4. RESULTS AND DISSCUSSION

4.1. Chemical composition of jojoba seeds and by-product:

Chemical composition of jojoba seeds grown at El-Kassassin and in El-Sheikh Zoied and their by-products are given in Table (2). The results show that moisture content ranges from 4.15±0.09 to 6.38±0.09% in the tested samples. Jojoba seeds grown at El-Kassassin under salinity stress (3000 ppm) contain the lower moisture content, while defatted meal contains the highest level. However, the initial moisture content is certainly important if the raw materials are going to be stored for long time.

Oil content in jojoba seeds ranges from 48.73±0.59 to 50.95±0.59%. Such results are almost similar to those reported by **Miwa (1984).**

Crude protein content in jojoba seeds and their by-products are as follows: jojoba seeds 15.35±0.22 to 16.34±0.22%, while in defatted meal is 30.58±1.12 to 32.19±1.12%. These results confirm the view that defatted meal of jojoba seeds is considered to be a source of protein.

Total carbohydrates of defatted meal gives the highest value 40.31±0.11 to 41.95±0.11% while jojoba seeds show the lowest value 15.35±0.08 to 16.34±0.08%. The total reducing and non-reducing sugars are about 6.21 to 7.00% of the seed.

The data in table (2) show that jojoba seeds and defatted meal contain 4.42±0.26 to 5.31±0.26% and 11.15±0.14% crude

fiber respectively. On the other hand ash content was found to be 1.61±0.03 to 1.88±0.03 and 3.20±0.05 to 3.33±0.05% for jojoba seeds and defatted meal respectively.

Simmondin, 2-Cyanomethylene–3-hydroxy–4,5 dimethoxy-cyclohexyl-β-D glucoside is the most important glucoside present in jojoba seeds, **Van Boven *et al.*, (1994a).** Data in Table (2) show that, the simmondsin levels in all samples of jojoba seeds are ~ 1.24±0.01% of the whole seed, while defatted meal contained the highest level of simmondsin 2.49±0.02%. This high toxicant level may also make these parts of the seeds unsuitable for animal in the food chain.

The major difficulty in the utilization of jojoba meals in animal diet is the presence of phenolic compounds in the meals. Phenolic compounds may be bounded with protein and gave an undersirable taste and color of the jojoba meal proteins. The total phenolic compounds content in jojoba seeds, jojoba meal and jojoba by- product were determined. The obtained results are shown in Table (2). Results indicate that the total phenolic compounds content of jojoba seeds, jojoba meal and jojoba by-product were 1.32-1.36%, 2.64-2.72% and 2.67% respectively.

Phytic acid or it's salt (phytate) is a cyclic derevative of inositol containing six phosphate radicals. It's physiological significance lies in the facts that it readily chelates with di- and tri- valent metal ions as calcium, magnesium, zinc and iron, the poorly solouble compounds that are not readily absorbed from the intestines. For this reason the presence of phytate as anti-nutritional factor which interferes with the bioavailability of minerals essential for optimal health. From the data presented in

Table (2) it can be seen that meal contains the highest amount of phytate (2.38% to 2.42) compared with jojoba seeds (1.19 %-1.22%). These results indicate that phytate might be characteristically present in the kernel and removing the oil will lead to an increase in the concentration of phytate on a unit weight bases.

These results are within the range of the results obtained by **Medina and Jrejo-Gonzalez (1990); Gaafer (2002) and Toliba (2004).**

4.1.1. Physical and chemical properties of jojoba oil:

Jojoba oil was subjected to the routine analysis of the oils and fats. Physicochemical properties of an oil or fat determine to some extent, its possible application in either industry or nutrition. Table (3) refers to the physicochemical properties of the samples, which included the refractive index, specific gravity, color, melting point, acid value, peroxide value, saponification value, iodine value and unsaponifiable matter percentage.

Refractive index of analysis of oils, its used for the estimation of their degree of saturation. From Table (3) it could be observed that the refractive index at 25°C of jojoba seed oil (grown under salinity stress, 3000 ppm at El Kassassin) is 1.4604 and jojoba seed oil (grown at El Sheihk Zoied) is 1.4634. The presence of a high concentration of unsaturated fatty acid and a greater amount of long chain fatty acids in the oils leads to an increase in it's refractive index as reported by **Parodi and Dunstan (1971).**

Specific gravity of the studied three samples of the oil were as follows: jojoba grown under salinity stress at El-Kassassin were 0.864 and 0.858 and jojoba grown at El-Sheikh Zoied was 0.855. These findings were found to be in harmony with those obtained by **Estefan (1983) and Salem (2003)**.

The color of lipids is of considerable commercial important for both food and industrial purposes. The color determination for jojoba oil was carried out with a fixed yellow slide of value 35 on the lovibond scale. The difference in colors between oils was assessed by matching its color against the red slides. These result were in agreement with those reported by **Abd El-Aziz (1989).**

Melting point range of any compound is a valuable index of purity. Therefore melting point of all oils under investigation was carried out. All studied oils have approximately the same value of melting point. Obtained results agree with those reported by **Wisniak (1994) and Gaafer (2002).**

The acid values of the investigated oils are low and this is understandable since the samples are quite fresh and well dried. Data in Table (3) indicate that the saponification value (mg KOH/g oil) of jojoba oil ranges from 93.19 to 94.21.

Autoxidation in the oils was estimated by peroxide value. Data in Table (3) show that the peroxide value found in all samples are relatively small. Such results clearly indicate that there is a little effect of autoxidation on the different oils.

