ELIMINATION OF TOXIC COMPOUNDS AND NUTRITIONAL EVALUATION OF JOJOBA MEAL PROTEINS

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ABSTRACT: The present study was conducted on jojoba meal and its protein isolate to evaluate its chemical and biological characteristics as an alternative source of protein materials. Defatted jojoba meal contained 31.89±1.12% crude protein, simmondsin 3.33±0.02%, total phenolic compounds 2.67±0.02%. Phytate content was found to be 2.39±0.05% in the defatted meal. Glutamic and aspartic acids were the most abundant amino acids. The total essential amino acids content was 37.1%.

Solvent extraction, heat and chemical methods have been used for detoxification of jojoba meal to make it palatable and nutritionally acceptable as a livestock feed ingredient. The better solvent for extraction was isopropanol-water (7:3), which eliminated 83.48% of simmondsin, 51.31% of total phenolic compound and 27.62% of phytic acid contained in defatted jojoba meal. Heating was less effective but more selective than isopropanol for elimination of antinutritional materials. Heating at 100°C for 3 h was the best heating treatment in removing the antinutritional factors.

Rats fed on untreated jojoba meal showed a significant increase in lipid profile e.g. triglycerides and total cholesterol. The activities of liver enzymes (ALT & AST) were significantly increased, such finding indicates the incidence of liver dysfunction. The level of serum creatinine and urea did not alter. On the other hand, the rats group which received 10 % jojoba protein isolate revealed improvement in weight gain and the investigated serum parameters.

INTRODUCTION: Jojoba is an oil seed shrub that grows naturally on arid lands in the southwest of the United States and in Mexico. Several thousand acres have been planted in Egypt as a basis for a new economic crop (**FAO, 2003**).. The principal product of jojoba is an unusual oil, the remaining meal is high in protein and is a potential livestock feed ingredient (**Abbott *et al.*, 1991**). Jojoba meal is the by-products remaining after the extraction of the oil. This material constitutes about 50% of the nuts. The protein content varies between 26-33% making the meal attractive as a livestock feed, but it is difficult to be used as animal

feed because of the presence of several anti-nutritional factors, such as simmondsin, phenol and phytic acid (**Erhan *et al.*, 1997**) which reduce food intake and body weight and deteriorate biochemical parameters and fertility (**Sobhy *et al.*, 2003**).

Several conventional processing methods such as soaking in different solvents, heat, chemical and biological treatments were tried to detoxify the jojoba meal to remove the undesirable components from jojoba meal for improving their nutritional quality (**Erhan *et al.*, 1997, Umoren *et al.*, 1998 and Abu-Foul *et al.*, 2004).**

York *et al.* (2000) stated that the high dose of simmondsin (0.5%) in the diet produced profound weight loss and death in rats. At autopsy, the kidney, heart and liver of the treated animals were larger than the pair-fed animals and there was a marked suppression of the bone marrow elements with severe anemia. **Sobhy *et al.*** (2003) found that rats group fed on defatted (3 or 6 %) jojoba meal showed non-significant decrease in serum total proteins, albumin and alpha and beta globulin. On the other hand, there were increase in cholesterol, triglycerides, total lipids, AST, ALT and urea. No change occurred in glucose, calcium and phosphorous contents.

The present work was undertaken to study the chemical composition to establish methods for removal the toxic compounds present in jojoba meal and to evaluate the detoxified products and their content of isolated proteins, concomitantly chemical constituents of blood. Hematological parameters and histopathological examination of albino rat tissues were also a matter of concern in the investigation.

MATERIALS AND METHODS: Jojoba meal by-product was obtained from Egyptian Natural Oil Co. Cairo, Egypt. A total of sixty male albino rats, weighing 60-70 g. Rats were divided into six groups, ten rats per each group, were employed in the experiment. All groups were fed with normal diet for two weeks for acclimation. The first group (control) was not treated with jojoba meal. The second, third, fourth and fifth groups received, jojoba meal or jojoba protein isolate as described in table (1) At the end of the 8^{th} week, they were scarified to detect any histopathological changes of liver and kidney.

Moisture, total lipids, crude protein, ash and crude fiber were determined according to the methods of **A.O.A.C. (1995).** Total carbohydrates was determined according to **Bernfeld (1955) and Miller (1959).** Simmondsin content in defatted jojoba meal was determined according to **Van Boven *et al.*** (1993). Total phenols were determined

according to **Gutfinger (1981)**. Phytic acid was determined according to **Latta and Eskin (1980)**. Quantitative determination of amino acids were carried out according to **Moore *et al.* (1958)**. Cystine was determined according to **Barton (1952)**. Tryptophan was determined according to **Blouth *et al.* (1963)**.

