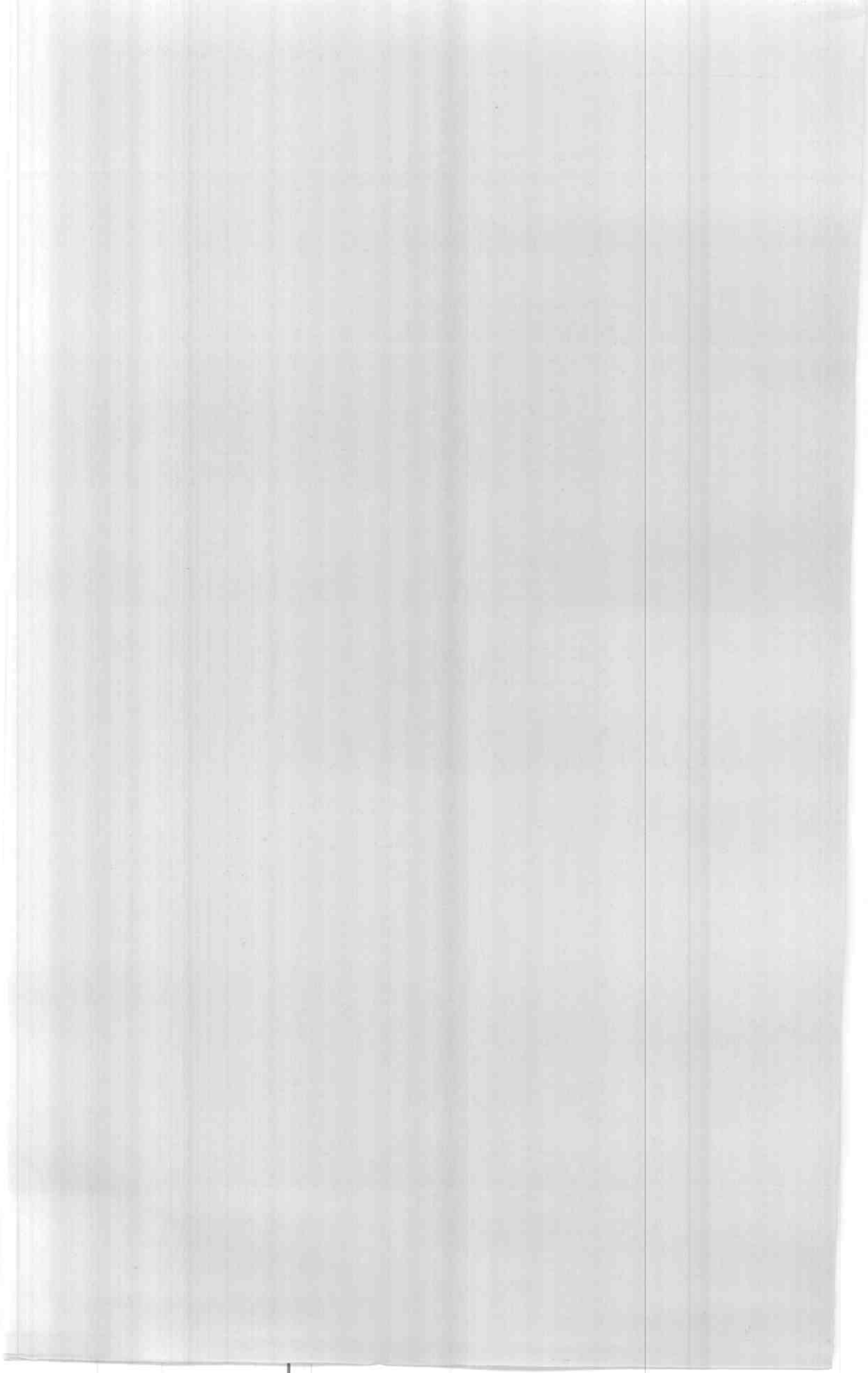
RESULTS AND DISCUSSION



4. RESULTS AND DISCUSSION

4.1. Chemical composition of sweet lupine and fenugreek seeds, defatted flours and their protein isolates:

The data presented in table (7) showed that moisture content in sweet lupine seed had lower than fenugreek seed. In contrast, moisture content in protein isolate of fenugreek had higher than that of sweet lupine. The mean values of moisture content were (6.85, 9.25, 2.53, 7.70, 9.01 and 7.12% for sweet lupine and fenugreek seeds, its flours and their protein isolates, respectively. These results agree with **Hussein, (1979) and King *et al.*, (1985).**

The whole sweet lupine seed and its defatted flour had significantly higher protein content than fenugreek seed and its flour. The protein content of lupine and fenugreek seeds was 37.94% and 30.25%, respectively. While their defatted flours contained 40.97 and 32.76%, respectively.

The protein content of protein isolate of sweet lupine and fenugreek was 93.72% and 91.48%, respectively. These results agree with **Rao and Sharma, (1987)** for fenugreek seeds and its flour, **Donangelo *et al.*, (1995)** for sweet lupine seed and **King *et al.*, (1985)** for isolate of lupine.

Sweet lupine seed contained higher percentage of total lipids (11.24%) compared with fenugreek seed (8.9%). While the lipids content of their defatted flours were 1.99% and 2.83%, respectively. Non significant differences were observed between isolates of sweet lupine and fenugreek in their contents for crude

lipids. These results agree with those reported by **Abdeen, (1987)** for *Lupinus termis* (9.8-11.7%), **Sauvaire *et al.*, (1976)** for fenugreek seed (7.1-8.8%).

Significant differences were noticed in crude fiber between sweet lupine and fenugreek seeds and their defatted flours. Meanwhile non-significant difference was observed between their isolates. These results agree with **Yanez *et al.*, (1983)** for lupine seeds (11.3%-16.80%), **Hussein, (1979)** for fenugreek seeds (9.02%).

Table (7): Chemical composition of sweet lupine and fenugreek seeds, defatted flours and protein isolates (gm/100gm sample on dry weight basis).

Chemical composition	Sweet lupine			Fenugreek			LSD at 0.
	Seed	(D.F) Flour	Protein isolate	Seed	(D.F) Flour	Protein isolate	
Total protein	37.94^{d}	40.97^{c}	93.72^{a}	30.25^{f}	32.76^{e}	91.48^{b}	0.7936
Crude lipids	11.24^{a}	1.99^{d}	1.13^{e}	8.90^{b}	2.83^{c}	1.41^{de}	0.6085
Crude fiber	11.48^{b}	14.52^{a}	1.17^{e}	8.36^{d}	9.96^{c}	1.63^{e}	0.8513
Total ash	3.34^{b}	3.93^{a}	1.60^{c}	3.21^{b}	3.49^{b}	1.95^{c}	0.3646
Total carbohydrates*	36.00^{d}	38.58^{c}	2.37^{e}	49.28^{b}	50.9^{a}	3.52^{e}	1.313
Moisture	6.85^{c}	9.25^{a}	2.53^{d}	7.70^{b}	9.01^{a}	7.12^{c}	0.5654

a,b,c,... There is no significant difference (P> 0.05) between any two means within the same row have the same letter.

*Total carbohydrates were calculated by difference. D.F = Defatted

From the same table, there were no significant differences found between sweet lupine and fenugreek seeds for ash content. Also, non-significant differences between isolates of lupine and fenugreek were noted while sweet lupine flour had higher ash content than fenugreek flour . The results agree with those reported by **King *et al.*(1985)**

Total carbohydrates in sweet lupine and its flour were significantly lower than that in fenugreek and its defatted flour. Total carbohydrates content of sweet lupine seed and its flour were 36.0% and 38.58%, respectively. While, fenugreek seed and its flour resulted in 49.28% and 50.90%, respectively. This increase in total carbohydrates may be due to the removal of total lipids during defatted process. These results agree with **Hussein, (1979)** for fenugreek seed (49.72%), **Abdeen, (1987)** for *Lupinus termis* varieties (32.16%-40.1%).

4.1.1. Electrophoretic pattern of sweet lupine and fenugreek protein isolates using (SDS- PAGE).

Protein fractions of sweet lupine and fenugreek protein isolates were separated using sodium dodecyl sulphate polyacrylamide gel electrophoresis SDS- PAGE. The molecular weights of the protein fractions were calculated using protein markers having known molecular weights. The obtained data are shown in Table (8) and illustrated in Fig (2).

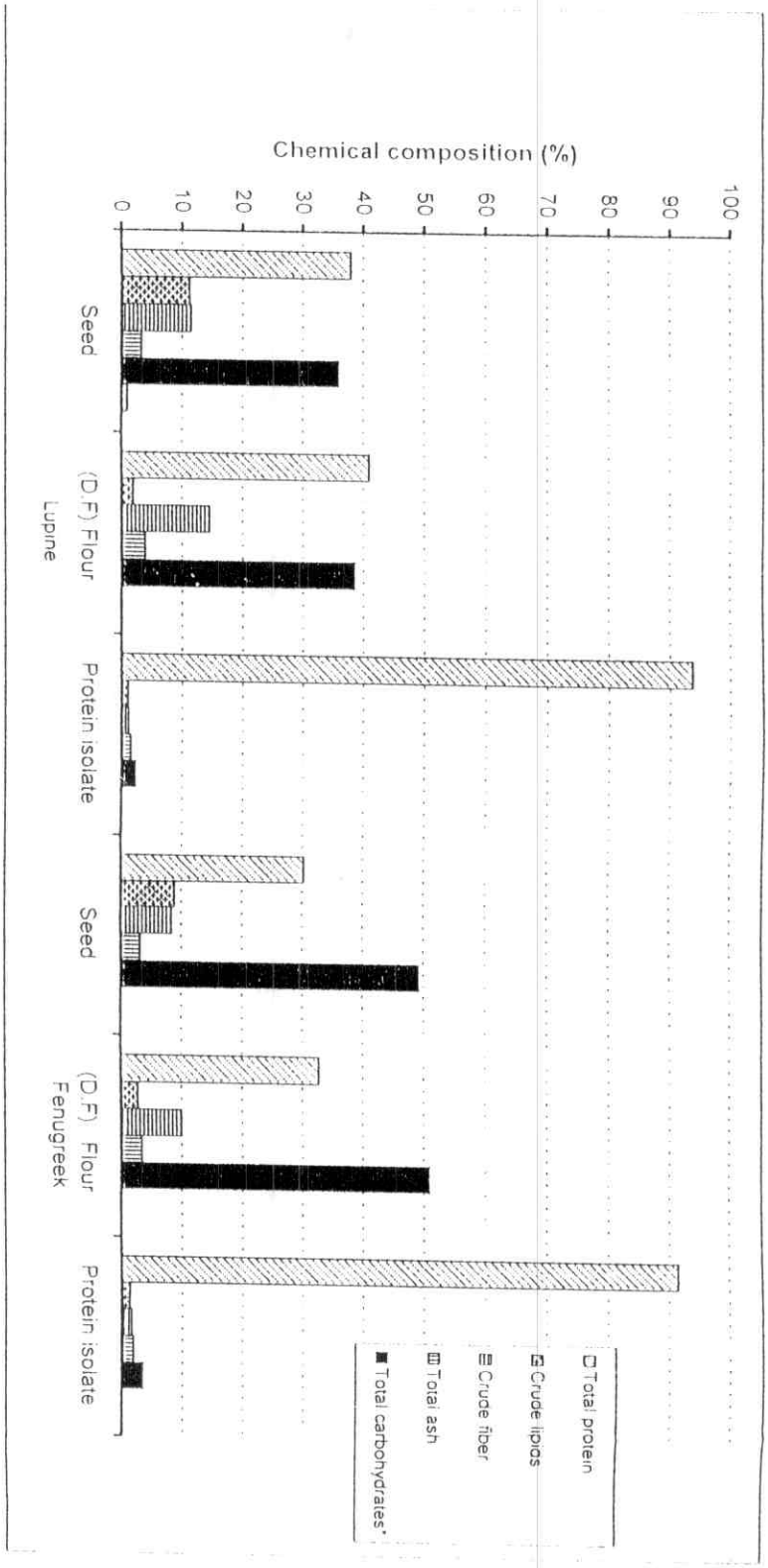
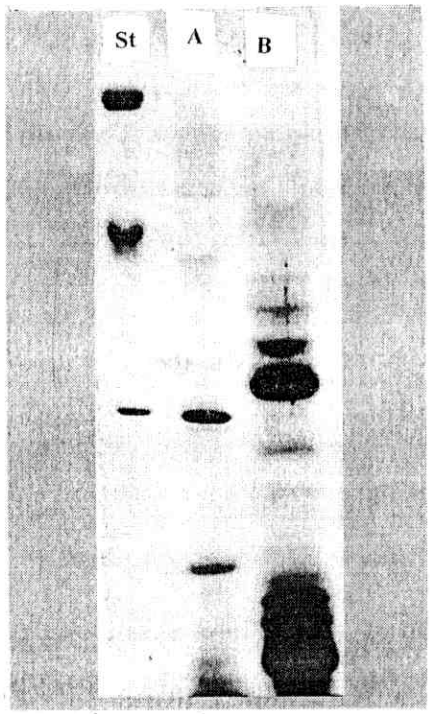


Fig (1): Chemical composition of sweet lupine and fenugreek seeds, defatted flour and protein isolate (gm/100gm sample on dry weight basis).



Fig(2):Sodium dodecly sulphate polyacrylamide electrophoresis (SDS- PAGE) of protein fractions of sweet lupine (B) and fenugreek (A) protein isolates.

The data presented in table (8) and illustrated in Fig (2) show that the protein of sweet lupine protein isolate is fractionated into 7 fractions with molecular weight of wide range from 5 to 81 KD_a whereas, protein of fenugreek protein isolate was fractionated into 6 fractions, with molecular weight of wide range from 2 to 78 KD_a.

From the results presented in table (8) and Fig (2) it could be observed that the amounts of protein fraction of sweet lupine protein isolate with molecular weight 5, 20, 24, 30, 35, 39 and 81 KD_a were 30.67%, 3.58%, 4.07%, 15%, 5.79%, 25.93% and 14.96%, respectively. Whereas the amounts of protein fraction of

fenugreek of protein isolate with molecular weight 2, 12, 28, 45, 58 and 78 KD_a were 18.96%, 10.06%, 18.43%, 13.26%, 20.29% and 19.19%, respectively.The presence of low molecular weight subunits means more solubility and activity which act as enzymes or hormones like substances. These results agree with **Oomah and Bushuk (1984)and Zacarias et al., (1990).**

Table (8):Electrophoretic pattern of sweet lupine and fenugreek proteins isolates using (SDS- PAGE).

Samples		Peak number						
		1	2	3	4	5	6	7
Marker	KD_a	66	48	29	2	-	-	-
	Amt%	32.88	36.27	14.79	16.06	-	-	-
Fenugreek	KD_a	78	58	45	28	12	2	-
	Amt%	19.19	20.09	13.26	18.43	10.06	18.96	-
Sweet lupine	KD_a	81	39	35	30	24	20	5
	Amt%	14.96	25.92	5.79	15.00	4.07	3.58	30.67

KD_a = 1000 Dalton (molecular weight of fraction) Amt % = Amount of fraction

4.2. Physical and chemical properties of oils:

Data presented in table (9) showed, the physicochemical properties of oils which included the refractive index, acid value, peroxide value and thiobarbaturic acid value.

From Table (9) it could be observed that refractive index (at 25°C) of cottonseed oil was 1.464, corn oil was 1.471, olive oil was 1.465 and sunflower seed oil was 1.4680. Refractive index

is used for the estimation of the degree of unsaturation of different oils.

Non-significant differences were observed between different oils for acid value except olive oil which had the highest acid value. The acid values of oils were 0.56, 0.75, 2.44 and 0.55 mg KOH/gm oil for cottonseed oil, corn oil, olive oil and sunflower oil, respectively.

Data in the same table showed that, olive oil had the highest significant of the peroxide value compared with the other oils followed by sunfiower oil, while non-significant difference was noticed between cottonseed oil and corn oil for peroxide value. The peroxide values of cottonseed oil, corn oil, olive oil and sunflower oil were 2.33, 2.10, 9.27, 4.55 meq/kg oil, respectively.