The iodine value serves as an indication of the degree of unsaturation. The iodine values of the different samples under investigation were determined. The obtained results are presented in Table (3). The iodine values of jojoba oils under investigation

ranges from 81.64±0.47 to 83.01±0.47. Such results indicate the presence of high concentration of unsaturated fatty acids in jojoba oils leads to an increase in its iodine values. Ester values of the oils are 92.32 to 93.51. Also, from results in Table (3), the unsaponifiable matter content of jojoba oil ranges from 49.51 to 52.42. These results are within the range of the results obtained by **El-Shamy *et al.* (2001); Gaafer (2002) and Toliba (2004).**

4.1.2. Fatty acid composition of jojoba oil:

Fatty acids are the intergral constituents of every fat or oil. The degree of complexity of the glycerides basically depends upon the number and amount of various fatty acids in it. Also, the physical and chemical characteristics of lipids are largely depend upon their fatty acid composition. Gas-liquid chromatography was used for the qualitative and quantitative determination of individual fatty acid methyl ester. Fatty acid composition of jojoba seed oil is presented in Table (4).

The obtained results show that jojoba seed oil contains high amount of unsaturated fatty acids. As for the unsaturated fatty acid contents of jojoba seed oil (grown under salinity stress 1000 and 3000 ppm, at El-Kassassin) are 99.86% and 99.20% and in jojoba seed oil (grown at El-Sheikh Zoied) it is 98.30% of total fatty acid. Gadoleic acid ($C_{20:1}$) represents the major fatty acid of jojoba oil and ranges from 68.60% to 71.80%, followed by erucic acid ($C_{22:1}$) that ranges from 12.58% to 14.27% and Oleic acid ($C_{18:1}$) that ranges from 12.49% to 15.37%. As well as the lowest amounts of saturated fatty acids, myristic ($C_{14:0}$), stearic ($C_{18:0}$) and arachidic ($C_{20:0}$), they are traces. These results are in agreement with those reported by **Wisniak (1994), Gaafer (2002) and Salem (2003).**

4.1.3. Hydrocarbons and sterols composition of unsaponifiable matter of jojoba oil:

Unsaponifiable matter consists of substances not related in structure to oils or fats such as: saturated hydrocarbons, squalene, sterols, aliphatic alcohols, terpene, alcohols and other phenolic compounds. Unsaponifiable matter was extracted from crude jojoba seeds oil and fractionated by Gas liquid chromatography against authentic compound. The obtained results are shown in Table (5).

From these results it could be observed that the unsaponifiable matter of crude jojoba seed oil consist of two groups. Hydrocarbons and sterols. The first group, hydrocarbons, content of the unsaponifiable matter of jojoba seed oil (grown under salinity stress 1000 and 3000 ppm at El-Kassassin) are 98.15% and 96.99% and in jojoba seed oil (grown at El-Sheikh Zoied) is 97.47% of total unsaponifiable matter, while the sterols are 1.24%, 1.69% and 1.11% of total unsaponifiable matter.

Also, from Table (5) it could be noticed that the unsaponifiable matter of crude jojoba seed oil contains 12 fractions ,only three of these fractions are sterols (cholesterol, stigmasterol and β- sitosterol) and the other 9 fractions are hydrocarbons. The hydrocarbons (C_{12}+C_{24}+C_{26}+C_{28}) ranges from 94.50% to 95.76% of total unsaponifiable matter. Stigmasterol ranges from 0.86% to 1.12% of the total unsaponifiable fraction and presents more than 80% of the sterols in the oil. However, C_{24}, C_{26} and C_{28} represented the major components of the total hydrocarbons in the oil. This results are in agreement with those reported by **Van Boven *et al*. (1997), El-Shamy (2001) and Gaafer (2002).**

4.2. Elimination of toxic compound of jojoba meal:-

The toxicity of jojoba meal is the major problem for its utilization as a feed source. This is attributed by most workers to the presence of simmondsin or unpalatable substances. Several processes, including solvent extraction, heat and chemical methods have been investigated for detoxification of jojoba meal. That process includes removing simmondsin or modifing of the cyano groups. However, these processes did not completely remove the bitter compounds.

Ammonical hydrogen peroxide is a good reagent for converting the toxicants in jojoba meal, namely simmondsin and related cyano compounds to the corresponding amides. Simmondsin amides are less toxic than the corresponding cyano compounds **(Verbiscar and Banigan, 1978).**

From the data presented in Table (6), it can be seen that the better ratio of solvents for extraction was isopropanol-water (7:3), which eliminated 83.48% of simmondsin, 51.31% of total phenolic compounds and 27.62% of phytic acid contained in defatted jojoba meal. Acetone is less effective but more selective than isopropanol for extracting simmondsin. Ammonical hydrogen peroxide is even more effective in removing simmondsin from defatted jojoba meal.

Removal of the toxicants from the meal by water washing is impractical unless the water-soluble protein is denatured. Coagulation of the jojoba proteins by brief boiling or acidification to PH 3.2 using acid greatly facilitates filtration. As shown in Table (6), water is less effective for removing

simmondsin but effective for total phenolic compound and for removing of phytic acid from jojoba meal.

Dry heating of jojoba meal at 100°C for 1, 2 and 3 h does have an effect on lowering levels of toxicants. However, this meal is highly toxic to rats. Microwave treatment for shorter time seems more effective on destruction of total phenolic and phytic acid compared with solvent treatments.