Detoxification of jojoba meal was conducted using different methods, namely extraction with isopropanol : water (7:3) was carried out according to **Medina *et al.* (1988)**, Extraction with boiling water was conducted according to **Verbiscar *et al.* (1980)**, Heat treatments include dry heating in open trays in an oven at 100°C for 1, 2 and 3 h were conducted according to **Verbiscar *et al.* (1980)**. Heating using the microwave was carried out using a Moulinex microwave oven (Serie FMI) generating 0.5 Kw power at 2, 450 MHZ according to the method of **Hiromi and Goro (1988)**. Jojoba meal with 25% moisture content was placed as a single layer in a pyrex petri dish, and then heated with microwave for 14 min. Jojoba meal protein was extracted and precipitated according to **Abbott *et al.* (1991)**.

Rat serum total cholesterol was determined using the enzymatic method as described by **Finely (1978)**, HDL-cholesterol (**Lopes-Virella *et al.*, 1977**), triglycerides (**Fossati and Precipe, 1982**) total proteins (**Doumas, 1975**), albumin (**Doumas *et al.*, 1971**). Globulin was calculated by subtracting the amount of albumin from total protein, uric acid (**Haisman and Muller, 1977**), blood urea (**Tabacco *et al.*, 1979**), creatinine (**Henery *et al.*, 1974**) and blood haemoglobin was determined according to **Van Kampen and Zijlstra, (1967)**. Alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined according to **Reitman and Frankel, (1957)**.

Histopathological examination was conducted according to **Drury and Wallington (1986)**.

Data were statistically analyzed using SAS program (SAS, 1996).

Table (1): Composition of the basal and treated diet.

Ingredient %	Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Group (6)
Corn oil	8.0	8.0	8.0	8.0	8.0	8.0
Vitamin premix	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix	5.0	5.0	5.0	5.0	5.0	5.0
Cellulose	1.0	1.0	1.0	1.0	1.0	1.0
Water	5.0	5.0	5.0	5.0	5.0	5.0
Cr_2O_3	0.2	0.2	0.2	0.2	0.2	0.2
Protein source						
Casein	10	-	-	-	-	-
Jojoba meal treated with isopropanol	-	31.25	-	-	-	-
Jojoba meal treated with heat at 100°C for 3 h.	-	-	31.25	-	-	-
Jojoba meal treated with water at pH 3.2	-	-		31.25	-	-
Jojoba protein isolated	-	-	-	-	10	
Untreated Jojoba meal	-	-	-	-	-	31.25
Starch + sucrose	69.8	48.55	48.55	48.55	48.55	69.8

RESULTS AND DISSCUSSION

Chemical composition of jojoba meal is given in Table (2). The results showed that moisture content was 7.18±0.09%. Crude protein was 31.89±1.12%, total carbohydrates 39.78±0.11%, crude fiber 10.20±0.14% and ash content 3.11±0.05%. These results confirm the view that defatted meal is considered to be an excellent source of protein.

Simmondsin level in defatted meal is 3.33±0.02%. This high toxicant level might also make these parts of the seeds unsuitable for animal feeding. Phenolic compounds may bind with protein leading an undesirable taste and color of the jojoba meal proteins. Results indicate that the total phenolic compounds content of meal was 2.67±0.02%, phytic acid or its salt (phytate) physiologically chelates di- and tri-valent metal ions such as calcium, magnesium, zinc and iron forming poorly soluble compounds not readily absorbed from the intestines. These results are in agreement with the results obtained by **Hassan *et al.* (2003) and Toliba (2004).**

Table (2): Chemical composition of jojoba meal.

Components	%
Moisture	7.18±0.09
Crude protein*	31.89±1.12
Total carbohydrates*	39.78±0.11
Crude fiber*	10.20± 0.14
Total ash*	3.11±0.05
Total simmondsin*	3.33±0.02
Total phenols*	2.67±0.02
Phytic acid*	2.39±0.05

* (g/100g dry weight)

The amino acids composition of jojoba meal was presented in Table (3). Results indicate that glutamic and aspartic acids are the most abundant amino acids followed by glycine. Cystine and tryptophan contents were found to be in minimum quantities 1.5% and 3.8% respectively. However, the total essential amino acids content was 37.1%. These results are in agreement with those reported by **Bodwell and Hopkins (1985) and Abou Foul *et al.* (2004)** who found that essential amino acids of oil seed protein ranged from 35 to 45% of their total amino acids.