Thiobarbaturic acid value in different oils was relatively small and non significant differences was observed.

Table (9): Physical and chemical properties of dietary oils.

Character / Sample	Refractive Index	Acid value	Peroxide value	Thiobarbaturic acid value
Cottonseed oil	1.464	0.560^{b}	2.33^{c}	0.017^{a}
Corn oil	1.471	0.747^{b}	2.10^{c}	0.013^{a}
Olive oil	1.465	2.44^{a}	9.27^{a}	0.022^{a}
Sunflower oil	1.468	0.547^{b}	4.55^{b}	0.017^{a}
LSD	-	0.3315	0.5191	N.S.

a,b,c,... There is no significant difference (P> 0.05) between any two means within the same coulmn have the same letter.

4.1.2. Fatty acid composition of dietary oils:

The fatty acids composition of cottonseed oil, corn oil, olive oil and sunflower oil are presented in table (10).

The obtained data showed that saturated fatty acids was 10.99%, 22.20%, 13.85%, and 45.02% for sunflower oil, olive oil, corn oil and cottonseed oil, respectively.

Palmitic acid ($c_{16:0}$) was the predominant saturated fatty acid in all oils which ranged from 8.44% (sunflower oil) to 42.51% (cottonseed oil), while stearic acid ($c_{18:0}$) was found in amounts ranged from 1.85% (corn oil) to 2.34% (olive oil). Also, arachidic acid ($c_{20:0}$) was 1.3% in olive oil.

From the results shown in table (10) it could be noticed that oleic acid ($c_{18:1}$) ranged from 25.60% to 58.16% for cottonseed oil and sunflower oil, respectively. In contrast linoleic acid ($c_{18:2}$) ranged from 28.98% to 57.44% for cottonseed oil and corn oil, respectively. while linolenic acid ($c_{18:3}$) was 0.22% in olive oil. On the other hand, olive oil characterized by the highest amount of monounsaturated fatty acids 60.43% followed by sunflower oil 58.16%, corn oil 28.72% and cotton seed oil 26.0%, while corn oil characterized by the highest amount of polyunsaturated fatty acid 57.44% followed by sunflower oil 30.85%, cottonseed oil 28.98% and olive oil 17.34%.

These results agree with **Pearson (1981); Di-Giovacchino (1996) and (Snyder and Mounts, 1990).**

Table(10): Fatty acids composition of cottonseed oil, corn oil, olive oil and sunflower oil.

Fatty acid	Cottonseed oil	Corn oil	Olive oil	Sunflower oil
$C_{14:0}$	0.26	-	0.15	0.33
$C_{16:0}$	42.51	12.00	18.35	8.44
$C_{16:1}$	0.40	0.21	3.23	-
$C_{18:0}$	2.25	1.85	2.34	2.22
$C_{18:1}$	25.60	28.51	56.35	58.16
$C_{18:2}$	28.98	57.44	17.12	30.85
$C_{18:3}$	-	-	0.22	-
$C_{20:0}$	-	-	1.36	-
$C_{20:1}$	-	-	0.85	-
SFA%	45.02	13.85	22.20	10.99
MUFA%	26.00	28.72	60.43	58.16
PUFA%	28.98	57.44	17.34	30.85

SFA= Saturated fatty acid.

MUFA= Monounsaturated fatty acid.

PUFA = Polyunsaturated fatty acid.

4.3. Biological evaluation:

4.3.1. Body weight gain:

The data in Table (11) showed that the body weight gain was 81.8% for the negative control, while it was 53.55% for the positive one. It could be seen that the highest significant decrease in weight gain for group fed on hypercholesterolemic diet containing defatted fenugreek flour compared with positive control, while the significant increase of weight gain ratio were found in groups fed on hyporcholesterolemic diets containing defatted lupine flour or protein isolate of fenugreek compared with positive control. On the other hand, dietary oils showed that significant increase in body weight gain by about 70.87%, 80.01% and 72.50% for groups fed on hypercholesterolemic diet containing corn oil, olive oil and sunflower oil, respectively. Mean while feeding on diets containing propolis (ethanol extract) showed significant increase in body weight compared with positive control.

It could be concluded that the increase in body weight gain may be due to the increased appetite which reflect the amount of high food intake of treated rats, by significant difference was found between cholesterol fed and negative control. The results in agreement with **Rao and Sharma (1987)**who showed that the food intake ,body weight gain and protein efficiency ratio of rats receiving fenugreek seeds alone was significantly lower than rats fed on casein diet. This effect may be due to the protein digestibility of fenugreek which is low due to gum in the seeds .

Table (11): Body weight, food intake, food efficiency and protein efficiency ratio of hyerpcholesterolemic rats fed on different experimental diets.

Animal group diet	Body weight				Food intake		
	Initial (gm)	Final (gm)	Gain %	Daily gain (gm)	Daily intake (gm)	Food efficiency ratio	Protein efficiency ratio
Negative control	a 159.83	a 290.57	a 81.80	a 1.45	a 15.42	a 9.42	a 0.942
Positive control	bc 131.30	e 201.87	d 53.75	d 0.78	i 11.22	c 6.99	c 0.699
D.F. lupine flour	b 139.12	bcd 226.33	c 62.70	c 0.97	d 11.73	b 8.26	b 0.826
Lupine protein solate	bc 129.50	e 201.84	d 55.86	d 0.80	j 10.72	c 7.49	c 0.749
D.F.fenugreek lour	bc 129.57	f 155.01	e 19.63	e 0.28	f 11.64	d 2.43	d 0.243
Fenugreek protein solate	bc 133.76	d 216.64	c 61.95	c 0.92	b 12.35	c 7.46	c 0.746
Corn oil	bc 135.66	bc 231.81	b 70.87	b 1.07	h 11.50	a 9.29	a 0.929
Olive oil	c 123.69	cd 222.65	a 80.01	b 1.10	e 11.68	a 9.42	a 0.942
unflower oil	bc 132.71	bcd 228.93	b 72.50	b 1.07	c 11.81	a 9.05	a 0.905
ropolis (ethanol xtract)	b 140.27	b 237.03	b 68.98	b 1.07	g 11.53	a 9.32	a 0.932
SD at 0.05	12.95	11.38	6.595	0.07617	0.01703	0.6258	0.05386

a,b,c,... There is no significant difference (P> 0.05) between any two means within the same column have the same letter.

Negative control: basal diet without cholesterol.

Positive control : basal diet with 1% cholesterol.

Data presented in Table (11) showed that, food intake was 15.4 gm/day for the negative control, while it was 11.22 gm/day for the positive one. The significant higher intake of foods in the negative control suggests the influence of the quality and ingredients of the diet on food intake, which may be related to differences in diet palatability. All groups fed on tested diets except group fed on lupine protein isolate were higher than positive control.

The food efficiency and protein efficiency ratios were (9.42 and 6.99)and (0.942 and 0.699) for the negative and positive control, respectively. It is clearly observed that rats fed on diet containing defatted lupine flour or protein isolate had higher food efficiency and protein efficiency ratios, while it lowered of rats fed on defatted fenugreek flour diet when compared with positive control. However, dietary oils (corn oil, olive oil and sunflower oil) or propolis ethanol extract increased both food efficiency and protein efficiency ratios.

In this respect, **Hassan and Said, (1999)** reported that kareesh cheese and casein diets at levels of 10% and 30% showed the highest increase in food intake and body weight gain when compared with the diets containing lupine (termis), soybean and meat.

4.3.2. Organs weight/body weight ratio:

Data concerning the calculation of organ weight/body weight ratio for the different tested diet groups with or without cholesterol for ten weeks are presented in Table (12).

From this table, the mean value of liver weight/body weight (%) of rats fed on positive diet (4.42%) showed significant increase compared with rats fed on negative diet (3.01%). This increase in relative ratio liver weight may be attributed to the increase in the amount of cholesterol in the diet. The mean of liver weight significantly increased with defatted lupine diet (4.87%) and with protein isolate from lupine and fenugreek diets (5.33%, 5.22% respectively) than with the control diet. The highest significant liver weight was found due to sunflower oil diet, and olive oil diet, which amounted in 5.69%, 5.65%, respectively, the increase of liver weight in rats fed on different diets generally may be due to accumulation of fats in the liver tissues, **Halhotra(1984)** compared with the positive diet.

Data presented in Table (12), showed that, kidneys weight/body weight ratio was affected by the different tested diet. Rats fed on defatted fenugreek diet had the highest kidney ratio (0.70%), while rats fed on negative diet (basal diet without cholesterol) had the lowest value of kidneys weight/body weight (0.52).Non- significant differences between other groups. This results agree with **Abd,El Salam and Abdel- Megeid (1998).**

Data obtained in Table (12) showed that, all rats fed on the tested diet with cholesterol had greater values of heart ratio when compared with negative diet. The data showed also that the average values of spleen ratio ranged from 0.167% to 0.270% with non- significant differnces between treatments. The mean values of testes ratio ranged from 1.04% for these fed on diet containing olive oil to 1.41% for these fed on diet containing

defatted fenugreek flour while the mean value of testes ratio was 0.86% for negative diet which significantly decreased than those of all diets under study.

Data in Table (12) showed that significant increase occured in brain weight/body weight ratio between control negative and control positive which fed on diets with or without cholesterol.

Table(12):Organs weight/body weight ratio of hypercholeste -rolemic rats fed on different experimental diets.

Animal group Diet	Final body weight	% Liver	% Heart	% Spleen	% Kidney	% Brain	% Lung	% Testes
Negative control	a 290.57	f 3.01	e 0.283	c 0.167	d 0.523	d 0.570	d 0.420	d 0.857
Positive control	e 201.87	e 4.42	d 0.330	abc 0.213	bc 0.633	c 0.713	d 0.403	a 1.287
D.F. lupine flour	bcd 226.33	cd 4.87	bcd 0.360	bc 0.197	bc 0.633	c 0.700	bc 0.527	a 1.293
Lupine protein isolate	e 201.84	b 5.33	cd 0.343	bc 0.207	ab 0.687	b 0.853	ab 0.563	1.307
D.F. fenugreek flour	f 155.01	d 4.76	a 0.430	ab 0.230	a 0.700	a 0.960	a 0.597	1.407
Fenugreek protein isolate	d 216.64	b 5.22	b 0.390	bc 0.200	abc 0.657	c 0.713	c 0.497	1.087
Corn oil	bc 231.80	bc 5.10	cd 0.343	ab 0.233	bc 0.637	c 0.703	abc 0.557	c 1.153
Olive oil	cd 222.65	a 5.65	bcd 0.363	ab 0.257	c 0.613	c 0.727	bc 0.530	c 1.040
Sunflower oil	bcd 228.93	a 5.69	bc 0.383	a 0.270	c 0.617	c 0.727	abc 0.547	1.050
Propolis (ethanol extract)	b 237.03	bc 5.10	bcd 0.353	ab 0.247	abc 0.673	c 0.667	abc 0.547	b 1.170
LSD at 0.05	11.38	0.2799	0.03808	0.05386	0.05386	0.07617	0.05386	0.120

a,b,c,... There is no significant difference (P> 0.05) between any two means within the same column have the same letter.

Negative control: basal diet without cholesterol. D.F.= Defatted.
Positive control : basal diet with 1% cholesterol.

4.3.3. Biochemical parameters of blood:

4.3.3.1. Serum total lipids:

The serum total lipids values of rats fed on different diets under investigation during feeding period (10weeks) are presented in Table (13) and illustrated by fig (3).

The data showed that, serum total lipids in negative control group ranged between 0.593 and 0.632 gm/dL during feeding periods with mean value of 0.617 gm/dL at the end of the experiment.

Also, the data in the same Table showed that a significant increase in serum total lipids in positive control group as compared with negative control. A significant decrease in serum total lipids for groups of rats fed on test diets compared with positive control at the end of experimental period.

It could be noticed that, the lowest values of total serum lipids were found in the group fed on either defatted lupine or its protein isolate at the end of the experimental period (0.734 and 0.807 gm/dL) followed by defatted fenugreek flour and corn oil containing diets (0.920 and 0.946 gm/dL),while the highest value was found in rats fed on olive oil containing diet (2.073gm/dL).The results in this table showed that total serum lipids in rats fed on propolis ethanolic extract containing diet raised gradually from 0.667gm/dL at zero time to reach 1.312gm/dL after six weeks, then reduced to 1.015gm/dLat the end of the exprimental perioed . It could be observed that significant increase in mean of total lipids after 6 weeks followed by significant decrease after 10 weeks in all treatments. On the

Table (13): Effect of dietary proteins, dietary oils and propolis ethanol extract on total lipids levels (gm/dL) in hypercholesterolemic rats during the feeding periods.

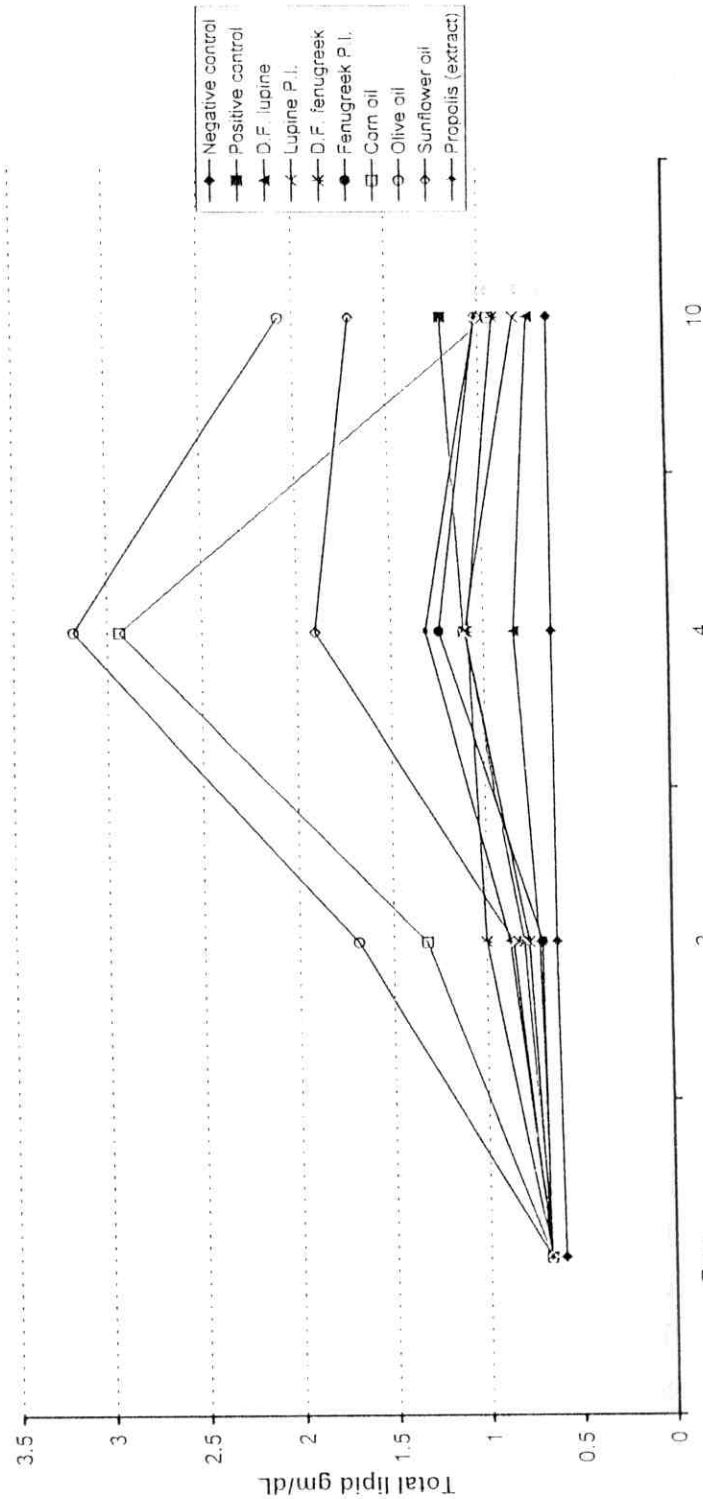
Animal group diet	Feeding periods(week)				Mean
	Zero	(2)	(6)	(10)	
Negative control	s 0.593	rs 0.618	qrs 0.632	qrs 0.627	g 0.617
Positive control	pqrs 0.667	l-r 0.793	gh 1.106	fg 1.204	d 0.942
D.F. lupine flour	pqrs 0.667	n-s 0.712	k-p 0.836	n-s 0.734	f 0.737
Lupine protein isolate	o-s 0.667	m-s 0.766	gh 1.100	l-q 0.807	e 0.835
D.F. fenugreek flour	pqrs 0.667	hijk 0.997	ghi 1.088	i-m 0.920	d 0.918
Fenugreek protein isolate	pqrs 0.667	n-s 0.694	fg 1.240	hij 1.013	de 0.904
Corn oil	o-s 0.667	f 1.318	b 2.934	h-l 0.946	b 1.466
Olive oil	o-s 0.667	e 1.685	a 3.179	c 2.073	a 1.901
Sunflower oil	o-s 0.667	j-o 0.852	d 1.903	e 1.697	c 1.280
Propolis (ethanol extract)	o-s 0.667	j-n 0.872	f 1.312	hij 1.015	d 0.967
Mean	0.660^{d}	0.931^{c}	1.533^{a}	1.104^{b}	

- Any two means have the same letter are non- significantly different at ($p > 0.05$).