Table (6): Effect of different treatments on the removal of anti-nutritional factors from jojoba meals:

Residual / Treatments	Simmondsin (%)	Total phenolic compounds (%)	Phytic acid (%)
Without treatment	100	100	100
Ammonical hydrogen peroxide	8.11	85.39	87.87
Isopropanol	16.52	48.69	72.38
Acetone	16.52	82.40	79.45
Water at pH 3.2	33.33	72.28	67.87
Heat 100°C for 1 h	43.24	66.83	62.76
Heat 100°C for 2 h	29.73	49.10	46.03
Heat 100°C for 3 h	23.12	31.64	29.71
Microwave	56.46	27.18	25.52

4.3. Amino acids composition of jojoba seeds:

The amino acids composition of Jojoba seeds are presented in Table (7). Results indicate that glutamic and aspartic acids are the most abundant amino acids followed by glycine. Cystine and tryptophan contents are found to be in minimum quantities 1.5% and 1.2% respectively. However, the total essential amino acids content is 37.1%. These results are in agreement with those reported by **Bodwell and Hopkins (1985).** They found that essential amino acids of oil seed protein ranges from 35 to 45% of their total amino acids. Also they reported that these levels of essential amino acids equal or exceed reference patterns that are based on human requirements.

The provisional amino acids scoring pattern proposed by **FAO/WHO (1973)** qualified an ideal protein as one in which 36% the total residues of essential amino acids. The data in Table (7) indicated that jojoba seed proteins had higher E/T ratio than proposed 36% for an ideal protein.

Table (7): Amino acids composition of jojoba seeds (g/100 g protein):

Amino acids	Jojoba seeds
Essential amino acids (E.A.A.)	
Lysine (Lys)	**4.70**
Leucine (Leu)	**6.30**
Isoleucine (lle)	**4.28**
Cystine+Methionine (Cys+Met)	**1.50+1.90**
Phenylalanine+Tyrosine (Phe+Tyr)	**4.90+3.80**
Tryptophan (Trp)	**3.80**
Threonine (Thr)	**1.20**
Valine (Val)	**4.72**
Total essential amino acids (T.E.A.A.)	**37.10**
Non essential amino acids (N.E.A.A.)	
Histidine (His)	**2.40**
Arginine (Arg)	**5.10**
Aspartic (Asp)	**10.20**
Glutamic (Glu)	**14.50**
Serine (Ser)	**5.40**
Proline (Pro)	**5.30**
Glycine (Gly)	**7.90**
Alanine (Ala)	**4.30**
Total Non essential amino acids (T.N.E.A.A.)	**55.10**
Total Amino Acid (T.A.A.)	**92.20**
E.A.A. / T.A.A.	**40.24**
E.A.A. / N.E.A.A.	**67.33**

4.4. Effect of pH on protein isolation from jojoba meal:

Several experiments are carried out in order to establish the proper pH values required for jojoba protein extraction.

The obtained results are presented in Fig. (2). From these results it is shown that the maximum jojoba protein extraction was achieved at pH 10. On the other hand, results show that on the acidic pH range, the percentage of the extracted protein was very low and reached its lowest amount at pH 4.8 (isoelectric point). However, at basic pH (10.0) the percentage of the protein extracted was found to be 94.13%. These results could be explained on the basis of the exhibited role of ionogenic groups in protein molecules in lyophilic colloidal systems of protein solutions. These proteins might be positively or negatively charged depending on the hydrogen ion concentration of the medium. The amount of NaOH bounds by the protein molecules depends on the equilibrium of hydrogen, **Wu and Sexson (1979).**

The ionogenic groups of proteins are present largely as zweitter-ions at isoelectric point. Thus at the alkaline pH values the base displace the hydrogen from ammonium groups $(-NH_3)^+$ of the zweitter-ion giving negative charges to the protein molecule that would increase as the normality of the base is increased **Samir (1976).**

Solubility of a specific protein reaches to its minimum at the isolectric point. **Wahba (1980)** noticed that the solubility of protein increases with increasing the acidity or alkalinity which might be attributed to the increase of repulsive electric forces induced by charges of same sign that might exist on protein molecules.

4.5. Determination of jojoba protein subunits molecular weight by using SDS-PAGE:

Polyacrylamide Gel Electrophoresis in the presence of detergent Sodium Dodecyl Sulphate (SDS-PAGE) was used for determining the subunit molecular weights (M.W.) of protein extracted by alkaline solutions from jojoba meal after different treatments.

The Pharmacia Low Molecular Weight (LMW) calibration kit provides six protein standards covering subunits M.W. ranging from 14.4 to 94 KD was used for constructing the calibration curve. The SDS-PAGE polypeptide patterns of the overall polypeptides in jojoba meal with protein standards are shown in Fig. (4).

The relative mobility (Rm) of each protein standard calculated from Fig. (3) and its molecular weights are shown in Table (8). A graph of log M.W. against Rm gave a straight line as shown in Fig. (3). From which the molecular weight of the subunits dissociation from both protein samples are determined (Table, 9). The obtained results show that jojoba meal protein dissociate into 7 subunits with molecular weight (MW) ranging from 57 to 14 KD. These results are in agreement with those reported by **Gaafer (2002)**.

Table (8): Molecular weights of standard proteins and their relative mobilities.

Standard proteins	Molecular weight M.W	Log M.W	Relative mobility (Rm)
Phosphorylase	94000	4.973	0.18
Albumin	67000	4.826	0.26
Pvalbumin	43000	4.633	0.45
Carbonic anhydrase	30000	4.477	0.56
Trypsin inhibitor	20100	4.303	0.74
Lactalbumin	14400	4.158	0.84