Jojoba meal protein extraction was conducted at different pH values to establish the proper one required for jojoba protein extraction. The obtained results are presented in Fig. (1). 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).

Table (3) Amino acids composition of jojoba meal.

Amino acids	g/100 g protein
Essential amino acids (E.A.A.):	
Lys	4.70
Leu	6.30
Ile	4.28
Cys + Met	1.5 + 1.9
Phe + Tyr	4.9 + 3.8
Trp	3.8
Thr	1.2
Val	4.72
Total essential amino acids (T.E.A.A.)	37.1
Non essential amino acids (N.E.A.A.):	
His	2.4
Arg	5.1
Asp	10.2
Glu	14.5
Ser	5.4
Pro	5.3
Gly	7.9
Ala	4.3
Total Non essential amino acids (T.N.E.A.A.)	55.1
Total Amino Acid (T.A.A.)	92.2
E.A.A. / T.A.A.	40.24
E.A.A. / N.E.A.A.	67.33

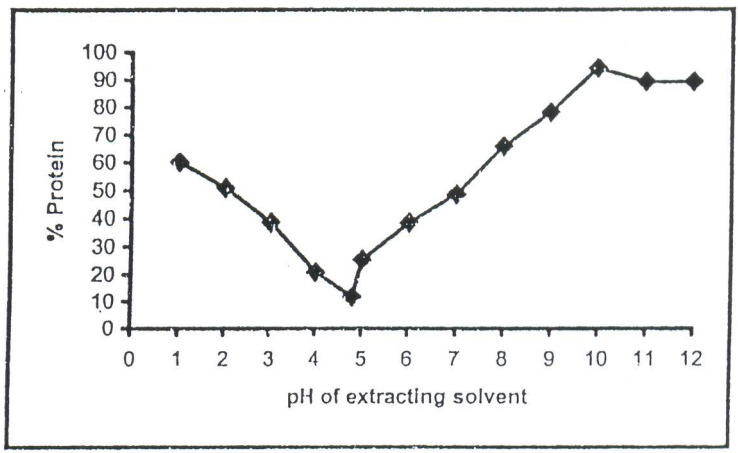

Fig. (1): Effect of pH on isolation of protein from jojoba meal.

Several processes, including solvent extraction and heat have been investigated for detoxification of jojoba meal. From the data presented in Fig. (2), it can be deduced that the better solvent 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.

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 Fig. (2), water seemed effective for removing simmondsin but less effective for removing total phenolic compound and for removing phytic acid from jojoba meal.

Dry heating of jojoba meal at 100°C for 3 h was of pronounced effect on lowering levels of toxicants.. Microwave treatment for short time seems more effective on destruction of total phenolic and phytic acid compared with solvent treatments.

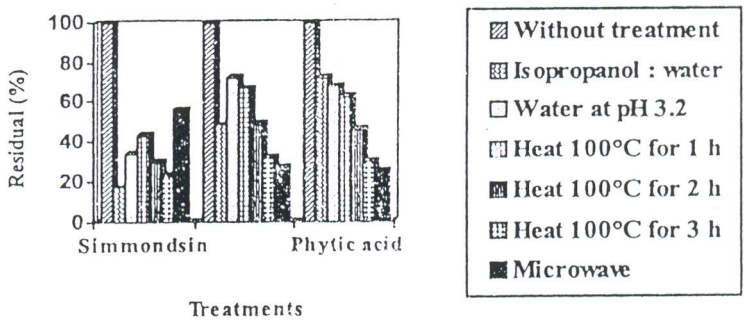

Fig. (2): Effect of different treatments on the removal of anti-nutritional factors from jojoba seeds:

Data presented in Table (4) illustrate that at end of the experimental period all the dietary jojoba meals, jojoba protein isolate or treated jojoba meals could significantly increase the body weight and weight gain of the investigated rats. The decrease in body weight and weight gain on the rats fed on untreated Jojoba meal could be attributed to the presence of antinutritional factors in Jojoba meal such as simmondsin, tannins, phenols and trypsin inhibitors (**Cokelaere *et al.*, 1993 b**). Likewise, all the dietary jojoba meals, except for the jojoba meal treated with isopropanol, exerted noticeable negative effect on feed consumption rate. Such negative effect seemed highest upon feeding the rats on the

untreated jojoba meal. This is probably due to the breakdown of simmondsin found in jojoba meal to aglycon in the intestinal tract by the intestinal bacteria. Aglycon is well known to be responsible for the food intake reduction (**Verbiscar *et al.*, 1980**). It is observed that rats fed on diet containing protein isolated of jojoba meal had greater feed efficiency than the corresponding ones of the rats fed on based diet (control). On the other hand, dietary untreated jojoba meal decreased feed efficiency, because the digestion of food constituents may be inhibited by simmondsin. These results agree with those reported by **Cokelaere *et al.* (1993 a)**.