LSD for time at $\alpha 0.05 = 0.04875$ D.F.=Defatted

LSD for treatment at $\alpha 0.05 = 0.07707$

LSD for treatment within time at $\alpha 0.05 = 0.1541$



Fig(3): Effect of dietary proteins, dietary oils and propolis ethanol extract on total lipid levels (gm/dL) in hypercholesterolemic rats during the feeding periods.

other hand significant differences in means of total lipid for treatments, the lowest value was 0.617gm/dL for negative control,while the highest value was 1.901gm/dL for olive oil.

These results are in the line with the findings of **Mikhail *et al.*, (1996)** who mentioned that, there was a significant drop in total lipids of hyperlipidemic rats fed on lupine, lupine/fenugreek and fenugreek protein diets compared with casein diet. This effect may be due to several factors such as: presence of dietary fiber, the action of amino acids **(Sautier *et al.*, 1982),** presence of saponins, presence of tannins **(Gamal and Hamed., 1991)** and **Kritchevsky *et al.*, (1984)** who found that serum and liver lipids were higher in rabbits fed on olive oil diet than groundnut oil diet.

4.3.3.2. Serum triglycerides (TG):

Elevated serum triglycerides (TG) levels were reviewed as an independent risk factor in coronary heart disease **(Pilch, 1987).**

Results obtained in Table (14) and illustrated graphically in fig (4) showed that serum triglycerides of rats fed on negative diet ranged from 36.38 mg/dLto 42.40 mg/dL with mean value of 39.32 mg/dL during the experimental period (10weeks). Significant increase in serum triglycerides by feeding on hypercholesterolemic diet, which raised from 95.15 mg/dL to 121.82 mg/dL at the end of feeding period. It could be noted that, there were significant increase in positive control group compared with all treated groups during feeding period.

Data presented in the same Table also showed that rats fed on diet containing defatted lupine or its protein isolate had the lowest values of triglycerides 43.68 mg/dL and 56.08 mg/dL, respectively. While, serum rats triglycerides fed on defatted fenugreek or its protein isolate decreased gradually to reach a minimum concentration 62.79mg/dL and 63.23 mg/dL, respectively at the end of feeding period (10weeks). On the other hand, rats fed on dietary oils significantly increased serum triglycerides to reach 124.07,118.95,119.76 mg/dL after 4 weeks followed by decrease gradually to reach 62.07, 73.67 and 94.19 mg/dL after 10 weeks for corn oil, sunflower oil and olive oil, respectively.

The data showed that mean of triglycerides significantly decreased from 89.29mg/dL at zero time to reach 67.77mg/dL at the end of the experimental period .On the other hand the highest mean value of triglycerides was 105.08mg/dL in groups fed on olive oil diet with 1% cholesterol ,while the lowest mean value was 69.12mg/dL for defatted lupine flour .

The results, also showed that serum triglycerides in rats fed on propolis ethanol extract decreased gradually from 95.15 mg/dL at zero time to reach 70.41 mg/dL at the end of feeding period. These results were in the line with the findings of **Olinescu *et al.*, (1983)** who found that propolis administration into rats reduced the triglycerides. These results are also agree with those of **Grundy and Denke, (1990)** who found that the elevation of serum triglycerides concentration in rats which fed on oils rich in oleic acid may be due to a stimulatory effect of oleic acid upon very low density lipoprotein synthesis or on

inhibitory effect of oleic acid upon very low density lipoprotein catabolism.

Table (14): Effect of dietary proteins, dietary oils and propolis ethanol extracts on triglycerides level (mg/dL) in hypercholesterolemic rats during feeding periods.

Animal group diet	Feeding period (week)						Mean
	Zero time	(2)	(4)	(6)	(8)	(10)	
Negative control	s 36.38	s 38.53	s 40.77	s 37.91	rs 42.40	s 39.74	39.32
Positive control	jkl 95.15	f-k 105.57	f-j 106.13	cdef 116.36	bc 125.69	bcd 121.82	111.8(
D..F. lupine flour	jkl 95.15	jklm 93.62	nop 70.41	pq 59.24	qr 52.62	rs 43.68	69.12
Lupine protein isolate	jkl 95.15	cdef 115.99	n 77.40	nop 70.19	opq 62.61	q 56.08	79.57
D.F.fenugreek flour.	jkl 95.15	a 137.75	ab 131.23	jkl 95.54	mn 82.22	qr 62.79	99.1
Fenugreek protein isolate	jkl 95.15	h-l 99.99	d-h 111.11	n 78.81	no 71.93	opq 63.23	86.7(
Corn oil	jkl 95.15	h-l 100.91	bc 124.07	lm 91.74	opq 64.40	opq 62.07	89.7
Olive oil	jkl 95.15	e-i 108.98	cde 119.76	c-g 113.94	g-l 103.08	jkl 94.19	105.8
Sunflower oil	jkl 95.15	g-l 102.86	cde 118.95	jkl 94.60	n 80.12	no 73.67	94.2
Propolis (ethanol extract)	jkl 95.15	klm 92.90	ijkl 98.38	lm 91.57	n 76.52	nop 70.41	87.4
Mean	89.29[b]	99.71[a]	99.82[a]	84.99[c]	76.16[d]	67.77[e]	

- Any two means have the same letter are non- significantly different at ($p > 0.05$).

LSD for time at $\alpha 0.05 = 3.258$ **D.F.**=Defatted

LSD for treatment at $\alpha 0.05 = 4.206$

LSD for treatment within time at $\alpha 0.05 = 10.30$

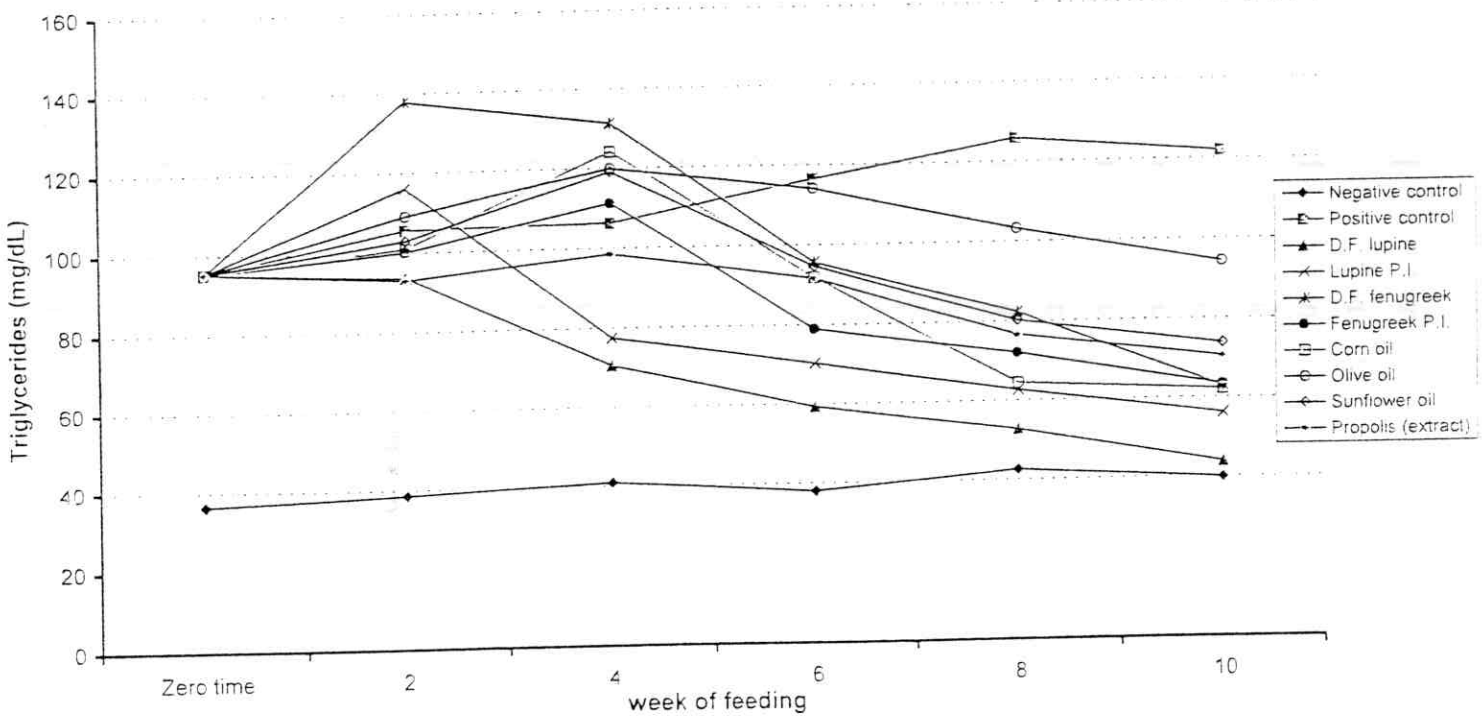


Fig (4): Effect of dietary proteins, dietary oils and propolis ethanol extracts on triglycerides level (mg/dL). in hypercholesterolemic rats during feeding periods

4.3.3.3. Serum total cholesterol:

Total serum cholesterol of hypercholesterolemic rats fed on the different diets during the experimental period is shown in Table (15) and fig (5).

The data showed that feeding cholesterol 1% and bile salts elevated serum total cholesterol at zero time to 128.72 mg/dL and reached to 225.47 mg/dL at the end of the experimental period in positive control. Serum total cholesterol in negative control group ranged between 72.59 and 78.32 mg/dL during feeding period. It could be observed that high significant increase of serum total cholesterol values in all groups after 4 weeks except defatted lupine and protein isolate of lupine groups compared with negative control.

Cholesterol of the rats fed defatted lupine, protein isolate of lupine and defatted fenugreek began to decrease significantly in serum while non significant difference was found due to feeding on protein isolate of fenugreek and propolis ethanol extract of serum total cholesterol level. In contrast, olive oil resulted in highest total cholesterol followed by sunflower oil and corn oil compared with positive control. Similar trend with gradual decreasing was found till the end of the experimental. From the data presented in the same table showed that significantly decrease of mean value of total cholesterol to reach 142.4mg/dL at the end of the experimental period (10weeks) .In contrast, significant differences between mean value of total cholesterol for treatments. The lowest mean value was 104.30mg/dL for defatted lupine flour ,while the highest mean

Table (15): Effect of dietary proteins, dietary oils and propolis ethanol extracts on total cholesterol level (mg/dL) in hypercholesterolemic rats during feeding periods.

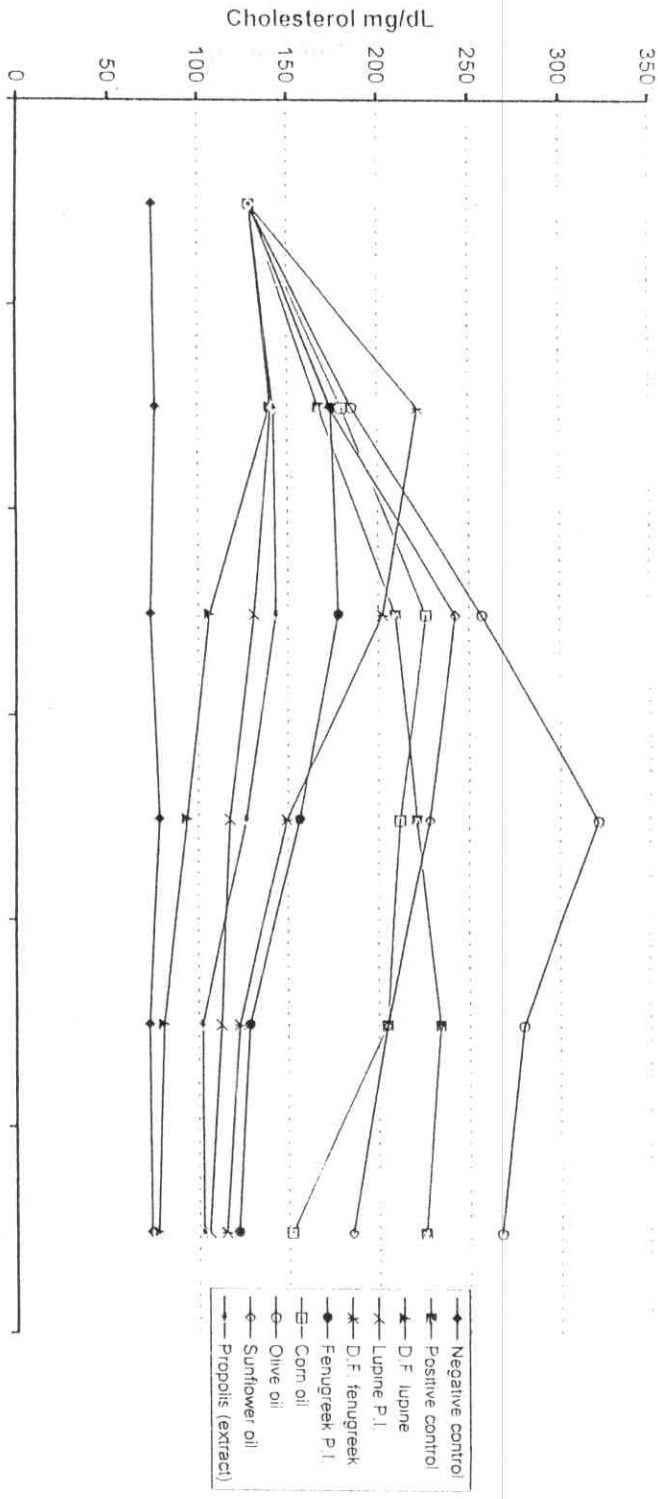
Animal group diet	Feeding period (week)						Mean
	Zero time	(2)	(4)	(6)	(8)	(10)	
Negative control	x 74.17	x 76.17	x 73.30	x 78.32	x 72.59	x 73.55	x 74.68
Positive control	opqr 128.72	jk 167.20	h 209.32	fg 221.11	de 233.77	ef 225.47	b 197.60
D.F. lupine flour	opqr 128.72	mnop 139.75	uv 106.02	w 93.63	x 80.56	x 77.28	g 104.30
Lupine protein isolate	opqr 128.72	mno 140.65	nopq 130.89	rstu 117.36	tuv 112.48	uvw 105.43	f 122.60
D.F.fenugreek flour	opqr 128.72	efg 221.94	h 202.38	lm 149.04	qrst 122.72	stu 114.95	d 156.60
Fenugreek protein isolate	opqr 128.72	ij 174.07	ij 177.81	kl 156.18	opqr 128.31	qrst 121.70	e 147.80
Corn oil	opqr 128.72	i 179.95	ef 226.25	gh 211.81	h 204.62	lm 151.54	c 183.80
Olive oil	opqr 128.72	i 185.66	c 256.94	a 320.94	b 279.48	c 267.08	a 239.70
Sunflower oil	opqr 128.72	ij 173.45	d 242.26	ef 228.15	h 204.74	i 185.25	b 193.80
Propolis (ethanol extract)	opqr 128.72	mn 142.24	mn 143.09	pqrs 126.51	vw 101.90	vw 102.20	f 124.10
Mean	123.30^{f}	160.1^{c}	176.8^{a}	170.3^{b}	154.1^{d}	142.4^{e}	

- Any two means have the same letter are non- significantly different at ($p > 0.05$).