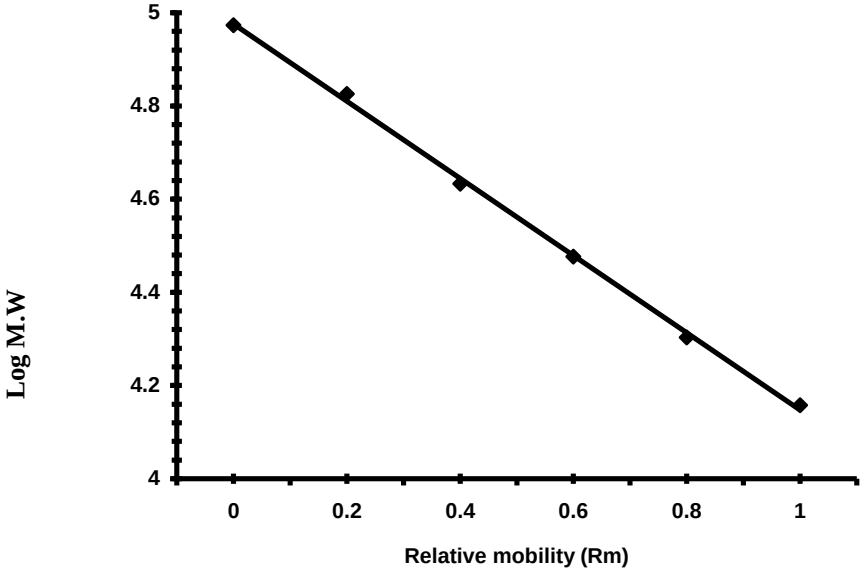


Fig. (3): Calibration curve for M.W. determination of subunits jojoba protein by SDS-PAGE.

Table (9): Molecular weights of jojoba meal protein.

M.W. of bands (K.D.)	Samples								
	1	2	3	4	5	6	7	8	9
1	58	58	58	58	58	58	58	58	58
2	56	56	56	56	56	56	56	56	56
3	42	42	42	42	42	42	42	42	42
4	31	31	31	31	31	31	31	31	31
5	24	24	24	24	24	24	24	24	24
6	17	17	17	17	17	17	17	17	17
7	14	14	14	14	14	14	14	14	14

1- Untreated
2- Treated with Isopropanol
3- Treated with Acetone
4- Treated with Ammonical hydrogen peroxide
5- Treated with water at pH 3.2
6- Treated with heat at 100°C for 1 h
7- Treated with heat at 100°C for 2 h
8- Treated with heat at 100°C for 3 h
9- Treated with Microwave

4.6. Biological evaluation of experimental diets:

The present studies were carried out to evaluate untreated jojoba meal, treated jojoba meal with different methods and jojoba protein isolate as food stuff and it's effect on body weight gain, food intake, feed conversion ratio, serum lipid profile, protein and liver, kidney functions of male rats.

4.6.1. Effect of different types of dietary on body weight gain, food intake and feed conversion ratio:

Data presented in Table (10) and illustrated graphically in Fig. (5) show the body weight gain (g) for experimental rats groups during a period of 8 weeks.

Analysis of variance shows significant effect ($P < 0.001$) of dietary jojoba meals on body weight gain of rats. At the end of experimental period, body weight gain of rats fed on jojoba meal treated with isopropanol or jojoba protein isolate was significantly greater ($P < 0.001$) than that of corresponding rats fed basal diet (control). Body weight gain of rats increases from 82.19±0.93g for control group to 124.01±0.81g, 133.63±1.33g, 98.76±0.71g and 93.44±0.88g for rats fed defatted jojoba meal treated with isopropanol, jojoba protein isolate, jojoba meal treated with ammonical hydrogen peroxide or acetone respectively. On the other hand, dietary 31.25% untreated jojoba meal reduced body weight gain to –16.27±1.52 g, respectively.

Daily average feed consumption in grams for each group are listed in Table (10) and illustrated graphically in Fig. (5). Results obtained show that, the rate of consumption differs according to the applied treatments. Analysis of variance shows

that variation in feed consumption due to dietary of untreated jojoba meal is high significant ($P < 0.001$). Jojoba meal Simmondsin is broken down to its aglycon in the intestinal tract by the intestinal bacteria and the aglycon is responsible for the food intake reduction **(Verbiscar *et al.*, 1980).**

One of parameters that used to determine the value of feeds for providing the necessary food amount required for one unit of growth. A lower value indicates an improved outcome. Feed conversion ratio is calculated by the equation: FCR = Feed ingested (g)/ Weight gain(g)

Data presented in Table (10) showed the average feed conversion ratio for experimental groups of rats. It is clearly observed that rats fed diet containing defatted jojoba meal treated with isopropanol had greater feed efficiency than those of the corresponding rats fed basal diet (control). However dietary untreated jojoba meal decreases feed efficiency. These results support the hypothosis that, the digestion of food constituents may be inhibited in certain manner by some additives (Simmondsin). The differences in body weight between control group and treated animals may be caused by a relative protein shortage in the jojoba supplemented rations, due to the presence of tannins, phenols and trypsin inhibitors **(Cokelaere *et al.*, 1993b).**

From the data presented in Table (10) it is clear that the addition of 31.25% untreated jojoba meal to the diet leads to a reduction in food intake by 21.75% and was associated with weight loss. However, after 12 days, death began occurring and the experiment was terminated before the planned 56 days.

These results are in agreement with those reported by **Cokelaere *et al.* (1993b)** they found that lower, growth rates, food intake and body weight gain of rats fed 3% untreated jojoba meal than that of rats fed on casein diet. Also, **Abbott *et al.* (1991)** reported that at levels up to 20% of the diet, jojoba meal treated with ammonia or sodium hydroxide showed no toxicity and supported growth equal to that of the control diet, whereas micro-biologically treated or ammonia plus steam treated meals supported a lower growth rates at 10 and 20% substitution.

4.6.2. Effect of different experimental diets on organs weight of rats:

The weights of liver, spleen, testis, kidney, lungs and heart expressed as percent of body weight for the different tested diet groups are summarized in Table (12) and illustrated graphically in Fig. (8).

The relative liver weight is non significantly lower with jojoba meal treated with ammonical hydrogen peroxide (1.84±0.74), meal treated with acetone (1.74±0.24) and jojoba meal treated with isopropanol diet (2.09±0.24) than that with the control diet (2.65±0.24). While the relative liver weight has a highly significant increase with untreated jojoba meal diet (5.02±0.24) than with the group fed control diet, the increase of liver weight in comparison to control experiment rats under the effect of different diets may be due to accumulation of fats in the liver tissues **Cokelaere *et al.* (1993b)** (Table, 13).