Values of the relative liver weight (Table 5) of the rats fed on jojoba meal treated with isopropanol or heating at 100°c for 3 h were lower than the corresponding one of the rats fed on the control diet. On the other hand, the relative liver weight of the rats fed on the untreated jojoba meal diets was significantly higher than the corresponding one of the rats group fed on the control diet. The increase in liver weight of the rats fed on the untreated jojoba meal in comparison to the control experimental rats may be due to accumulation of fats in the liver tissue (**Cokelaere *et al.*, 1993 b**).

From the data presented in Table (5) it is clear that kidneys, lungs and heart were not-significantly affected by the different experimental diets except in rats fed on untreated jojoba meal which have the highest weights of kidneys, lungs and heart. These results are in agreement with those reported by **Cokelaere *et al.* (2000)**.

The different treated jojoba meals or jojoba protein isolate did not affect significantly triglycerides and HDL-cholesterol levels (Table 6). On the other hand, a significant effect could be noticed on cholesterol and LDL-cholesterol. The untreated jojoba meal caused the highest increase on both total cholesterol and LDL-cholesterol. The increase in serum value of cholesterol may be due to enhanced synthesis by the liver and/or decreased excretion of cholesterol and its metabolites in the feces (**Sobhy *et al.*, 2003**).

Total serum proteins, albumin and globulins, as well as blood hemoglobin of rats fed on different diets expressed as mg/100 ml are presented in Table (7). All nutrients types except for the untreated jojoba meal led to a non-significant effect on the average value of serum total protein. The lowest value of total proteins was observed in rats fed on untreated jojoba meal. The anti-nutritional factors present in untreated jojoba meal could inhibit the specific enzymes responsible for lipogenesis

Table (4): Body weight gain, food intake, feed consumption and feed conversion ratio of rats fed different experimental diets for eight weeks.

Treatment	Initial body weight (g)	Final body weight (g)	Body weight gain (g) [A]	Feed consumption (g/group) [B]	Feed conversion ratio [A/B]
Control (Basal diet)	60.94±1.31	143.13±1.98	82.19±0.93	578.33±7.52	0.150
Jojoba meal treated with isopropanol	60.55±0.79	184.56±0.96	124.01±0.81	559.91±6.95	0.221
Jojoba meal treated with heated at 100°C for 3 h	60.75±0.90	159.51±1.90	98.76±0.71	512.49±7.24	0.193
Jojoba meal treated with water at pH 3.2	60.91±0.83	154.35±1.63	93.44±0.88	461.91±6.88	0.202
Jojoba protein isolate	60.29±1.28	193.92±1.88	133.63±1.33	456.49±5.78	0.293
*Untreated jojoba meal	61.25±0.82	44.98±0.64	-16.27±1.52	107.99±7.84	-0.151
L.S.D. at 0.05	n.s.	3.58	2.96	25.68	
L.S.D. at 0.01	n.s.	4.86	4.02	34.84	

* Data obtained before 12 days.

Table (5): Effect of different experimental diets on organs weight of rats (g/100 g body weight).

Treatment	Final weight (g)	Liver weight	Spleen weight	Testis weight	Kidney weight	Lung weight	Heart weight
Control (Basal diet)	143.13±1.31	2.65±0.24	0.30±0.06	0.98±0.23	0.84±0.94	0.53±0.04	0.31±0.04
Jojoba meal treated with isopropanol	184.56±0.79	2.09±0.18	0.37±0.07	1.41±0.26	0.83±0.90	0.46±0.03	0.29±0.03
Jojoba meal treated with heated at 100°C for 3 h	159.51±0.90	1.84±0.21	0.33±0.05	1.20±0.20	0.84±0.88	0.46±0.04	0.28±0.03
Jojoba meal treated with water at pH 3.2	154.35±0.83	1.74±0.16	0.29±0.05	1.13±0.18	0.78±0.84	0.45±0.05	0.44±0.04
Jojoba protein isolate	193.92±1.28	1.95±0.25	0.29±0.04	1.55±0.28	0.76±0.91	0.41±0.04	0.28±0.03
*Untreated jojoba meal	44.92±0.82	5.02±0.36	0.40±0.08	0.85±0.15	1.85±0.96	1.33±0.06	0.67±0.05
L.S.D. at 0.05	4.61	1.47	0.36	1.41	0.56	0.22	0.18
L.S.D. at 0.01	6.37	2.04	0.50	1.95	0.78	0.31	0.25

* Data obtained before 12 days.