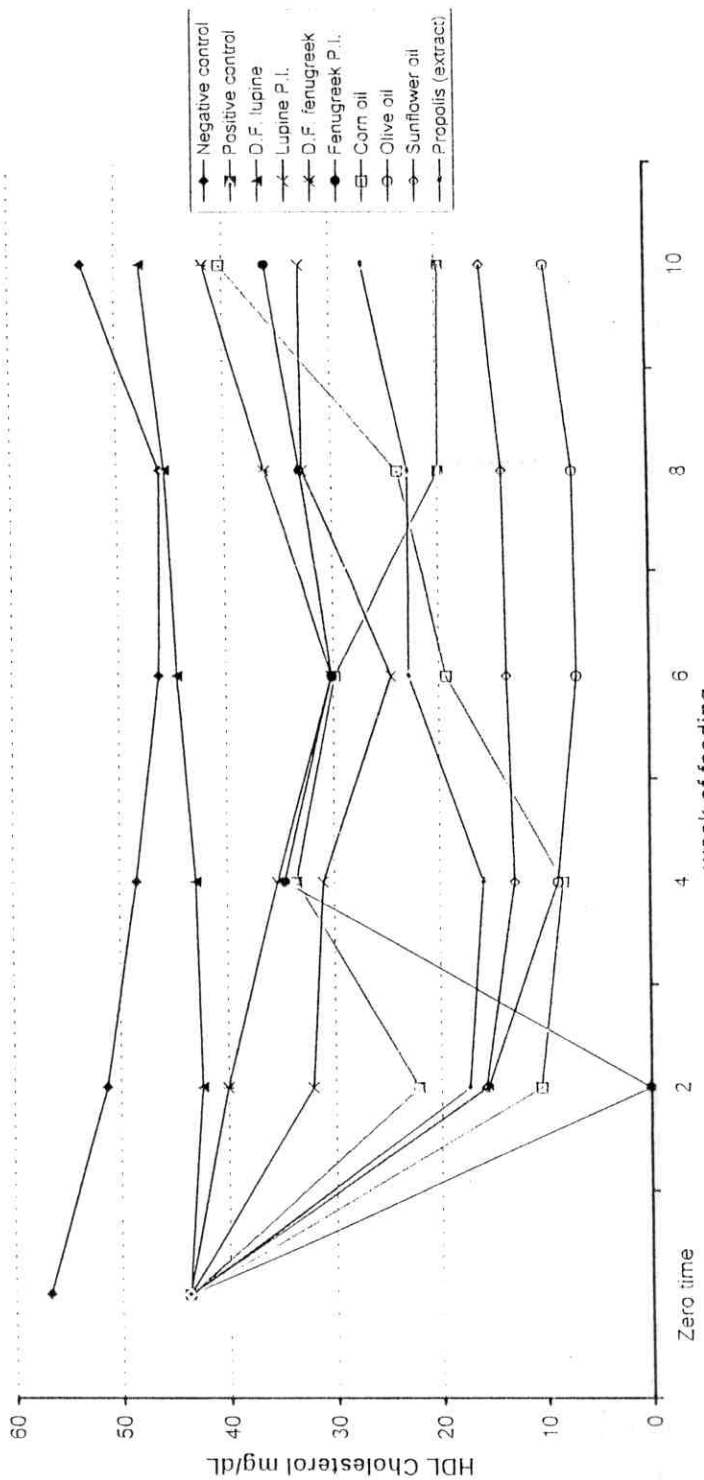
LSD for time at $\alpha 0.05 = 3.504$ **D.F.**=Defatted

LSD for treatment at $\alpha 0.05 = 4.523$

LSD for treatment within time at $\alpha 0.05 = 11.08$



Fig(5): Effect of dietary proteins, dietary oils and propolis ethanol extracts on total cholesterol level (mg/dL) in hypercholest- erolemic rats during feeding periods.



Fig(6): Effect of dietary proteins, dietary oils and propolis ethanol extract on high density lipoprotein (HDL-C) (mg/dL) in hypercholesterolemic rats during feeding periods.

There were non-significant differences between defatted lupine flour group and negative control group during experimental period. Groups fed on diets containing plant i.e. protein showed higher HDL-C level than positive control group. At the end of experimental period the values of HDL-C in serum rats which fed on defatted lupine, defatted fenugreek, protein isolate of fenugreek or protein isolate of lupine were 47.67, 41.80, 36.00 and 32.85 mg/dL, respectively. In contrast, rats fed on diets containing propolis ethanol extract, corn oil, sunflower oil and olive oil showed serum HDL-C amounted in 26.90, 40.27, 15.70 and 9.56 mg/dL respectively. The data showed that, the mean value of HDL-Cwas 44.98mg/dL at zero time and reached to 32.35 mg/dL after (10 weeks), while the highest mean value was 44.44 mg/dL for defatted lupine flour and the lowest mean value was 15.23mg/dL for olive oil diet compared with positive control.

These results agree with **Mikhail *et al.*, (1996)** who found that HDL-fraction showed marked elevation in serum hyperlipidemic rats fed on diets containing fenugreek flour, lupine flour and their mixture at 10% level of lyophilyzed protein. The results also agree with **Shen and Hg, (1995)** who showed that royal jelly reduced serum cholesterol and increased HDL-cholesterol levels.

4.3.3.5. Serum Low Density Lipoprotein cholesterol (LDL):

Consumption of vegetable proteins, vegetable oils and bee products (such as royal jelly, pollen, propolis) have been hypothesized to be protective against coronary heart disease and atherosclerosis because they lowers LDL-cholesterol in the

serum **(Keys, 1975** and **Janice, 1987).** The results in Table (17) and illustrated by fig (7) showed that LDL-C level was found in the negative control ranged from 10.16 mg/dL to 24.73 mg/dL with a mean value of 16.58 mg/dL compared with these fed on hypercholesterolemic diet (positive control) ranged from 64.23 mg/dL at zero time to 181.47 mg/dL with a mean value 146.1 mg/dL at the end of feeding period.

Serum LDL-C increased significantly in all treated groups after 2 weeks of feeding. The LDL cholesterol decreased after feeding on diets containing defatted lupine and fenugreek flours and their protein isolates while significant increase in LDL-C level was found in groups fed on dietary oil after 4^{th} week compared with positive control.

At 8^{th} and 10^{th} weeks there was significant decrease in serum LDL-C level in all treated groups, but groups fed on vegetable protein sources and propolis ethanol extract showed highly significant decrease in LDL-C than groups fed on dietary oils compared with positive control. Rats fed on plant proteins showed non-significant difference compared with negative control. Mean values of LDL-cholesterol concentration were 45.55, 73.58, 95.21 and 98.65 mg/dL for defatted lupine, protein isolate of lupine, protein isolate of fenugreek and defatted fenugreek groups, respectively. While mean values of LDL-C for groups fed on propolis ethanol extract, corn oil, sunflower oil and olive oil were 81.44, 141.30, 155.4 and 203.0 mg/dL, respectively. These results appears to be in agreement with those of **Duane (1997)** who found that legumes lower serum LDL-C by partially interrupting the enterohepatic circulation of bile acid

Table (17): Effect of dietary proteins, dietary oils and propolis ethanol extract on low-density lipoprotein level (LDL) (mg/dL) of hypercholesterolemic rats during feeding periods.

Animal group diet	Feeding period(week)					
	Zero time	(2)	(4)	(6)	(8)	(10)
Negative control	i 10.16	yzi 17.26	yzi 16.76	y 24.73	xzi 18.06	zi 12.53
Positive control	uv 64.23	n 123.99	k 154.58	j 168.03	gh 184.39	hi 181.47
D.F.lupine flour	uv 64.23	rst 78.66	w 49.07	x 36.18	y 24.27	xz 20.88
Lupine protein isolate	uv 64.23	r 85.37	r 84.39	rst 78.79	uv 67.36	v 61.36
D.F. fenugreek flour	uv 64.23	k 154.40	lm 140.85	q 99.76	tuv 70.05	uv 62.60
Fenugreek protein isolate	uv 64.23	n 123.73	no 118.97	op 110.31	rs 80.96	stu 73.06
Corn oil	uv 64.23	kl 149.37	fg 193.24	ij 174.16	j 167. 96	q 98.86
Olive oil	uv 64.23	kl 148.40	d 224.32	a 290.76	b 251.73	c 238.69
Sunflower oil	uv 64.23	m 137.21	e 205.67	f 195.71	hij 174.89	k 154.79
Propolis (ethanol extract)	uv 64.23	pq 106.41	pq 107.60	r 85.34	uv 63.82	v 61.22
Mean	58.82e	112.5c	129.5a	127.38b	110.35c	96.55d

- Any two means have the same letter are non- significantly different at ($p > 0.05$).

LSD for time at $\alpha 0.05 = 2.902$

LSD for treatment at $\alpha 0.05 = 3.746$ **D.F.** =Defatted

LSD for treatment within time at $\alpha 0.05 = 9.176$

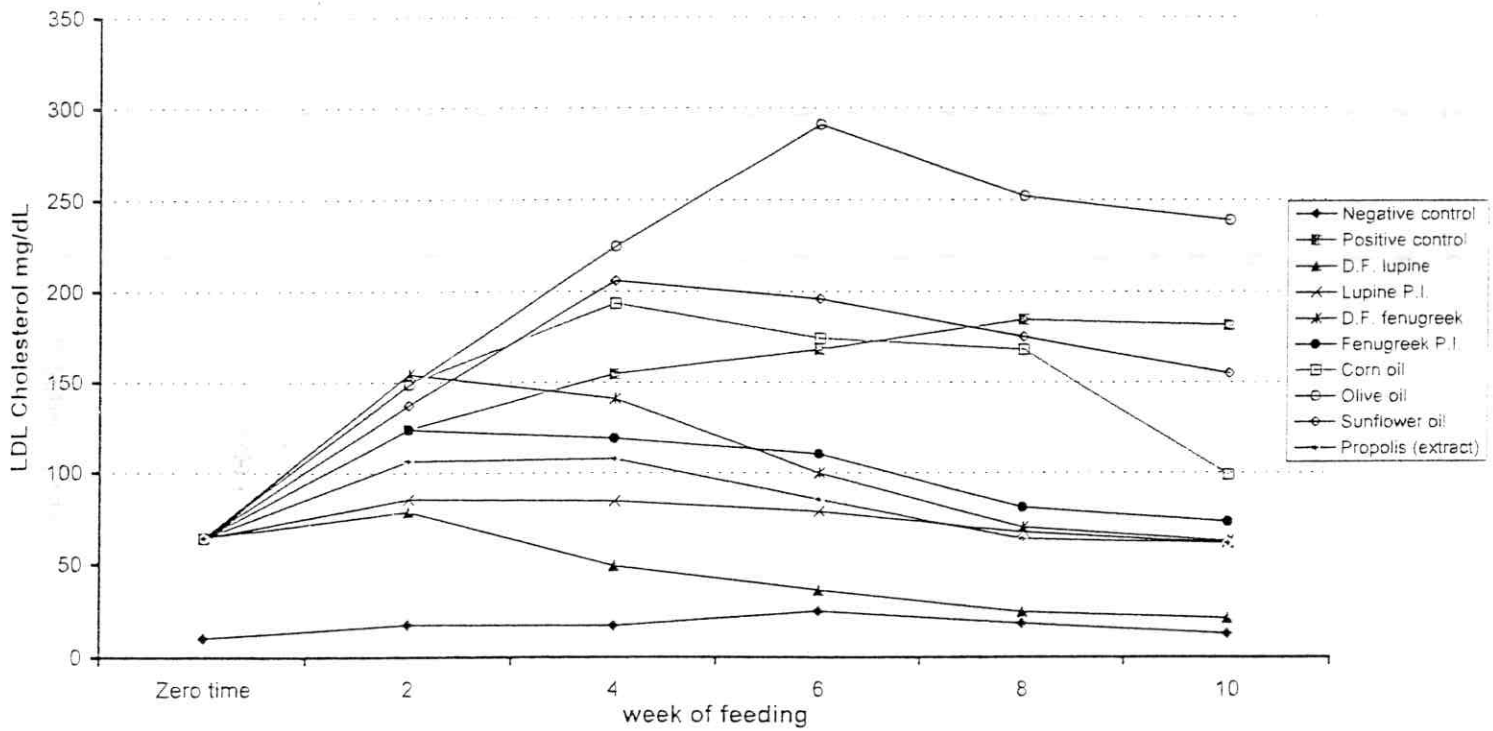


Fig (7): Effect of dietary proteins, dietary oils and propolis ethanol extract on low-density lipoprotein level (LDL) (mg/dL) of hypercholesterolemic rats during feeding periods.

and increased cholesterol saturation of bile by increasing hepatic secretion of cholesterol. These data also agree with **Pedersen *et al.*, (2000)** who concluded that, rapeseed oil and sunflower oil had more favorable effects on blood lipids and LDL subfractions compared with olive oil. Some of the differences may be attributed to differences in polyunsaturated/saturated fatty acid ratio, squalene and phytosterol contents of the oils.

4.3.3.6. Serum very low density lipoprotein cholesterol (VLDL-C):

The results in Table (18) and illustrated by fig (8) showed that the highly significant increase was found in rats fed on hypercholesterolemic diet (positive control), which ranged from 19.03 mg/dL at zero time to 24.36 mg/dL after 10 weeks compared with negative control, which ranged from 7.31-8.52 mg/dL. Significant decrease in the mean values of VLDL-C concentration was noticed in all treated groups compared with positive control. The highly significant decrease of serum VLDL-C was found for rats fed on defatted lupine flour followed by defatted fenugreek flour compared with the positive control and non significant difference between defatted lupine group and negative control. On the other hand, rats fed on diet containing corn oil showed highly significant decrease in serum VLDL-C from 19.03 mg/dL to reach 12.41 mg/dL after 10 weeks compared with positive control followed by sunflower oil and olive oil which showed a highly and significant decrease respectively compared with positive control. On the other hand ,propolis extract resulted in highly significant decrease than that of positive control .From the aforementioned data, it could be

Table (18): Effect of dietary proteins, dietary oils and propolis ethanol extract on very low-density lipoprotein level (VLDL-C) (mg/dL) of hyper-cholesterolemic rats during feeding periods.