Results obtained in Table (12) and illustrated graphically in Fig. (8) show that, the mean values of spleen weight ranges

from 0.29±0.06 to 0.40±0.06 (g/100 g body weight) for rats fed basal diet or for rat fed 31.25% treated jojoba meal.

The influence of the jojoba meal on the testis weight is apparently due to its food restriction effect, because the increase of testicular weight is seen both in rats fed jojoba meals treated with isopropanol, ammonical hydrogen peroxide, or untreated jojoba meal.

From the data presented in Table (12) and Fig. (8), it is clear that kidneys, lungs and heart weights are non-significantly affected by the different experimental diets except in animal fed on untreated jojoba meal. Rats fed on diet containing jojoba meal treated with isopropanol, ammonical hydrogen peroxide or basal diet almost have the same values of kidneys, lungs and heart weight, while rats fed on untreated jojoba meal have the highest kidneys, lungs and heart weights. These results are in agreement with those reported by **Cokelaere *et al.* (1993b)**.

4.6.3. Effect of different experimental diets on serum lipids profile:

The effect of dietary treated jojoba meals, jojoba protein isolate and untreated jojoba meal on serum triglyceride, cholesterol, LDL and HDL-cholesterol of rats were studied.

Results obtained in Table (14) and illustrated graphically in Fig (9) show that serum triglycerides of rats fed on basal diet is 143.83±3.54 mg/100 ml serum. It decreases to 140.45±3.54 and 139.48±3.54 mg/100 ml by feeding with jojoba meal treated with acetone and jojoba protein isolate, respectively. While, rats fed diet containing untreated jojoba meal have the highest value of triglyceride 154.80±3.54 mg/100 ml.

The elevation of the serum triglyceride concentration after feeding of untreated jojoba meal may be due to the inhibitory effect of toxic compounds which presents in untreated jojoba meal upon very low density lipoprotein catabolism.

The obtained results show that serum cholesterol has non-significantly increase by dietary treated jojoba meal or jojoba protein isolate, it ranged from 120.09±2.46 to 124.39±2.46 mg/ 100 ml serum. While, feeding with dietary untreated jojoba meal leads to an increase in serum cholesterol by 11.71%. The increase in the serum value of cholesterol may be due to their enhancement synthesis by the liver as well as decreased excretion of cholesterol and its metabolites in the feces (Table, 15).

From the data presented in Table (14), it can be seen that, rats fed diet containing jojoba meal treated with isopropanol or with ammonical hydrogen peroxide leads to an increase both low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol but the effect on the former is substantially greater. While, rats fed diet containing un-treated jojoba meal may actually reduce HDL-cholesterol. These results are in agreement with those reported by **Cokelaere *et al.* (1993b) and Sobhy *et al.* (2003).**

4.6.4. Effect of different experimental diets on serum total proteins, albumin, globulin and hemoglobin in rats:

Total proteins, albumin, globulin and hemoglobin of rats serum fed on tested diets expressed as mg /100 ml are presented in Table (16) and Fig. (10). Results show that the average value of total proteins in rats fed a control diet is 6.15±0.27 mg/100 ml. From the same results it's clear that all nutrient type except untreated jojoba meal lead to a non-significant effect in the average value of serum total protein. The lowest value of total protein was observed in rats fed on untreated jojoba meal. The anti-nutritional factors present in untreated jojoba meal may inhibit the protein biosynthesis to produce the specific enzyme for lipogenesis process and also to inhibit hormones excretion which regulated protein metabolism.

From the above mentioned results, it's clear that serum albumin and globulin of rats fed different experimental diet have a non-significantly increase compared with control except rats received untreated jojoba meal, which shows the lowest serum albumin and globulin 5.33±0.27 and 1.88±0.20 mg/100 ml.

Data concerning albumin/globulin (A/G) ratio in rats serum after feeding on different diets show that there is significant decrease in this ratio. Albumin/ globulin ratio is decreased by 19.16%, 8.98%, 20.96% and 23.95% after feeding with jojoba meal treated with isopropanol, ammonical hydrogen peroxide, acetone or feeding with jojoba protein isolate, respectively (Table, 17).

Table (16) shows the value of hemoglobin at the end of the experiment. Compared to control, all treated groups show a non-significant decrease in hemoglobin concentration except rats received jojoba protein isolate which gives the highest hemoglobin concentration 14.00 mg/100 ml. these results are in agreement with those reported by **Cokelaere *et al.* (1993b) and Sobhy *et al.* (2003).**

4.6.5. Effect of different experimental diets on liver and kidney function of rats:

Plasma transaminases activities of AST and ALT were determined as indicators of liver functions, since the increase in these activities means that the liver become abnormal case.

The mean values of plasma transaminases activites of alanine transferase (ALT) and aspartate transaminase (AST) are presented in Table (18) and illustrated in Fig. (11). Data show a non-significant elevation in ALT and AST for rats fed treated jojoba meals (with isopropanol, ammonical hydrogen peroxide or with acetone), while rats fed untreated jojoba meal have a higher activities of the ALT and AST. The release of specific tissue enzyme into the blood stream may be dependent on both the degree and type of damage exerted by simmondsin which present in untreated jojoba meal.

