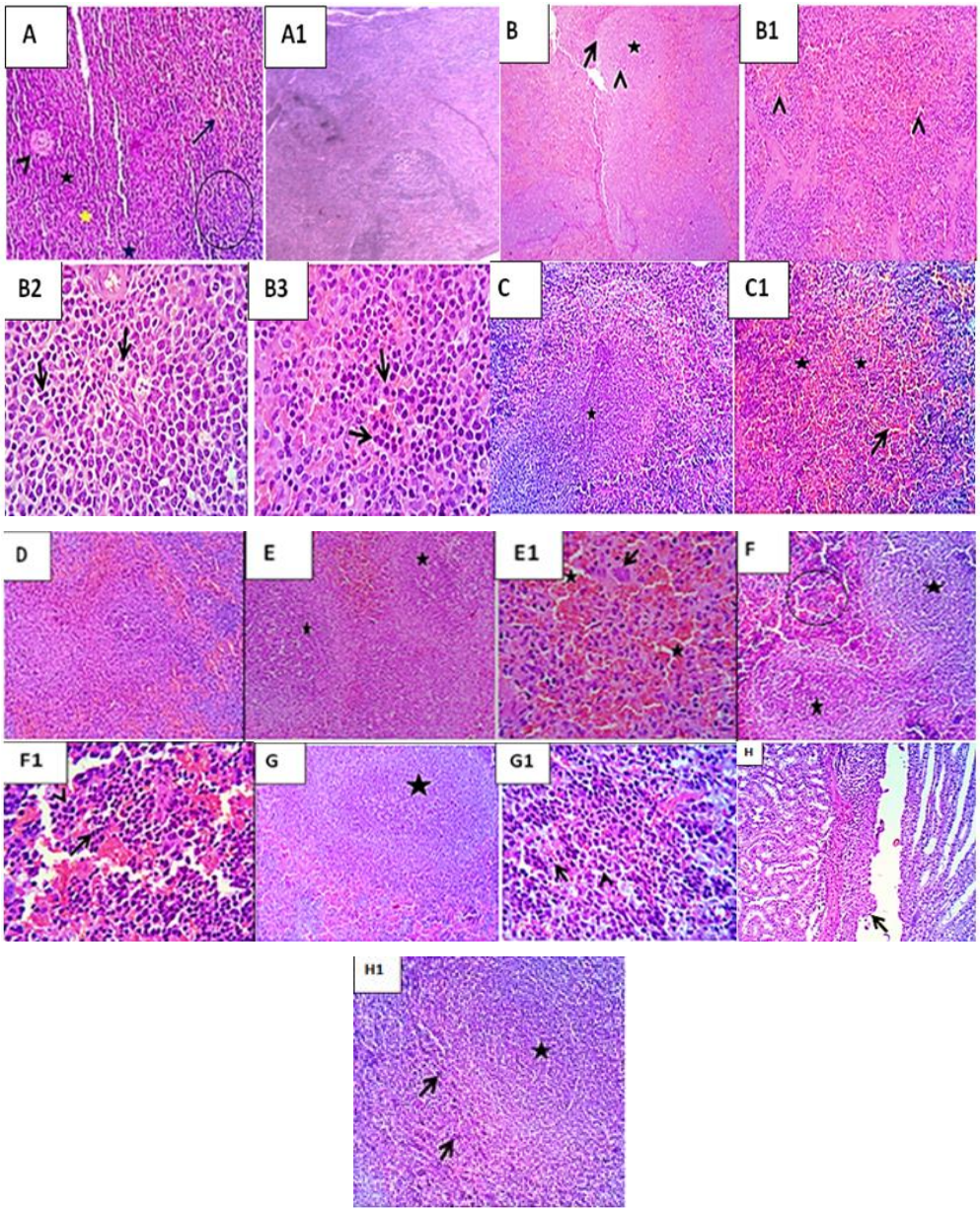

Figure 5. Spleen photo micrograph of male rats: (Photo A&A1) of -ve group, (Photo B,B1,B&B3) +ve group, (Photo C&C1) chicory roots (5%) group, (Photo D) chicory roots (10%) group, (Photo E&E1) chicory roots (20%) group, (Photo F&F1) pure inulin (0.5%) group, (Photo G&G1) pure inulin (1.0%) group and (Photo H&H1) pure inulin (2.0%) group.

CONCLUSIONS

In conclusion, results showed that chicory roots group (10%) is the best results in all treatment groups for the following chemical analyzes which include (Fasting serum-glucose, Kidney functions, Red Blood Cells, HB, Platelets, white Blood Cells, immunoglobulins (IgM, IgG and NO) and Protein electrophoresis (Alpha-1-Globulin, Alpha-2-Globulin, Beta Globulin, Gamma Globulin, T.P, ALB, Globulin and A/G Ratio). We can say that the improvement of results probably refer to a presence inulin and many chemical compounds such as flavonoids found in chicory roots the beneficial of the immune system**.**

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