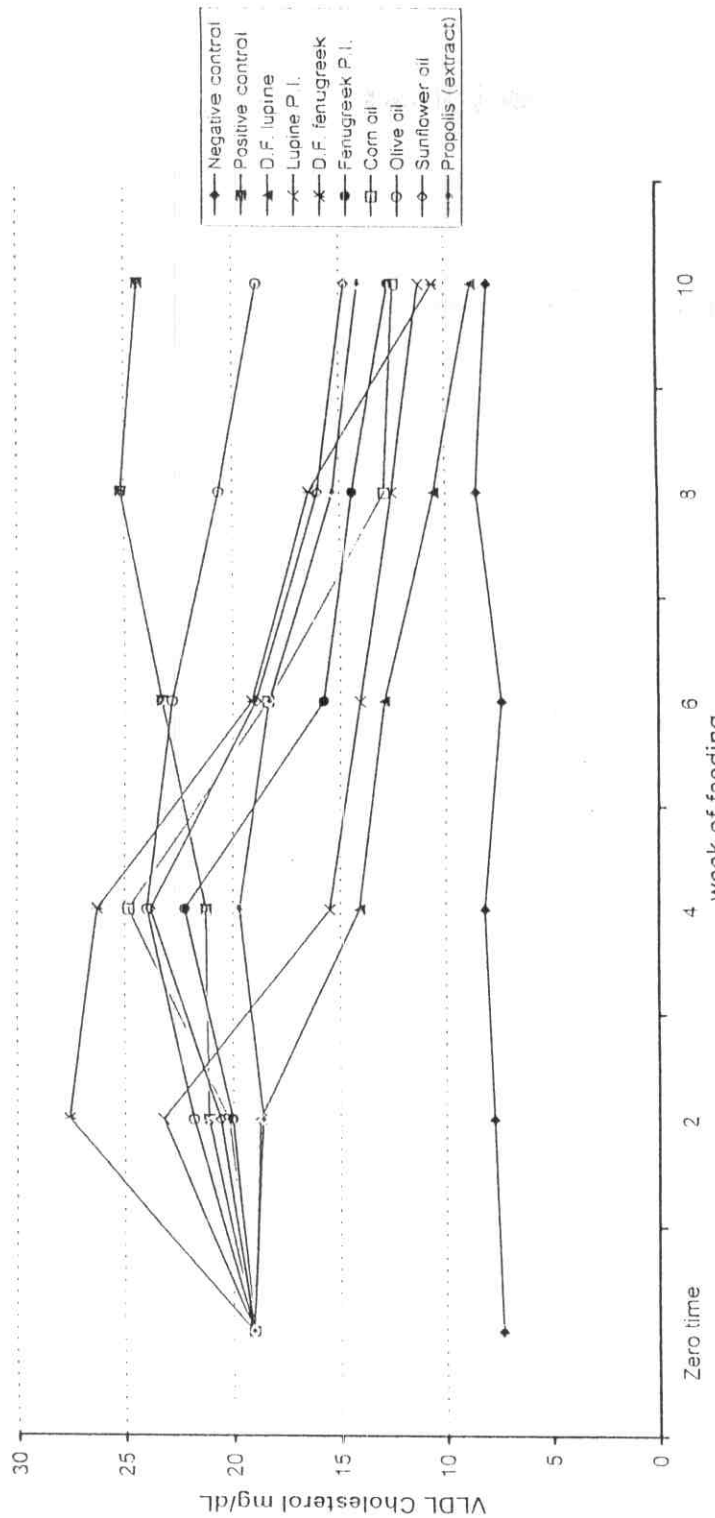
Animal group diet	Feeding period (week)						Mean
	Zero time	(2)	(4)	(6)	(8)	(10)	
Negative control	s 7.31	s 7.71	s 8.15	s 7.38	rs 8.52	s 7.95	h 7.84
Positive control	jkl 19.03	f-j 21.11	f-j 21.23	cdef 23.27	bc 25.14	bcd 24.36	a 22.36
D.F. lupine flour	jkl 19.03	jkl 18.72	nop 14.08	opq 12.91	qr 10.53	rs 8.74	g 14.00
Lupine protein isolate	jkl 19.03	cdef 23.20	n 15.48	nop 14.04	pq 12.52	q 11.21	f 15.91
D.F. fenugreek flour	jkl 19.03	a 27.55	ab 26.24	jk 19.11	lmn 16.44	qr 10.56	c 19.82
Fenugreek protein isolate	jkl 19.03	hijk 20.00	d-h 22.22	n 15.76	nop 14.39	pq 12.65	e 17.34
Corn oil	jkl 19.03	hijk 20.18	bc 24.81	klm 18.35	opq 12.88	pq 12.41	e 17.94
Olive oil	jkl 19.03	e-i 21.79	bcde 23.95	c-g 22.79	g-k 20.61	jkl 18.84	b 21.17
Sunflower oil	jkl 19.03	g-k 20.57	cde 23.79	jkl 18.92	mn 16.02	nop 14.73	d 18.84
Propolis (ethanol extract)	jkl 19.03	jkl 18.58	ijk 19.67	klm 18.31	no 15.31	nop 14.08	e 17.05
Mean	17.86^{b}	19.94^{a}	19.96^{a}	17.08^{c}	15.24^{d}	13.55^{e}	

- Any two means have the same letter are non- significantly different at ($p > 0.05$).

LSD for time at $\alpha 0.05 = 0.6769$

LSD for treatment at $\alpha 0.05 = 0.8738$

LSD for treatment within time at $\alpha 0.05 = 2.140$



Fig(8): Effect of dietary proteins, dietary oils and propolis ethanol extract on very low-density lipoprotein level (VLDL-C) (mg/dL) of hypercholesterolemic rats during feeding periods.

concluded that defatted lupine flour showed a superior effect in decreasing VLDL-c followed by deffated fenugreek flour and their corresponding protein isolate. Meanwhile corn oil reduced VLDL-C to be about one half as that of positive control followed by sunflower and olive oil . Propolis extract equal in its action as that of sunflower oil . The statistical analysis confirmed the present data which showed that the interaction between the feeding period and treatments was in parallel with the finding. The data were in the line with those of **Macarulla *et al.*,(2001)**who found that the whole seed or protein isolate of faba bean diets induced a significant reduction in plasma (LDL+VLDL) cholesterol .They added that, the effectiveness of the whole bean was higher than of the protein isolate. Also this data agree with **Wanger (2001)** who reported that, the polyunsaturated fatty acids-rich corn oil diet was able to reduce very low density lipoprotein than mixed oil diet .

4.3.3.7. Serum lipoprotein cholesterol and atherosclerosis:

Several studies indicted that elevated level of serum total cholesterol and low-density lipoprotein cholesterol represented a risk factor for the development of coronary heart disease (CHD). Serum high-density lipoprotein cholesterol (HDL-C) levels are inversely related to the incidence of CHD **Janic *et al.*, (1987).**

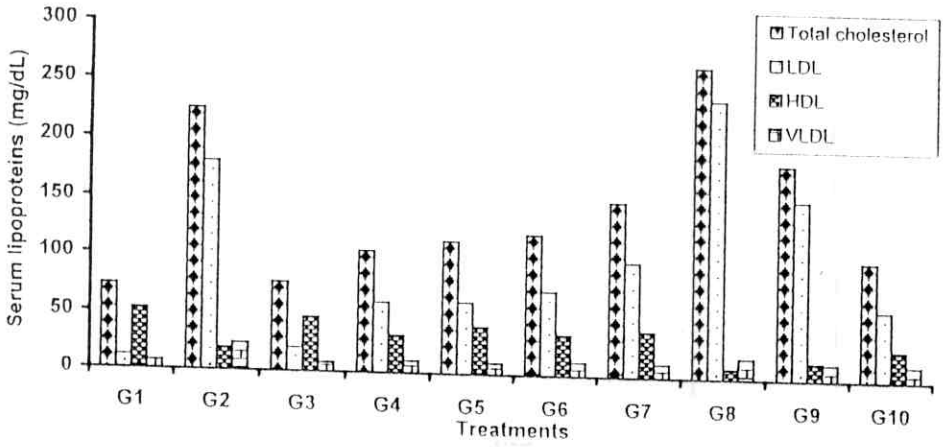
The data presented in Table (19) and illustrated by fig (9) showed the higher increase in serum LDL/HDL ratio (9.24) in positive control group than negative control which showed a value of 0.236. All groups fed on different legumes as source of proteins have the lower values of LDL/HDL than positive control where, rats fed on defatted lupine flour diet had the

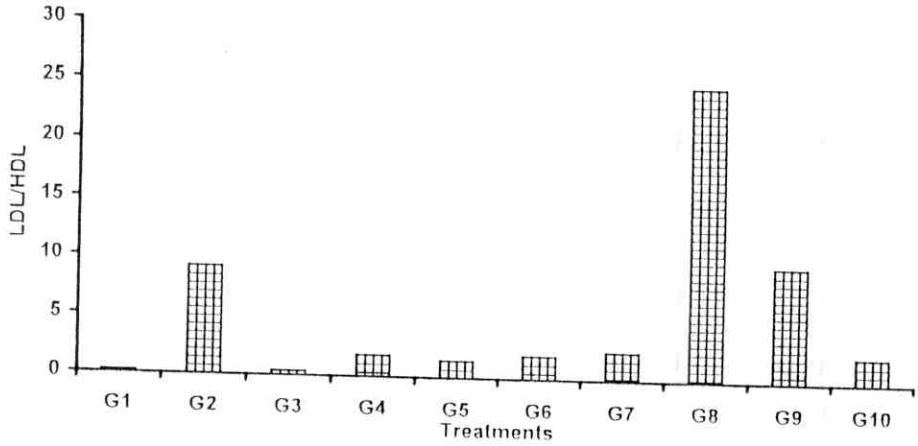
Table (19): Effect of dietary proteins, dietary oils and propolis ethanol extract on serum lipoproteins levels (mg/dL) in hypercholesterolemic rats, after feeding period (10 weeks).

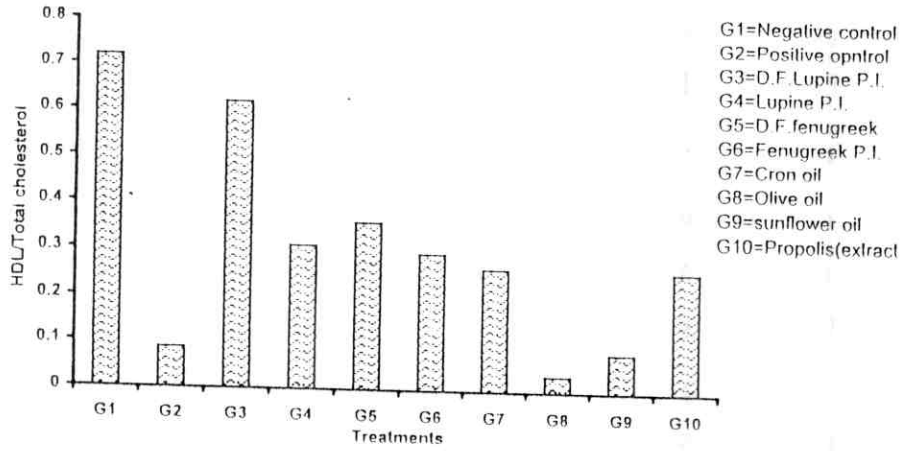
Animal group diet	Parameters					
	Total cholesterol	LDL	HDL	VLDL	LDL/ HDL	HDL/Total cholesterol
Negative control	73.55	12.53	53.07	7.95	0.236	0.720
Positive control	225.47	181.47	19.64	24.36	9.240	0.087
D.F. lupine flour	77.28	20.88	47.67	8.74	0.438	0.620
Lupine protein isolate	105.43	61.36	32.85	11.21	1.868	0.310
D.F. fenugreek flour	114.95	62.60	41.80	10.56	1.497	0.360
Fenugreek protein isolate	121.70	73.06	36.00	12.65	2.029	0.296
Corn oil	151.54	98.86	40.27	12.41	2.45	0.266
Olive oil	267.08	238.69	9.56	18.84	24.967	0.036
Sunflower oil	185.25	154.79	15.70	14.73	9.859	0.085
Propolis(ethanol extract)	102.20	61.22	26.90	14.08	2.276	0.263

- Any two means have the same letter are non- significantly different at ($p > 0.05$).

D.F. =Defatted







g(9) Effect of dietary proteins, dietary oils and propolis ethanol extract on serum lipoproteins (mg/dL) in hyper cholesterolemic rats.

lowest ratio (0.438) followed by defatted fenugreek flour diet (1.497). On the other hand, the highest ratio of LDL/HDL was (24.97) for rats fed on olive oil diet compared with positive control. These results agree with **Stark and Madar, (1993)** who reported that, saponins in fenugreek as hypocholester- olemic agents which appear to be interact with bile salts in the digestive tract. Also, **Pedersen et al., (2000)** reported that PUFA-rich diet had more influence on lipoprotein metabolism than the MUFA-rich diet.

It could be observed that the HDL/total cholesterol ratio ranged between 0.087 and 0.72 for positive and negative control, respectively. The present results showed that the LDL/HDL ratio was lower in groups of rats fed on defatted lupine diet containing 1% cholesterol, than other groups.

These results agree with **Moral *et al.*,(1997)**who used some atherogenic indexes(HDL/TC;HDL/LDL;HDL/TC-HDL)as a risk factor for atherosclerosis .These indexes were significantly higher in rats fed on sunflower oil diet when compared to rats fed on olive diet.

4.3.3.8. Serum glucose:

Serum glucose level of hypercholesterolemic rats fed on different diets during feeding period is presented in Table (20) and illustrated by fig (10).

The data showed that feeding on cholesterol 1% and bile salts elevated gradually serum glucose level from 79.24 mg/dL at zero time to 180.19 mg/dL after 10 weeks in positive control. While rats fed on basal diet without cholesterol (negative

control) serum glucose ranged between 59.60 and 66.85 mg/dL during the experimental period. Serum glucose significantly increased in all treated groups after two weeks and reached a maximum level after 4 weeks for groups fed on sunflower oil, corn oil and propolis ethanol extract diets. After 6 weeks all treated groups showed significant decrease in serum glucose level.

The statistical analysis confirmed the present data which showed that, significantly increase in mean value of serum glucose reached to 146.9 mg/dL and 145.3 mg/dL after 4 and 6 weeks followed by significantly decrease reached to 114.60 mg/dL after 10 weeks. On the other hand, found that the highest mean value was 152.70 mg/dL for olive oil, while the lowest mean value was 110.50 mg/dL for defatted lupine flour compared with positive control.

The results showed that feeding on legumes as source of plant protein, dietary oil and propolis ethanol extract reduced serum glucose level in hypercholesterolemic rats. The most effective in decreasing serum glucose was found in rats fed on defatted lupine, propolis ethanol extract and corn oil diets.

These findings agree with **Trevisan *et al.*, (1990)**and **Hassan and Said, (1999)** who reported that the level of 10% protein content for all tested animal and plant protein diets significantly decreased plasma glucose in diabetic rats.

Table (20): Effect of dietary proteins, dietary oils and propolis ethanol extract on serum glucose level (mg/dL) in hypercholeste- rolemic rats during feeding periods.

Animal group diet	Feeding period (week)						Mean
	Zero time	2	4	6	8	10	
Negative control	w 61.95	vw 66.85	w 60.68	w 62.03	w 59.60	w 64.34	h 62.57
Positive control	uv 79.24	uv 81.52	nopq 113.44	fgh 156.14	ef 168.07	de 180.19	de 129.80
D.F. lupine flour	v 79.24	b 202.92	ghi 149.13	ijk 140.58	o-t 105.66	tu 93.17	e 128.40
Lupine protein isolate	uv 79.24	ghi 148.88	hijk 142.59	klm 129.81	o-t 105.80	qrst 100.52	f 117.80
D.F. fenugreek flour	uv 79.24	ghi 148.95	mno 119.48	n-r 112.43	o-t 104.83	rst 98.06	g 110.50
Fenugreek protein isolate	v 79.24	hij 144.74	fg 161.44	lmn 123.31	nop 115.51	n-s 109.42	f 122.30
Corn oil	uv 79.24	fgh 155.24	bc 194.77	ef 166.58	fgh 156.14	p-t 103.66	b 142.60
Olive oil	uv 79.24	nopq 112.89	ijk 137.89	a 223.13	bc 194.37	ef 168.65	a 152.70
Sunflower oil	v 79.24	st 96.61	b 202.03	cd 183.05	ijkl 135.83	jklm 131.95	bc 138.10
Propolis (ethanol extract)	uv 79.24	de 178.65	cd 187.43	fgh 155.83	n-s 110.78	st 96.34	cd 134.70
Mean	77.51^{e}	133.7^{b}	146.9^{a}	145.3^{a}	125.7^{c}	114.6^{d}	

- Any two means have the same letter are non- significantly different at ($p > 0.05$).