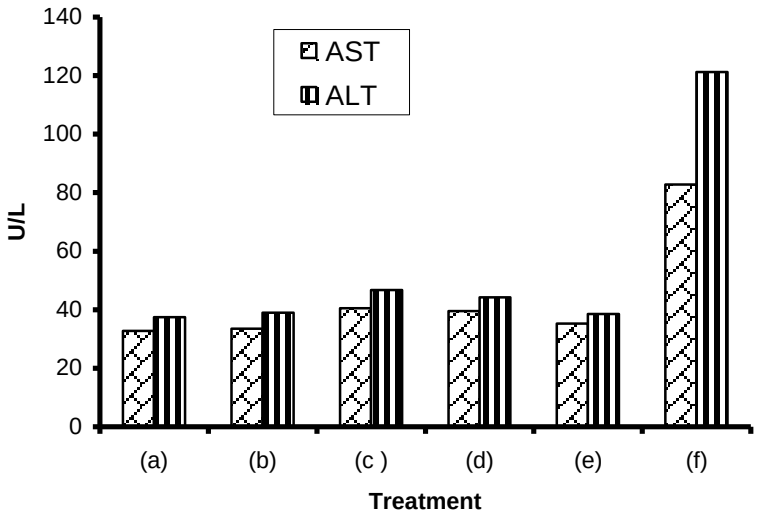
Serum urea and creatinine are determined as indicators of kidney function, since the increase in these components means that the kidney is less active or abnormal case.

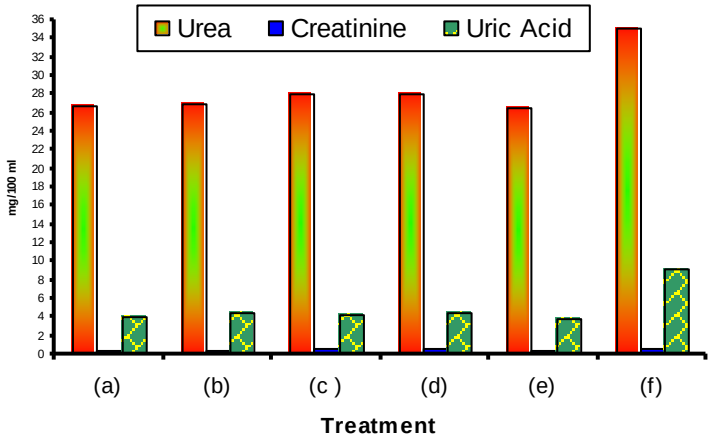
Mean values of serum urea and creatinine are elevated throughout the experiment. The significant urenimia is noticed in rats received untreated jojoba meal. The elevation of blood urea

and creatinine in treated rats may be attributed to the toxic effect of simmondsin which leads to disorders of the kidney which reduces the glomerular filtration rate and consequently retention of urea in the blood.

The results in Table (18) show that the values of serum uric acid in rats fed 3% treated jojoba meal with isopropanol, ammonical hydrogen peroxide or with acetone have a non-significantly increase when compared with rats fed basal diet (control). While rats fed untreated jojoba meal have a higher level of serum uric acid.

The results of the present study are in agreement with that of **Cokelaere *et al.* (1993a) and Cokelaere *et al.* (2000).**





(a) Control (Basal diet)
(b) Jojoba meal treated with isopropanol
(c) Jojoba meal treated with ammonical hydrogen peroxide
(d) Jojoba meal treated with acetone
(e) Jojoba protein isolate (f) Untreated jojoba meal

Fig. (11): Effect of different experimental diets on liver and kidney functions in rats.

4.7. Histopathological Findings:

4.7.1. Kidneys:

Control group:

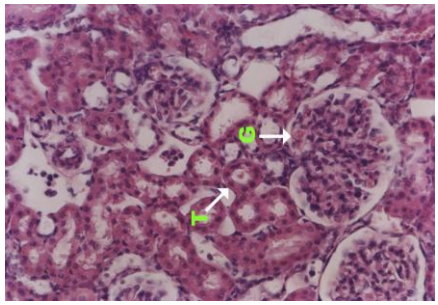
The microscopic examination of the kidneys of rats fed on Basal diet revealed nearly normal histological structure of the renal tissues. The renal tubules were lined by simple cuboidal epithelium with rounded nuclei and eosinophilic cytoplasm. The glomeruli were formed from gromerular tufts and Bowman's capsule with clear Bowman's space (Fig. 12 a).

Jojoba meal treated with isopropanol group:

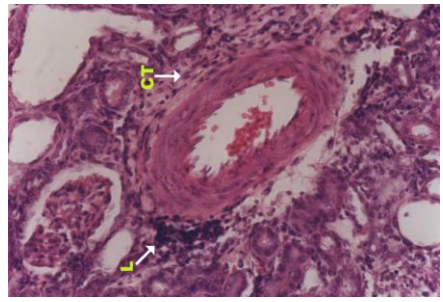
Congestion of the renal blood vessels and intertubular blood capillaries was microscopically observed. Thickening in the wall of blood vessels due to perivascular fibrous connective tissue proliferation infiltrated with inflammatory cells mostly lymphocytes was seen (Fig. 12b). Multiple areas of lymphocytic cellular aggregation in the interstitial tissues were noticed. Moreover, focal fibrous connective tissue proliferation infiltrated with eosinophils was also detected.

Jojoba meal treated with ammonical hydrogen peroxide group:

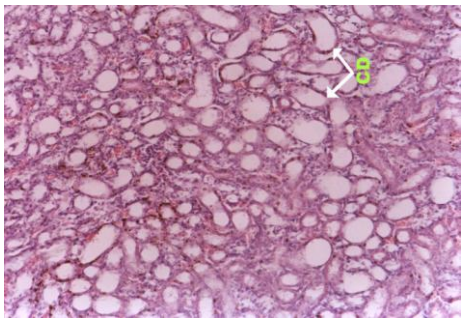
The histopathological examination of the kidneys of rats fed on jojoba meal treated with ammonical hydrogen peroxide group showed cystic dilatation of some renal tubules with flattening of their lining epithelium (Fig. 12c). Congestion of the blood capillaries and vessels with perivascular mononuclear cellular aggregation were seen. Moreover, focal mononuclear cellular aggregation replaced some damaged renal tubules was also found.