Table (6): Effect of different experimental diets on serum triglyceride and cholesterol type of rats.

Treatment	Triglyceride (mg/100ml)	Cholesterol (mg/100ml)	LDL-cholesterol (mg/100ml)	HDL-cholesterol (mg/100ml)
Control (Basal diet)	143.83±3.54	120.09±2.46	57.96±2.39	33.36±0.92
Jojoba meal treated with isopropanol	148.22±3.62	124.36±2.66	60.55±2.18	34.16±0.90
Jojoba meal treated with heated at 100°C for 3 h	144.21±3.50	124.39±2.52	60.39±2.27	35.16±0.88
Jojoba meal treated with water at pH 3.2	140.45±2.98	120.29±2.40	56.86±2.39	35.34±0.91
Jojoba protein isolate	139.48±3.12	121.77±2.48	56.92±2.41	36.95±0.98
* Untreated jojoba meal	154.80±4.02	136.03±2.88	72.18±2.56	32.88±0.85
L.S.D. at 0.05	6.56	7.42	7.20	2.32
L.S.D. at 0.01	8.90	10.26	9.96	3.15

* Data obtained before 12 days.

Table (7): Effect of different experimental diets on serum total protein, albumin, globulin and hemoglobin of rats.

Treatment	Total protein (g/100ml)	Albumin (g/100ml)	Globulin (g/100ml)	Albumin / globulin ratio	Hemoglobin (g/100ml)
Control (Basal diet)	6.15±0.27	3.80±0.13	2.35±0.20	1.67±0.11	13.77±0.08
Jojoba meal treated with isopropanol	6.55±0.26	3.72±0.11	2.83±0.21	1.35±0.10	13.60±0.09
Jojoba meal treated with heated at 100°C for 3 h	6.18±0.25	3.72±0.11	2.46±0.20	1.52±0.11	12.86±0.07
Jojoba meal treated with water at pH 3.2	6.14±0.27	3.49±0.12	2.66±0.22	1.32±0.10	12.52±0.08
Jojoba protein isolate	6.10±0.26	3.38±0.13	2.68±0.22	1.27±0.10	14.00±0.09
*Untreated jojoba meal	5.33±0.24	3.45±0.12	1.88±0.18	1.85±0.13	12.36±0.08
L.S.D. at 0.05	0.98	n.s.	0.59	0.34	0.23
L.S.D. at 0.01	133	n.s.	0.83	0.47	0.32

* Data obtained before 12 days.

process and also inhibit hormones excretion which regulate protein metabolism. Serum albumin of rats fed on the different experimental diet were significantly decreased compared with that of the control. Serum globulins increased due to the different dietary jojoba meals, except for the untreated jojoba meal which caused significant decrease in serum globulins as compared with the control treatment. Data concerning albumin/globulin (A/G) ratio in serum of rats after feeding on different diets showed a significant decrease in that ratio. Compared to control, all treated groups showed a non-significant decrease in hemoglobin concentration except for the rats that received jojoba protein isolate which gave the highest hemoglobin concentrations. These results are in agreement with those reported by **Cokelaere *et al.* (1993 b)** and **Sobhy *et al.*, (2003).**

Plasma transaminases activities of AST and ALT were determined as indicators of liver functions, since the increase in these activities means that the liver became in an abnormal case. The mean values of plasma transaminases activities of alanine (ALT) and aspartate transaminase (AST) were presented in Table (8). A non-significant elevation occurred in ALT and AST in liver of rats fed on treated jojoba meals. On the other hand, rats fed on untreated jojoba meal showed higher ALT and AST values. 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 is present in untreated jojoba meal. Serum urea and creatinine were determined as indicators of kidney function, since the increase in these components means that the kidney are less active or in abnormal case. A significant increase in urea was noticed in rats received untreated jojoba meal. The elevation of blood urea and creatinine in these rats may be attributed to the toxic effect of simmondsin which leads to disorders of kidney causing a reduction in glomerular filtration rate and consequently retention of urea in the blood. Likewise rats fed on untreated jojoba meal have a higher level of serum uric acid (**Cokelaere *et al.*, 1993a and Cokelaere *et al.*, 2000).**

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 esinophilic cytoplasm. The glomeruli were formed from glomerular tufts and Bowman's capsule with clear Bowman's space (Fig. 3a). Thickening in the wall of blood vessels occurred in kidneys of the rats fed on jojoba meal treated with isopropanol : water (7:3) due to