LSD for time at $\alpha 0.05 = 3.925$ **D.F**.= Defatted

LSD for treatment at $\alpha 0.05 = 5.067$

LSD for treatment within time at $\alpha 0.05 = 12.41$

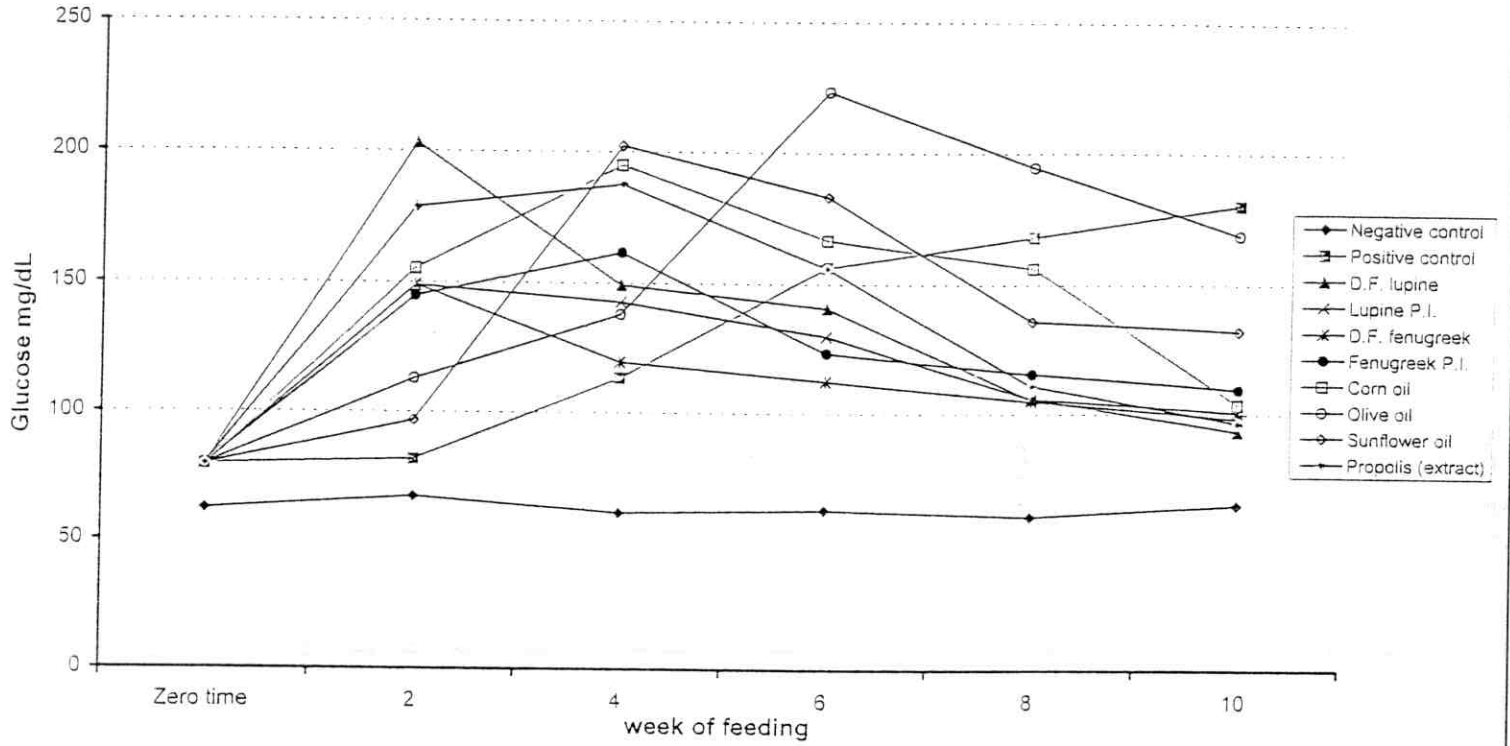


Fig (10): Effect of dietary proteins, dietary oils and propolis ethanol extract on serum glucose level (mg/dL) in hypercholesterolemic rats during feeding periods.

4.3.3.9. Serum aspartate amino transferase (AST):

The serum AST values of rats fed on different diets under investigation during the experimental period are summarized in Table (21) and illustrated by fig (11).

The results in Table (21) showed that, AST activity in negative control group ranged between 32.40 and 41.02 IU/L. Also this Table showed that a significant increase in serum AST activity in positive control group which elevated gradually to reach its maximum level (85.07 IU/L) after 10 weeks compared with negative control.

The data presented in table (21) indicated a significant increase in AST values after 6 weeks in all treated groups while a significant decreases in AST values was found after 10 weeks for all treated groups. It could be observed that defatted lupine, defatted fenugreek have the lowest values of serum AST (52.29 and 56.40 IU/L, respectively) followed by corn oil diet (68.25 IU/L). at the end of the experimental period.

These results agree with **Abdel-Rahim *et al.*, (1995)** who reported that AST and ALT activities in serum were significantly stimulated by feeding on hypercholesterolemic diet. Also, the results were in line with the findings of **Abd El-Salam and Abd- El-Megeid, (1998)** who reported that, feeding on fenugreek diets decreased serum AST and ALT in hyperglycemic rats.

Table (21): Effect of dietary proteins, dietary oils and propolis ethanol extract on AST level (IU/L) in hypercholesterolemic rats during of feeding periods.

Animal group diet	Feeding periods(week)				Mean
	Zero Time	(2)	(6)	(10)	
Negative control	n 32.40	n 33.60	lmn 41.02	lmn 38.37	f 36.35
Positive control	mn 36.72	lmn 40.75	jklm 47.50	ef 85.07	e 52.51
D.F. lupine flour	mn 36.72	lmn 39.04	fg 76.11	ijk 52.29	e 51.04
Lupine protein isolate	mn 36.72	jklm 46.59	gh 67.98	hi 61.37	e 53.17
D.F. fenugreek	mn 36.72	jklm 47.88	e 93.90	ij 56.40	d 58.72
Fenugreek protein isolate	mn 36.72	jkl 48.95	b 148.44	fg 77.44	b 77.89
Corn oil	mn 36.72	klmn 43.54	d 115.31	gh 68.25	c 65.96
Olive oil	mn 36.72	ij 55.57	a 209.95	b 141.58	a 111.00
Sunflower oil	mn 36.72	ijk 52.27	c 126.45	f 81.76	b 74.30
Propolis (ethanol extract)	mn 36.72	lmn 39.34	b 148.96	fg 74.97	b 75.00
Mean	36.29^{d}	44.75^{c}	107.60^{a}	73.75^{b}	

- Any two means have the same letter are non- significantly different at ($p > 0.05$).

LSD for time at 0.05 = 3.064

D.F.= Defatted

LSD for treatment at 0.05 = 4.844

LSD for treatment within time at 0.05 = 9.689

Fig (11): Effect of dietary proteins, dietary oils and propolis ethanol extract on AST level (IU/L) in hypercholesterolemic rats during of feeding periods.

4.3.3.10. Serum alanine amino transferase (ALT):

The activity of serum ALT of rats fed on tested diet were determined and the results are presented in Table (22) and illustrated by fig (12).

The obtained data in table (22) showed that, there was non-significant differences in ALT values in negative control which ranged between 19.22 and 23.12 IU/L during feeding period compared with rats fed on positive control diet except that after 10 weeks of feeding which resulted in significant increase compared with other period .

The data summarized in Table (22) showed that non-significant increases in serum ALT after 6 weeks for all treated groups except the group fed on defatted fenugreek flour, its protein isolate, corn oil and olive oil which showed highly significant increase .Similar trend with slight decrease in serum ALT were observed after 10 week in all groups compared with positive control. The highest value was 49.39 IU/L for rats fed on olive oil while the lowest value was 21.1 IU/L for rats fed on defatted lupine. These results are agree with **Abdel-Rahim *et al.*, (1995)** and **Abd El-Salam and Abd-El- Megeid, (1998).**

It is worth to mention that lupine flour and its protein isolate acted at ALT to be constant during the feeding period with non -significant (10 weeks) differences

Table (22): Effect of dietary proteins, dietary oils and propolis ethanol extract on ALT level (IU/L) in hypercholesterolemic rats during of feeding periods.

Animal group diet	Feeding periods(week)				Mean
	Zero time	(2)	(6)	(10)	
Negative control	ef 21.22	ef 23.12	f 19.22	ef 21.17	d 21.18
Positive control	ef 22.35	ef 23.60	ef 23.29	cd 30.34	bc 24.89
D.F. lupine flour	ef 22.35	ef 20.32	ef 22.08	ef 21.01	d 21.44
Lupine protein isolate	ef 22.35	ef 21.14	ef 22.27	ef 21.86	d 21.91
D.F. fenugreek flour	ef 22.35	ef 22.08	b 36.33	ef 22.74	b 25.87
Fenugreek Protein isolate	ef 22.35	ef 22.49	dg 26.30	ef 22.96	bcd 23.53
Corn oil	ef 22.35	f 19.06	de 26.53	ef 23.51	cd 22.86
Olive oil	ef 22.35	bc 33.99	a 54.38	a 49.39	a 40.03
Sunflower oil	ef 22.35	ef 23.14	de 25.96	ef 24.04	bcd 23.87
Propolis(ethanol extract)	ef 22.35	ef 22.41	ef 21.74	ef 21.68	cd 22.04
Mean	22.24^{c}	23.14^{c}	27.81^{a}	25.87^{b}	

- Any two means have the same letter are non- significantly different at ($p > 0.05$).

LSD for time at $\alpha 0.05 = 1.650$ **D.F.**= Defatted

LSD for treatment at $\alpha 0.05 = 2.608$

LSD for treatment within time at $\alpha 0.05 = 5.217$

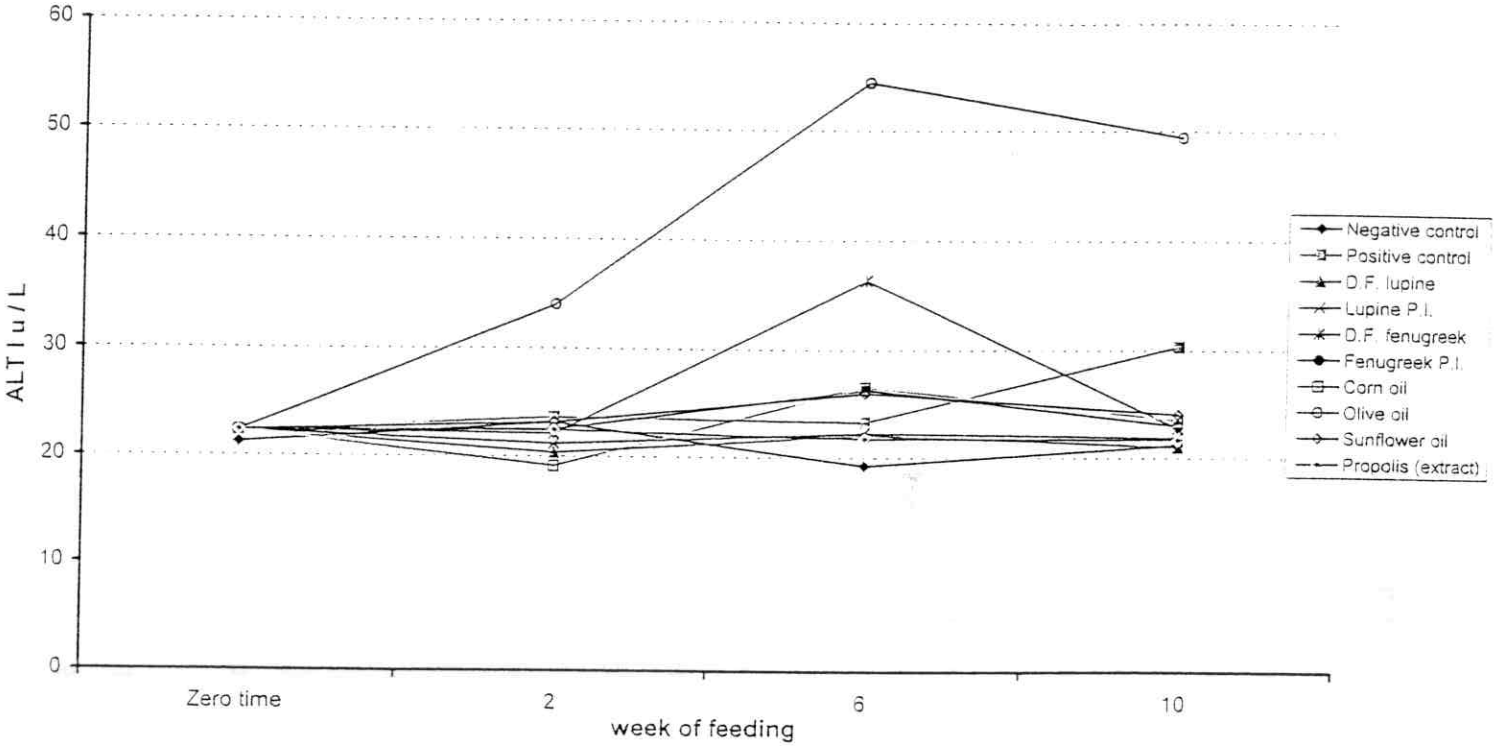


Fig (12): Effect of dietary proteins, dietary oils and propolis ethanol extract on ALT level (IU/L) in hypercholesterolemic rats during of feeding periods.

4.3.3.11. Serum creatinine:

The findings in Table (23) and illustrated by fig (13) showed that the relative increase in serum creatinine was observed in the group of rats fed on hypercholesterolemic diet ranged 0.540-0.847 mg/dL compared with negative control. Also non-significant increases in serum creatinine were observed in all groups except rats fed on olive oil which had significantly increased.

The highest values of serum creatinine was 2.197 mg/dl for rats fed on olive oil diet while the lowest values was 0.513 mg/dl followed by 0.547, 0.567 and 0.630 mg/dl for rats fed on defatted fenugreek, propolis ethanol extract, sunflower oil and defatted fenugreek, respectively.

4.3.3.12. Serum uric acid:

Data presented in Table (24) and illustrated by fig (14) showed the mean values of serum uric acid of rats fed on different diets under investigation during the feeding period.

The data indicated that, the serum uric acid in negative control group ranged from 3.08 to 3.90 mg/dL. Also, data showed a significant increase in serum uric acid reached to 9.97 mg/dL after 10 weeks in positive control group. Also, rats fed on olive oil had higher increase in serum uric acid level (11.77 mg/dL) than other groups.

It could be seen that a significant decrease in serum uric acid level (4.38 mg/dL) for rats fed on propolis ethanol extract diet while significant increases in serum uric acid level were observed in other groups.

The results were in the line with the finding of **Abdel-Rahim, *et al.*, (1997)** who reported that, serum uric acid level was elevated by hypercholesterolemic diet but this increase was reduced by feeding on hypocholesterolemic agents.

Table (23): Effect of dietary proteins, dietary oils and propolis ethanol extract on serum creatinine level (mg/dL) in hypercholesterolemic rats during of feeding periods.

Animal group diet	Feeding periods(Week)				Mean
	Zero time	(2)	(6)	(10)	
Negative control	f 0.473	def 0.517	cdef 0.673	def 0.543	b 0.552
Positive control	def 0.540	def 0.567	cdef 0.623	c 0.847	b 0.644
D.F. lupine flour	def 0.540	def 0.553	ef 0.503	cdef 0.630	b 0.557
Lupine protein isolate	def 0.540	cdef 0.640	cdef 0.603	cdef 0.670	b 0.613
D.F. fenugreek flour	def 0.540	cd 0.783	cdef 0.607	def 0.513	b 0.611
Fenugreek protein isolate	def 0.540	cdef 0.700	def 0.537	cdef 0.720	b 0.624
Corn oil	def 0.540	cdef 0.620	cdef 0.687	cde 0.750	b 0.649
Olive oil	def 0.540	c 0.847	b 1.617	a 2.197	a 1.300
Sunflower oil	def 0.540	def 0.570	def 0.513	def 0.567	b 0.547
Propolis(ethanol extract)	def 0.540	def 0.530	def 0.577	def 0.547	b 0.548
Mean	0.533^{c}	0.633^{b}	0.694^{b}	0.798^{a}	

- Any two means have the same letter are non- significantly different at ($p > 0.05$).