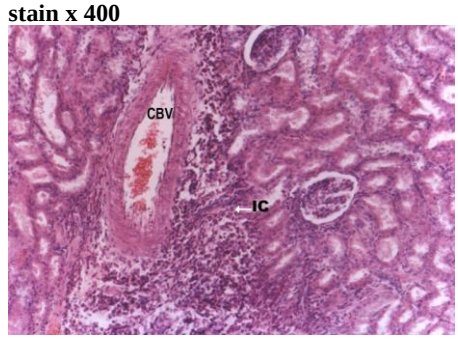
(a): Kidney of rat fed on basal diet showing normal histological structure of renal tubules (T) and glomeruli (G). H & E stain x 400



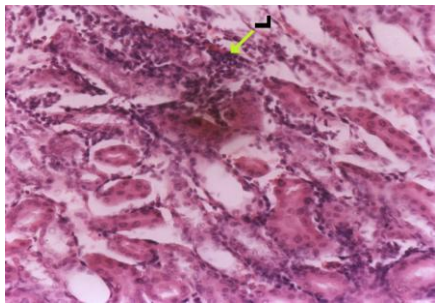
(b): Kidney of rat fed on jojoba meal treated with isopropanol showing perivascular C.T proliferation (CT) infiltrated with lymphocytes (L). H&E stain x 400



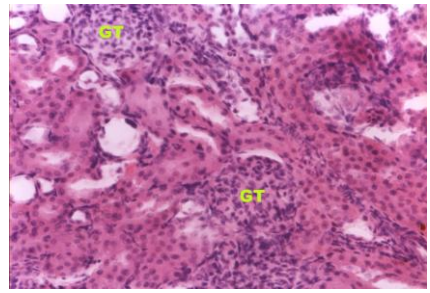
(c): Kidney of rat fed on jojoba meal treated with ammonical hydrogen peroxide showing cystic dilatation of some renal tubules (CD). H&E stain x 200



(d): Kidney of rat fed on jojoba meal treated with acetone showing congestion of blood vessels (CBV) with perivascular inflammatory cellular aggregation (IC). H & E stain x 200



(e): Kidney of rat fed on jojoba protein isolate showing aggregation of few lymphocytes (L) in the interstitial tissues. H&E stain x 200.



(f) Kidney of rat fed on jojoba meal showing proliferation of the glomerular tufts (GT) and occlusion of Bowman's spaces. H&E stain x 400

Fig. (12a,b,c,d & f): Photomicrographs showing histopathological effects of different dietary on kidney of rats.

Jojoba meal treated with acetone group:

The histopathological examination of the kidneys of rats fed on Jojoba meal treated with acetone revealed degenerative changes in the renal tubules particularly the proximal tubules. These degenerative changes manifested by cloudy swelling represented by swelling of the lining epithelium and narrowing of their lumens (Fig. 12 d). These changes were accompanied with inflammatory reaction in the form of inflammatory cellular infiltration in between the renal tubules and a round congested blood vessels. Moreover, focal areas of extravasation of blood replaced the renal tissues were also detected.

Jojoba protein isolated group:

The microscopic examination of the kidneys of rats fed on Jojoba protein isolated revealed slight pathological changes evidenced by focal lymphocytic cellular infiltration of the interstitial tissues (Fig. 12 e).

Untreated jojoba meal group:

The histopathological examination of the kidneys of rats fed on jojoba meal showed extensive damage of the renal tubules. These renal damages were in the form of severe cloudy swelling, vacuolar and hydropic degeneration of the lining epithelium of renal tubules (Fig. 12f). Proliferation of the gromerular tufts with occlusion of the Bowman's space of some glomeruli was recorded. Cystic dilatation of the renal tubules with flattening of their lining epithelium was observed. Moreover, multiple areas of inflammatory cellular infiltration of the interstitial tissue and around the blood vessels were also noticed.

4.7.2. Liver:

Control group:

The microscopic examination of the liver of rats in control group revealed nearly histologic hepatic tissues, where normal hepatocytes were arranged in cords around central vein. Moreover, small bile ducts lined by cuboidal epithelium with portal vessels were observed in the portal ared (Fig. 13 a).

Jojoba meal treated with isopropanol group:

The histopathological examination of the liver rats fed on Jojoba meal treated with isopropanol revealed no pathological changes in the hepatocytes. However, Congestion of central veins, with perivascular lymphocytic cellular aggregation were seen (Fig. 13 b). Moreover, multiple inflammatory cellular infiltration of hepatic parenchyma mostly lymphocytes were also noticed.

Jojoba meal treated with ammonical hydrogen peroxide group:

Congestion of the central veins and sinusoids with focal areas of hydropic degeneration of hepatocytes were the main microscopic lesions observed in the examined liver of rats fed on jojoba meal treated with ammonical hydrogen peroxide.

Pyknosis of the nuclei of some hepatic cells was seen. Moreover, hyperplasia of the lining epithelium of the bile duct with periductal fibrous connective tissues proliferation mixed with few lymphocytes were also found (Fig. 13 c).

Jojoba meal treated with acetone group :

The microscopic examination of the liver of rats fed on jojoba meal treated with acetone showed congestion of blood vessels and sinusoide with focal extravasation of erythrocytes which replaced the hepatic parenchyma (Fig. 13 d). Degenerative changes in the hepatic cells manifested by cloudy swelling and hydropic degeneration were detected. Focal lymphocytic cellular aggregations among the hepatic tissues were recorded.