Table (8): Effect of different experimental diets on liver and kidney functions in rats.
* Data obtained before 12 days. AST : Aspartic transaminase
ALT : Alanine transaminase

Treatment	AST (U/L)	ALT (U/L)	Urea (mg/100ml)	Creatinine (mg/100ml)	Uric Acid (mg/100ml)
Control (Basal diet)	32.75±2.70	37.50±3.08	26.81±0.25	0.48±0.01	3.97±0.31
Jojoba meal treated with isopropanol	33.50±2.60	39.00±3.19	27.01±0.26	0.50±0.02	4.54±0.34
Jojoba meal treated with heated at 100°C for 3 h	40.50±2.88	46.75±3.38	27.99±0.25	0.56±0.02	4.26±0.33
Jojoba meal treated with water at pH 3.2	39.50±2.80	44.25±3.27	28.13±0.26	0.58±0.03	4.40±0.34
Jojoba protein isolate	35.25±2.72	38.50±3.02	26.57±0.24	0.47±0.01	3.87±0.29
*Untreated jojoba meal	82.75±2.68	121.25±4.22	35.19±0.28	0.71±0.03	9.24±0.38
L.S.D. at 0.05	8.14	9.30	0.77	0.03	0.94
L.S.D. at 0.01	11.27	12.86	1.06	0.04	1.30

perivascular fibrous connective tissue proliferation infiltrated with inflammatory cells mostly lymphocytes was seen (Fig. 3b). Multiple areas of lymphocytic cellular aggregation in the interstitial tissues were noticed. Moreover, focal fibrous connective tissue proliferation infiltrated with esinophils was also detected. The histopathological examination of the kidneys of rats group fed on jojoba meal treated by heating at 100°C for 3 h showed cystic dilatation of some renal tubules with flattening of their lining epithelium (Fig. 3c). Congestion of the blood capillaries and vessels with perivascular mononuclear cellular aggregation was noticed. Moreover, focal mononuclear cellular aggregation replaced some damaged renal tubules.

The histopathological examination of the kidneys of rats group fed on Jojoba meal treated with water at pH 3.2 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. 3d). 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. 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. 3e). The histopathological examination of the kidneys of rats fed on untreated 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. 3f). Proliferation of the glomerular 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.

The microscopic examination of livers of the rats in the 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 area (Fig. 4a). The histopathological examination of liver of the rats fed on Jojoba meal treated with isopropanol (Fig. 4b) revealed no pathological changes in the hepatocytes. However, there was congestion of central veins, with perivascular lymphocytic cellular aggregation besides multiple

inflammatory cellular infiltration of hepatic parenchyma mostly lymphocytes. Congestion of the central veins and sinusoids with focal areas of hydropic degeneration of hepatocytes and pyknosis of the nuclei of some hepatic cells besides hyperplasia of the lining epithelium of the bile duct with periductal fibrous connective tissues proliferation mixed with few lymphocytes were the main microscopic lesions observed in the examined liver of rats fed on jojoba meal treated by heating at 100°C for 3 h.

Liver of rats fed on Jojoba meal treated by water at ph 3.2 showed congestion of blood vessels and sinusoide with. focal extravasation of erythrocytes which replaced the hepatic parenchyma (Fig. 4d). Degenerative changes in the hepatic cells manifested by cloudy swelling and hydropic degeneration and focal lymphocytic cellular aggregations among the hepatic tissues were observed. In the portal areas, some bile ducts were dilated and filled with homogenous esinophilic secretion and same others showed hyperplasia of their lining epithelium. Moreover, fibrous connective tissue proliferation infiltrated with lymphocytes was also noticed. Liver of the rats fed on jojoba isolated protein revealed some degenerative changes in the hepatocytes evidenced by vacuolation of their cytoplasm (Fig. 4e). Moreover, hyperplasia of biliary epithelium with presence of homogenous esinophilic substance in lumen and periductal fibrous connective tissue proliferation were also detected. Liver of the rats fed on untreated jojoba meal revealed massive degenerative changes in the hepatocytes. These changes were manifested by diffuse vacuolar, hydropic, and fatty degeneration of hepatocytes (Fig. 4f). Focal inflammatory cellular aggregation mainly lymphocytes were 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 could be concluded that, toxic effect of untreated jojoba meal was more prominent on the endothelial lining of blood vessels. Such effect was evidenced by vasodilatation of blood vessels with extravasation of erythrocytes which could be attributed to its irritant effect on the endothelium.