LSD for time at $\alpha 0.05 = 0.06894$ **D.F.**= Defatted

LSD for treatment at $\alpha 0.05 = 0.1090$

LSD for treatment within time at $\alpha 0.05 = 0.2180$

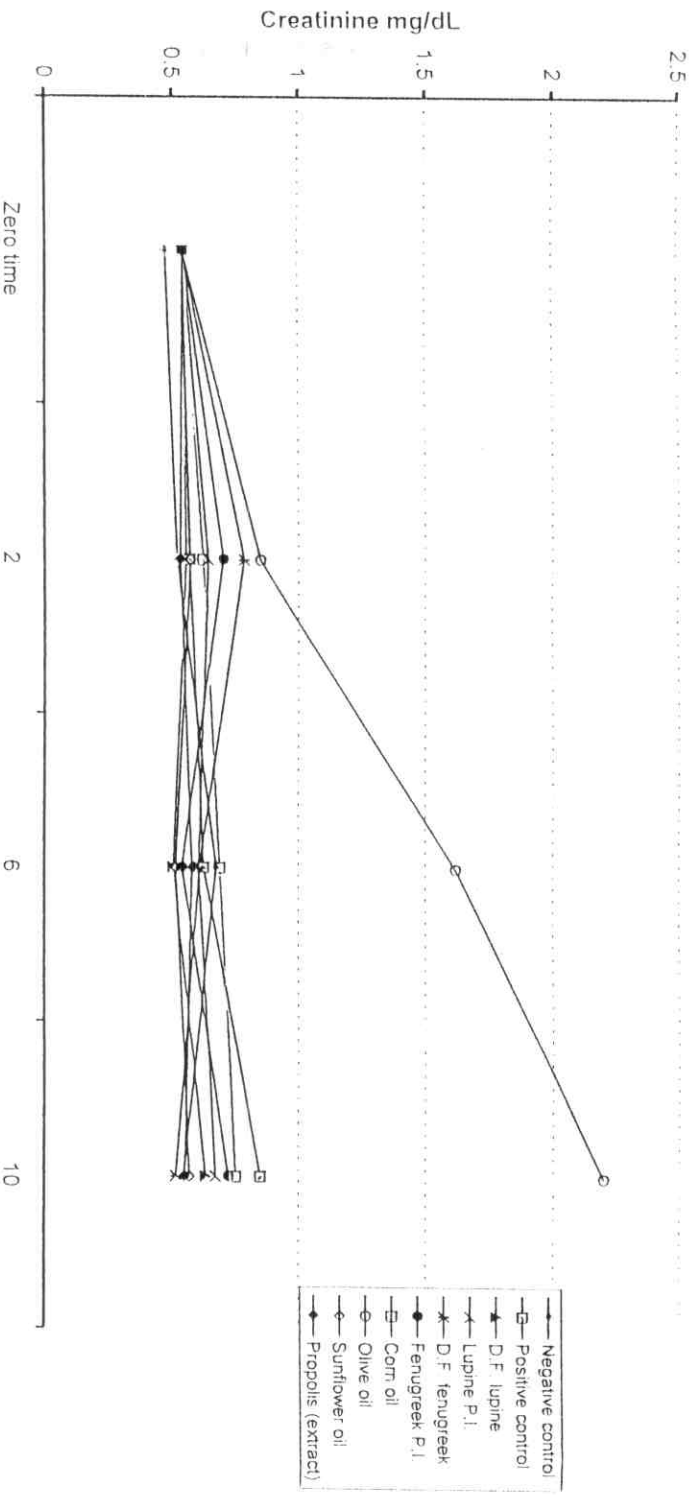


Fig (13): Effect of dietary proteins, dietary oils and propolis ethanol extract on serum creatinine level (mg/dL) in hypercholesterolemic rats during of feeding periods

Table (24): Effect of dietary proteins, dietary oils and propolis ethanol extract on uric acid level (mg/dL) in hypercholesterolemic rats during of feeding periods.

Animal group diet	Feeding periods (weeks)				Mean
	Zero time	(2)	(6)	(10)	
Negative control	fg 3.08	fg 3.25	defg 3.90	efg 3.86	de 3.52
Positive control	fg 3.33	fg 3.35	de 5.03	b 9.97	b 5.42
D.F. lupine flour	fg 3.33	fg 3.05	cd 4.52	defg 4.17	cd 3.99
Lupine protein isolate	fg 3.33	fg 3.12	efg 3.56	efg 3.81	de 3.45
D.F. fenugreek flour	fg 3.33	fg 2.91	defg 4.00	defg 4.09	de 3.58
Fenugreek protein isolate	fg 3.33	fg 3.31	efg 3.60	defg 4.26	de 3.63
Corn oil	fg 3.33	fg 2.88	defg 3.91	defg 4.08	de 3.55
Olive oil	fg 3.33	fg 3.01	a 12.57	a 11.77	a 7.67
Sunflower oil	fg 3.33	g 2.73	fg 3.08	efg 3.86	e 3.25
Propolis (ethanol extract)	fg 3.33	defg 4.19	c 6.62	def 4.38	c 4.63
Mean	3.31^{b}	3.18^{b}	5.17^{a}	5.43^{a}	

- Any two means have the same letter are non- significantly different at ($p > 0.05$).

LSD for time at $\alpha 0.05 = 0.4043$ **D.F.** =Defatted

LSD for treatment at $\alpha 0.05 = 0.6392$

LSD for treatment between time at $\alpha\ 0.05 = 1.278$

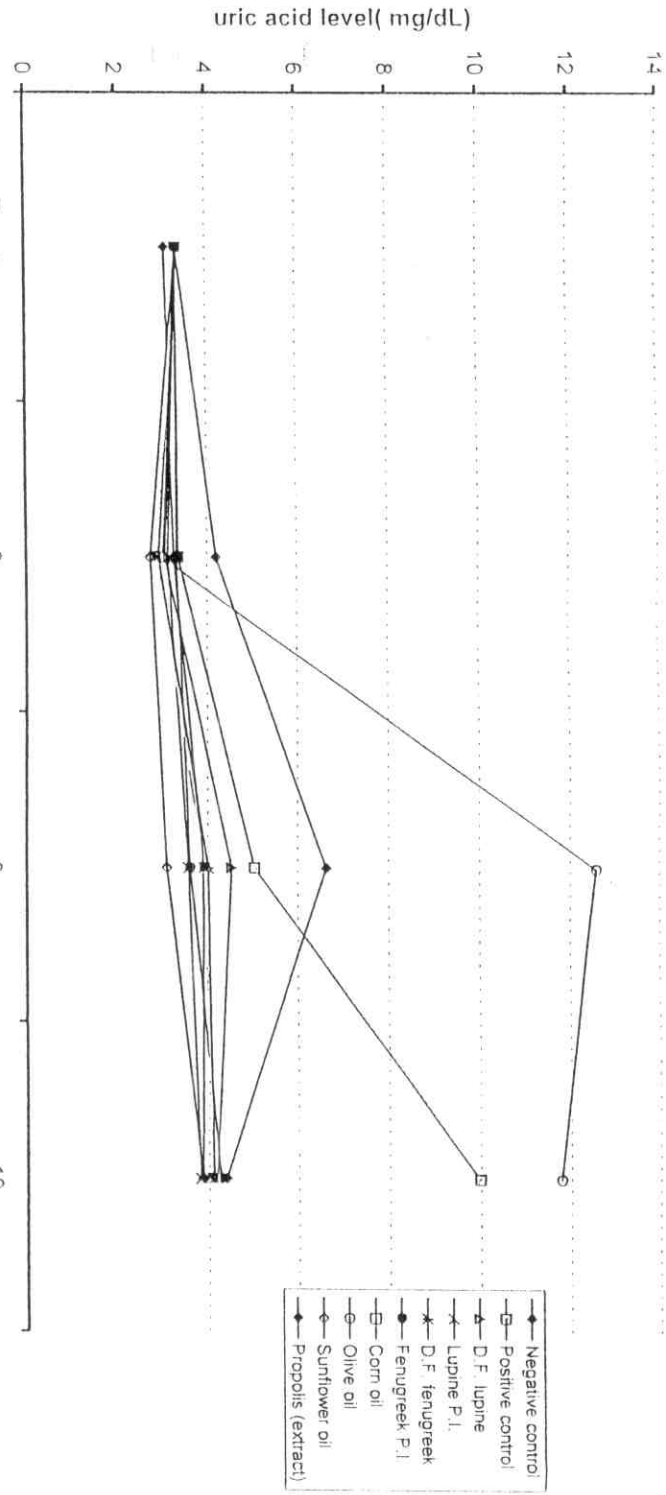


Fig (14): Effect of dietary proteins, dietary oils and propolis ethanol extract on uric acid level (mg/dL) in hypercholesterolemic rats during of feeding periods.

4.3.4. Histopatholgical results:

Group (A): fed on basal diet without cholesterol

1-Aorta:

Cross section in the wall of the aorta showed normal structure of the tunica intema that covered with endothelial cells and tunica media that consists of fenestrated elastic lamina and tunica adventitia consists of connective tissue and vasa vasorum Fig(1)

2-Heart:

Cross section in the heart wall showed normal orientation in the cardiac muscle with multinucleated fibres, Fig(6).The endocardium and epicardium appeared normal .Covered with endothelium and the purkinje fibres appeared in the deep area of endocardium.

3-Liver:

Cross section in the liver showed central vein surrounded by hepatic cords consists of hepatocytes contain central nucleus. Fig (9)

4-Kidney :

Cross section in the cortex and medulla of the kidney showed normal nephron and tubules with normal glomeruli and proximal and distal convoluted tubules. Fig(13)

5- Testes :

Cross section in the testes showed normal seminephrous tubules containing sertoli cells and spermatogonium. Spermatid

and spermatozoa was present in the center of the seminephrous tubules. Fig(22)

6-Brain:

Cross section in the cerebrum showed normal structure and distribution of the pyramidal and nerve cells and fibres. Fig(19)

7-Lung:

Cross section in the lung showed normal alveoli covered with endothelial cells. Tertiary bronchioles appeared wide lined by low columnar cells. Fig(16)

GROUP (B):Fed on basal diet +1% cholesterol.

1-Aorta:

Thickening in the endothelial cells of the tunica intema with some desquamation of few cells that appeared the nucleus only present.Fig(2)

Lymphocytic infiltration around the medium size artery.

2-Heart:

Hyaline degeneration "hyalinization" in the wall of the heart

"myocardium". The cardic muscle appeared loss of structure and homogenous with pyknotic nucleus. Fig (7)

3-Liver:

The hepatocyte showed multiple changes including vacular and hydrobic degeneration with lymphocytic infiltration.

Large fat globules accumulated in the hepatcytes that showed fatty changes. Fig (10)

4-Kidney:

Destructive changes appeared in the most details of the kidney that lead to obscure most structure. Massive lymphocytic infiltration. Fig(14)

5-Testes :

No characterstic changes were observed in the normal structure of the testes.

6-Brain:

Hammorrhge in the brain artery and gliosis in the cells of the brain .Fig(20)

7-Lung:

Active hypermia arround the wall of the small artery with some inflamatory cells around the tertiary bronchioles. Fig(17)

Group(C):Fed on defatted lupine flour+1% cholesterol.

1-Aorta:

The wall of the aorta appeared without characterstic changes

The other organs showed no significant changes in their structure.

GROUP(D):Fed on corn oil +1% cholesterol.

1-Aorta:

Very small thickening in the endothelial cells of the tunica intema Fig(3).

The other organs showed very mild changes only includes congestion in their blood vessles.

Group(E):Fed on olive oil +1% cholesterol.

1-Aorta:

Thickening in the endothelial cells of the tunica intema of the aorta with desqumation observed. Some cells of the tunica adventitia showed destruction. Fig(4)

2-Heart:

Hyaline degeneration "hyalinization" in the wall of the heart

"myocardium". The cardic muscle appeared loss of structure and homogenous with pyknotic nucleus. Fig (8)

3-Liver:

The hepatocyte showed multiple changes including vacular and hydrobic degeneration with lymphocytic infiltration. Large fat globules accumulated in the hepatocytes that appeared fatty changes. Fig (11)

4-Kidney :

Cloudy swelling in the cuboidal cells of the proximal convoluted tubules and pyramidal cells of the distal convoluted tubules. Fig(15)

Congestion in the blood vesseles of the kidney and some inflmatory cells around it.

5-Testes:

Degeneration in the spermatogonium cells, loss structure of the interstitial cells and connective tissue. Azospermia Fig(23)

6-Brain:

Lymphocytic infiltration in the cells of the meninges and desqumation in their cuboidal cells.Fig(21).Congestion in the blood vessles of the brain.

7-Lung:

Perivascular lymphocytic infiltration. Diffuse inflmatory cells between the alveoli. Emphysema in most of the alveoli. Fig(18)

Group(F):Fed on sunflower oil +1% cholesterol.

1- Aorta:

Mild thickening in the endothelial cells of the tunica intema of the Aorta. No desqumation observed fig (5).

2- liver:

Cross section in the liver showed vacular degeneration fig (12). And other organs showed very mild changes only includes congestion in their blood vessles.

Table (25): Histopathological changes in Aorta of rats fed on different diets

Changes / Group	Thickening	Desquamation of some cells	Lymphocyte infiltration	Destruction of some cells
Negative control Fig (1)	-	-	-	-
Positive control Fig (2)	+++	++	++	-
Corn oil Fig (3)	+	-	-	-
Olive oil Fig (4)	+++	+++	-	+
Sunflower oil Fig (5)	++	-	-	-

(-) No changes (+) very mild (++) Mild (+++) sever

Table (26): Histopathological changes in Heart, Brain, lung and Testes of rats fed on different diets:

Changes / Group	Heart	Brain	Lung	Testes
Negative control	- Fig (6)	- Fig (19)	- Fig (16)	- Fig (22)
Positive control	Hyaline degeneration Fig (7)	Hemorrhage and gelilosis Fig (20)	Active hyperemia and inflammatory cells Fig (17)	-
Olive oil	Hyaline degeneration Fig (8)	Lymphocytic infiltration and congestion Fig (21)	Emphysema and inflammatory cells Fig (8)	Azospermia Fig (23)

(-) No changes

Table (27): Histopathological changes in liver of rats fed on different diets:

Changes / Groups	Hydrobic degeneration	Vacuolar degeneration	Fatty changes
Negative control Fig (9)	-	-	-
Positive control Fig (10)	+++	+++	+++
Olive oil Fig (11)	+++	+++	+++
Sunflower oil Fig (12)	++	++	++

Table (28): Histopathological changes in kidney of rats fed on different diets :

Changes / Groups	Congestion	Degeneration	Inflammation cells	Swelling
Negative control Fig (13)	-	-	-	-
Positive control Fig (14)	++	+++	-	-
Olive oil Fig (15)	+++	+	+++	+++

(-) No changes (+) very mild (++) mild (+++) sever

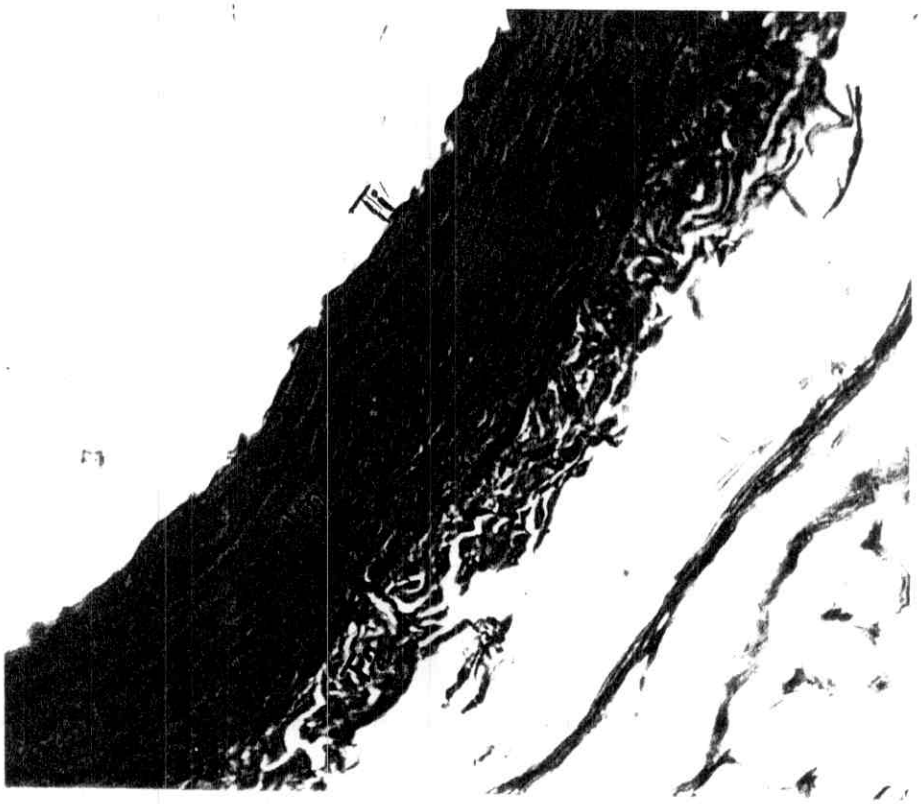


Fig (1) Cross section in the wall of the Aorta of group (A) showing : Tunica intema (Ti) , Tunica media (Tm) and Tunica adventitia (Ta).
HX&E X100