In the portal areas, some bile ducts were dilated and filled with homogenous eosinophilic secretion and other showed hyperplasia of their lining epithelium. Moreover, fibrous connective tissue proliferation infiltrated with lymphocytes was also noticed in the portal areas.

Jojoba protein isolated group :

The histopathological examination of the liver of rats fed on jojoba protein isolated revealed some degenerative changes in the hepatocytes evidenced by vacuolation of their cytoplasm (Fig. 13e). Moreover, hyperplasia of biliary epithelium with presence of homogenous eosinophilic substance in lumen and periductal fibrous connective tissue proliferation were also detected.

Untreated jojoba meal group:

The pathological examination of the liver of rats fed on jojoba meal revealed massive degenerative changes in the hepatocytes. These changes manfasted by diffuse vacuolar, hydropic, and fatty degeneration of hepatocytes (Fig. 13 f). Focal inflammatory cellular aggregation mainly lymphocytes were

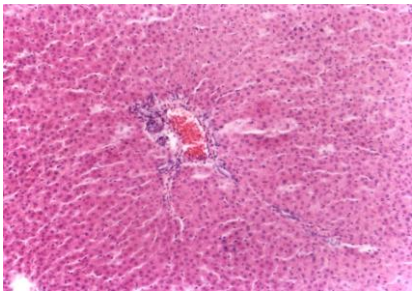
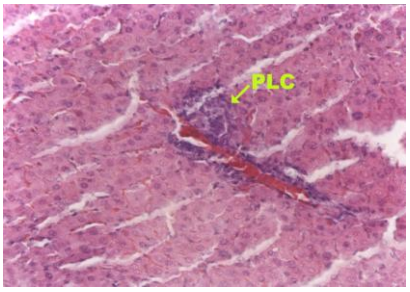
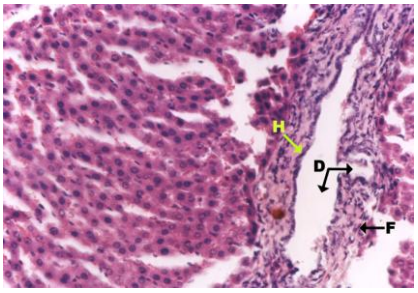
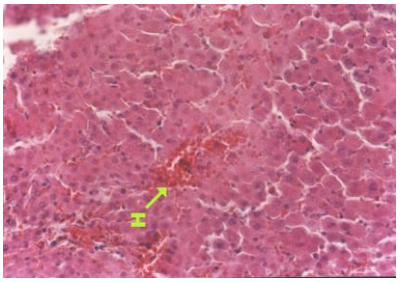
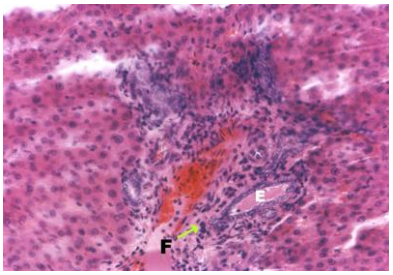
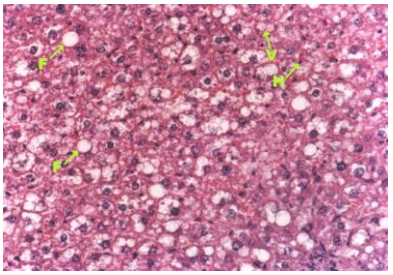
	
(a): Liver of rat fed on basal diet showing normal histologic structure of hepatic parenchyma. H&E stain x 200	**(b): Liver of rat fed on jojoba meal treated with isopropanol showing congested blood vessel with perivascular lymphocytic cellular aggregation (PLC). H&E stain x 400**
	
(c): Liver of rat fed on jojoba meal treated with ammonical hydrogen peroxide showing hyperplasia of bile ductal epithelium (H) with dilatation of the lumen (D) and periductal fibrosis (F). H&E stain x 400	**(d): Liver of rat fed on jojoba meal treated with acetone showing small area of hemorrhage (H). H&E stain x 400**
	
(e): Liver of rat fed on jojoba protein isolate showing periductal fibrosis (F) with presence of homogenous eosinophilic substance (E) in lumen of bile duct. H&E stain x 400	**(f): Liver of rat fed on jojoba meal diffuse vacuolar (V) hydropic (H) and fatty (F) degeneration of the hepatocytes. H&E stain x 400**

Fig. (13a,b,c,d,e & f): Photomicrographs showing histopathological effects of different dietary on liver of rats.

prevalent. Moreover, hyperplasia of bile ductal epithelium and peribiliary fibrous connective tissue proliferation infiltrated with mononuclear inflammatory cells mostly lymphocytes were also noticed.

From the recorded histopathological findings, it was concluded that, toxic effect of jojoba meal was more prominent on the endothelial lining of blood vessels. This effect was evidenced by vasodilatation of blood vessels with extravasation of erythrocytes which could be attributed to its irritant effect on the endothelium. Our openion was supported by the result of **Cokelaere *et al.* (1993b) and Sobhy *et al.* (2003).**

The hepatorenal toxic effect of jojoba meal was manifested by degenerative changes of hepatocytes and renal tubules. These findings were in a harmony with those of **Sobhy *et al.* (2003).**

These histopathological changes were reflected on the serum analysis of rats fed on untreated jojoba meal where elevations of serum AST, ALT, urea and uric acid levels were recorded. The detoxifing effect of most treatments of jojoba meal with different materials was limited as observed in microscopic examination of the liver and kidneys of rats. However, using of jojoba protein isolated was only treatment limited the toxic effect of jojoba.

Moreover, administration of jojoba meal stimulated inflammatory reaction in the form of lymphocytic cellular infiltration of both hepatic and renal parenchyma. These histopathological changes were in agreement with **Sobhy *et al.* (2003).**