The hepatorenal toxic effect of jojoba meal was manifested by degenerative changes of hepatocytes and renal tubules. These histopathological changes seemed to be reflected on the serum analysis of rats fed on untreated jojoba meal where elevations of serum activities of AST, ALT and levels of urea and uric acid. The detoxifying 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 jojoba isolated protein was the only treatment that 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. Similar findings were attained by Cokelaere *et al.* (1993b) and Sobhy *et al.* (2003).

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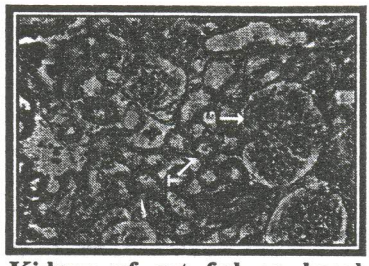
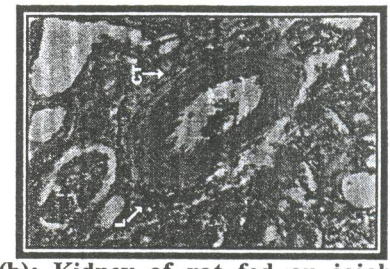
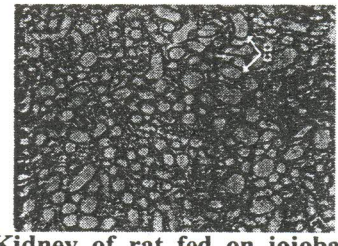


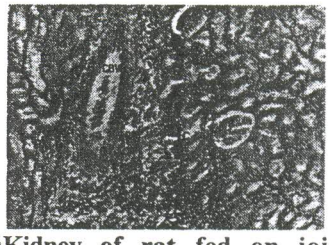
(a): Kidney of rat fed on basal diet showing normal histological structure of renal tubules (T) and glomeruli (G). H & E stain x 400



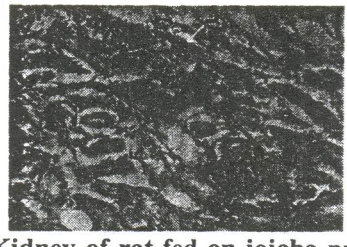
(b): Kidney of rat fed on jojoba meal treated with isopropanol showing perivascular C.T proliferation (CT) infiltrated with lymphocytes (L). H&E stain x 400



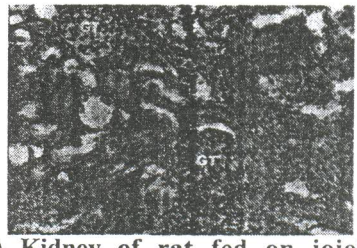
(c): Kidney of rat fed on jojoba meal treated with ammoniacal hydrogen peroxide showing cystic dilatation of some renal tubules (CD). H&E stain x 200



(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

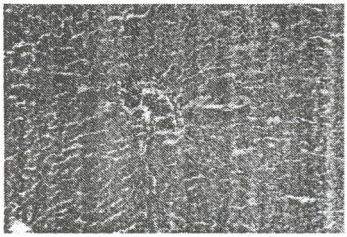


(e): Kidney of rat fed on jojoba protein isolate showing aggregation of few lymphocytes (L) in the interstitial tissues. H&E stain x 200.

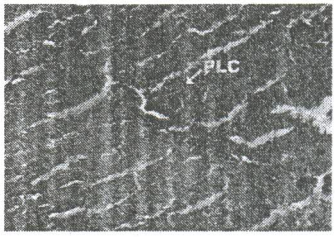


(f) Kidney of rat fed on jojoba meal showing proliferation of the glomerular tufts (GT) and occlusion of Bowman's spaces. H&E stain x 400

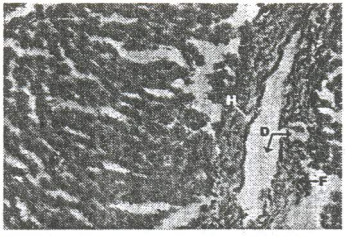
Fig. (3a,b,c,d & f): Photomicrographs showing histopathological effects of different dietary on kidney of rats.



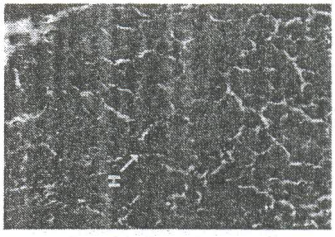
(a): Liver of rat fed on basal diet showing normal histologic structure of hepatic parenchyma. H&E stain x 200



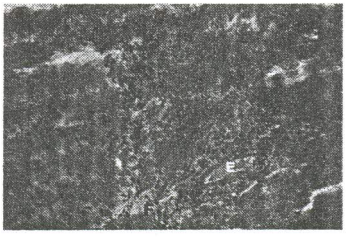
(b): Liver of rat fed on jojoba meal treated with isopropanol showing congested blood vessel with perivascular lymphocytic cellular aggregation (PLC). H&E stain x 400



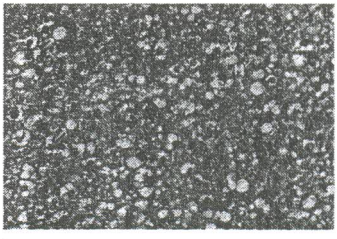
(c): Liver of rat fed on jojoba meal treated with ammonical hydrogen peroxide showing hyperplasia of bile ductal epithelium (H) with dilatation of the lumen (D) and periductal fibrosis (F). H&E stain x 400



(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 esinophilic 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. (4a,b,c,d,e & f): Photomicrographs showing histopathological effects of different dietary on liver of rats.

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الملخص العربي

التخلص من المركبات السامة والتقييم الغذائى لكسب الجوجوبا ومعزوله البروتينى

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عبدالنبى السيد الديب عبدالله السيد حسين
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يهدف البحث إلى الاستفادة من مخلف بذور الجوجوبا وكذلك التقييم الكيميائى والبيولوجى لها كمصدر للبروتين. وقد تم عمل محاولات لإزالة العوامل المضادة للتغذية باستخدام المذيبات أو المعاملات الحرارية من كسب البذرة.

كما تم عمل تجربة تغذية على حيوانات تجارب (ذكور فئران) للتقييم الغذائى لكسب الجوجوبا ومعزوله البروتينى. وأوضحت النتائج ما يلى:

- التركيب الكيمائى لكسب الجوجوبا: البروتين الخام ٣١,٨٩±١,١٢، الكربوهيدرات ٣٩,٧٨±٠,١١، الألياف الخام ١٠,٢٠±٠,١٤، الرماد ٣,١١±٠,١١% كما احتوى الكسب على مضادات التغذية وهى السيموندسين بنسبة ٣,٣٣±٠,٠٢، الفينولات الكلية ٢,٦٧±٠,٠٢ وحمض الفيتيك ٢,٣٩±٠,٠٥% على أساس الوزن الجاف.
- وجد أن أعلى نسبة لاستخلاص بروتين الجوجوبا عند درجة حموضة ١٠ حيث كانت نسبة البروتين المستخلص ٩٤,١٣% بينما عند درجة حموضة ٤,٨ (نقطة التعادل الكهربائى) كانت اقل نسبة للبروتين المستخلص ٢٠,٧٦%.
- احتوى الكسب على نسبة عالية من حمض الجلوتاميك والأسبارتيك يليها الجليسين، علاوة على ذلك كان إجمالى الأحماض الأمينية الأساسية ٣٧,١%.
- وقد أظهرت النتائج أن أحسن معاملة للتخلص من السيموندسين هى الأيزوبروبانول : الماء (٧ : ٣) حيث تم التخلص من ٨٣,٤٨% منها. كما وجد أن أفضل معاملة حرارية للتخلص من مضادات التغذية كانت على درجة ١٠٠°م لمدة ٣ ساعات.
- وفى التجربة البيولوجية وجد أن الفئران المغذاة على عليقة تحتوى على كسب الجوجوبا الغير معامل حدث لها نفوق تام بعد ١٢ يوم من التجربة وبعد إعادة هذه التجربة أخذت عينات الدم والأعضاء قبل ١٢ يوم وأظهرت النتائج حدوث نقص فى وزنها وقد حدث فيها ارتفاع ملحوظ فى دهون الدم المختلفة (الجليسريدات الثلاثية – الكوليسترول الكلى) مع زيادة في نشاط إنزيمات الكبد ووظائف الكلية، وانخفاض بسيط فى مكونات بروتينات الدم (البروتين الكلى – الألبيومين – الجلوبيولين). كما حدث انخفاض بسيط فى هيموجلوبين الدم.
- كما لوحظ أن استخدام بروتين الجوجوبا المعزول ، وكذلك المخلف المعامل كيميائياً فى التغذية له تأثير جيد حيث اتضح حدوث زيادة فى الوزن وتحسن ذو قيمة إحصائية في كفاءة تحويل الغذاء. كذلك تحسن فى نسبة الدهون فى الدم. كذلك تحسن ملحوظ فى بروتينات الدم وكذلك وظائف الكبد والذى انعكس فى خفض معدل الزيادة الواضحة فى نشاط الإنزيمات الناقلة لمجموعة الأمين.