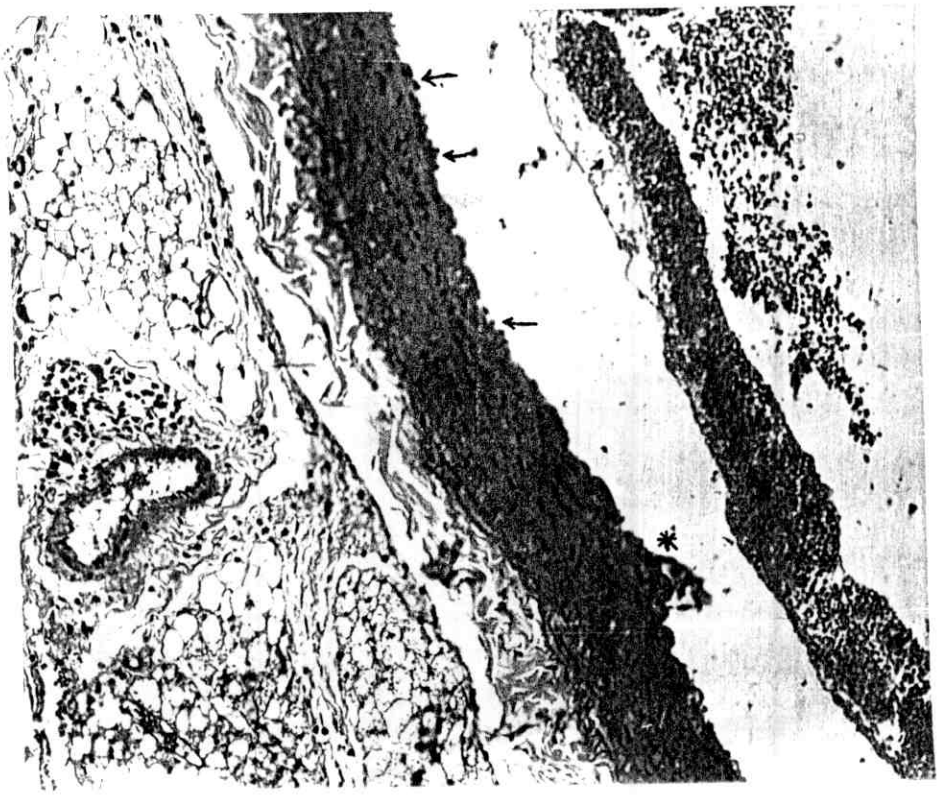


Fig (2) Cross Section in the Aorta of the group (B) showing thickening in the endothelial Cells (arrow) and desquamation of some Cells. (stars)

HX&E X100

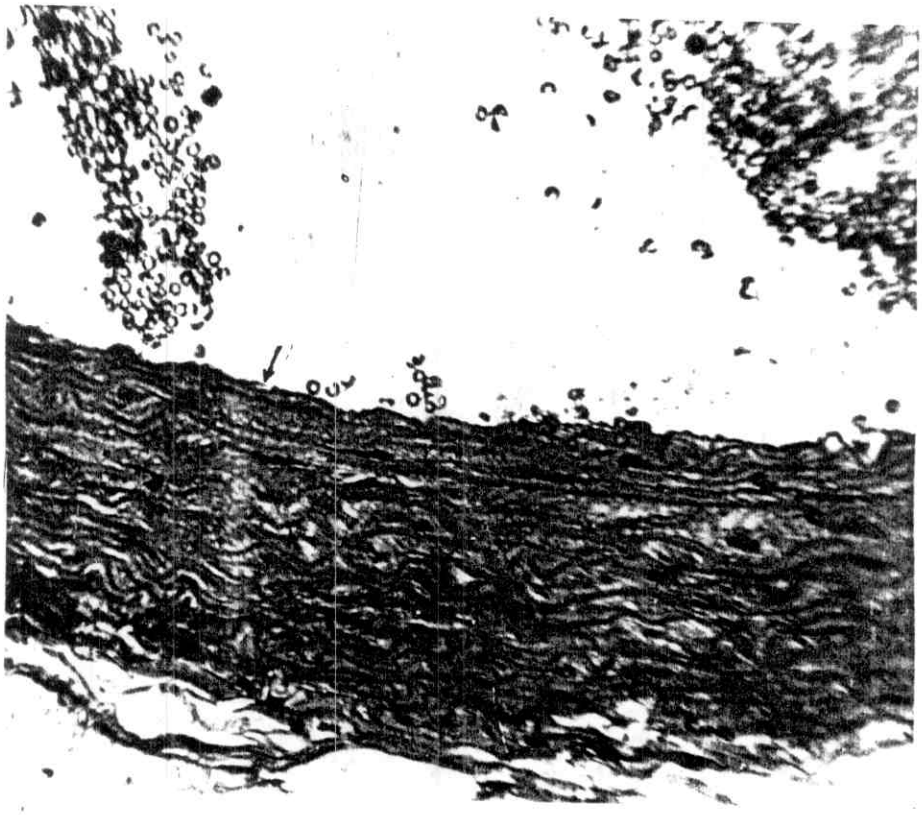


Fig (3) Cross section in the wall of the Aorta of group (D) showing very mild intimal thickening in the endothelial cells (arrows) .
HX&E X100

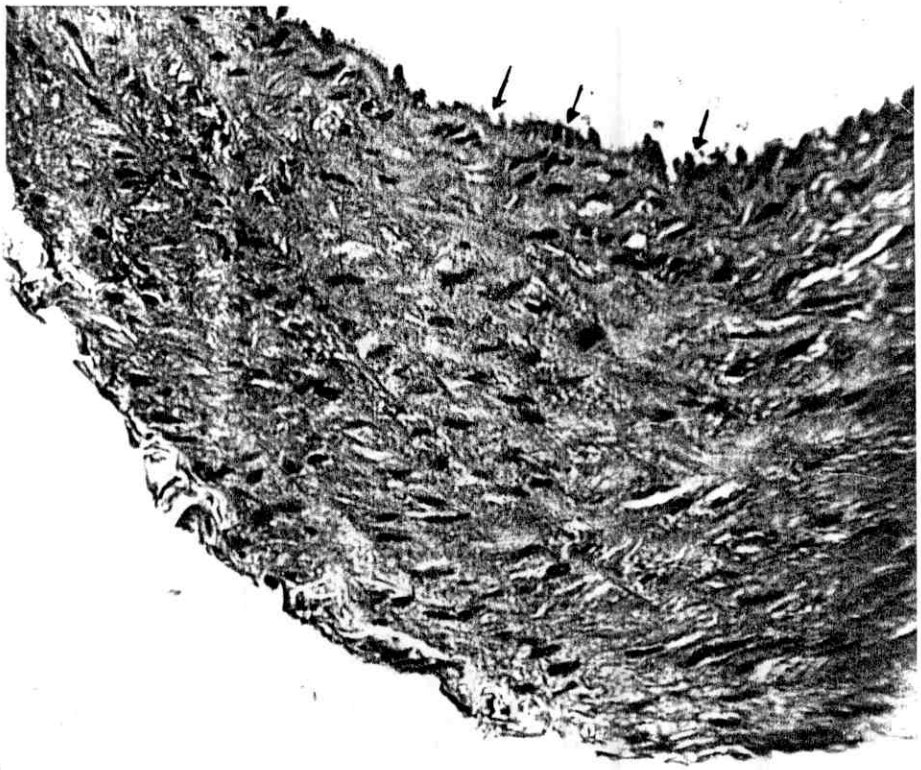


Fig (4) Cross section in the wall of the Aorta of group (E) showing thickening in the endothelial cells (arrows) and desquamation of the cells (stars)
HX&E ..... X400.

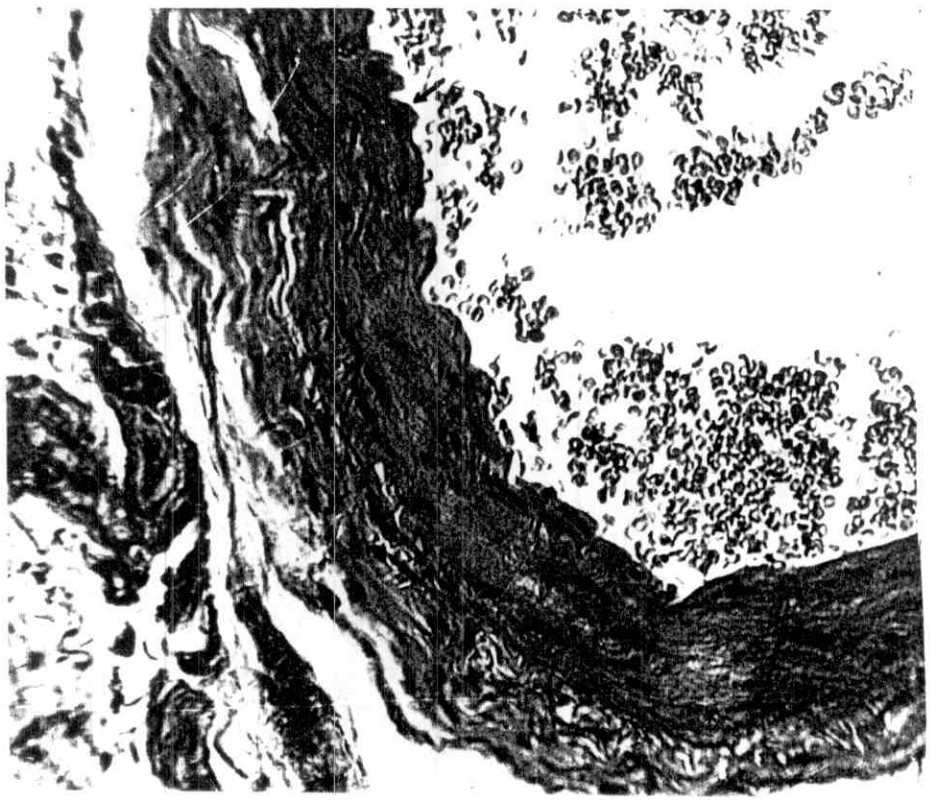


Fig (5) Cross section in the wall of the Aorta of group (F) showing mild thickening in the endothelial cells (arrows). HX&E X100

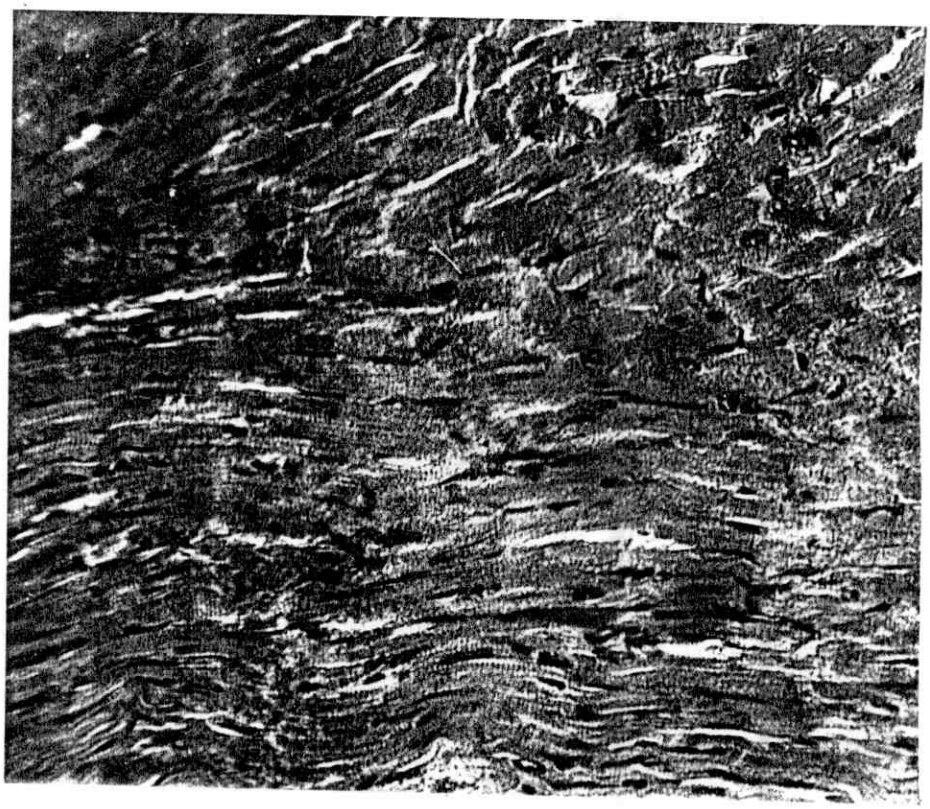


Fig (6) Longitudinal section in the wall of the heart of the group (A) showing cardiac muscle (arrows).
HX&E .....X100.

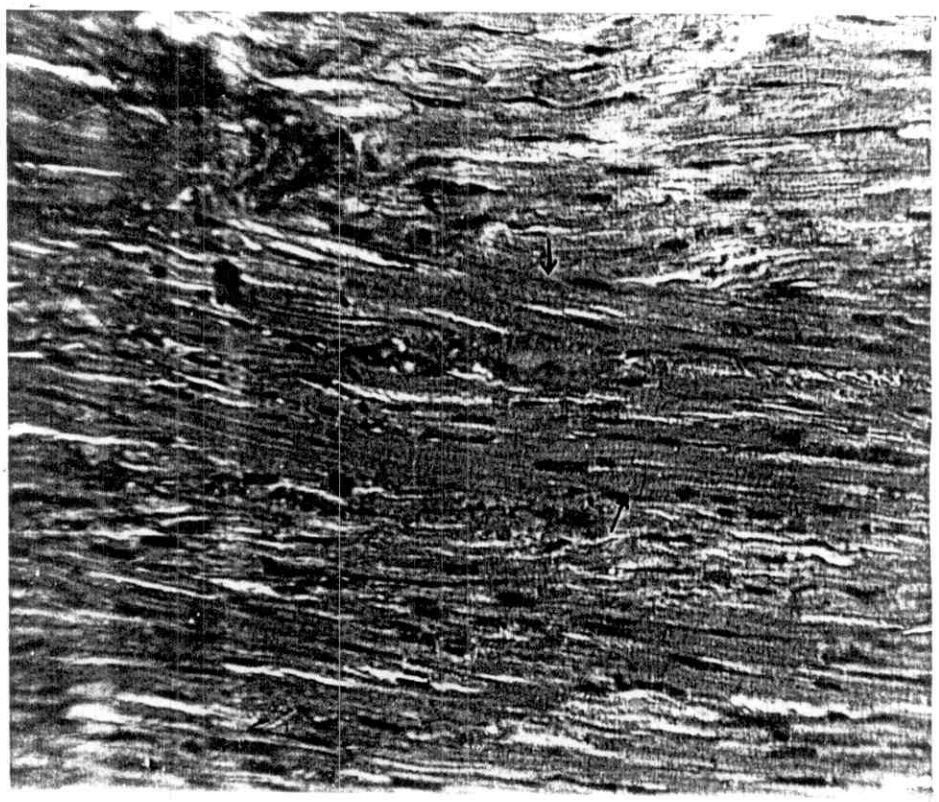


Fig (7) L.S in the wall of the heart of the group (B) showing hyaline degeneration in the cardiac muscle (arrows) HX&E.....X100.

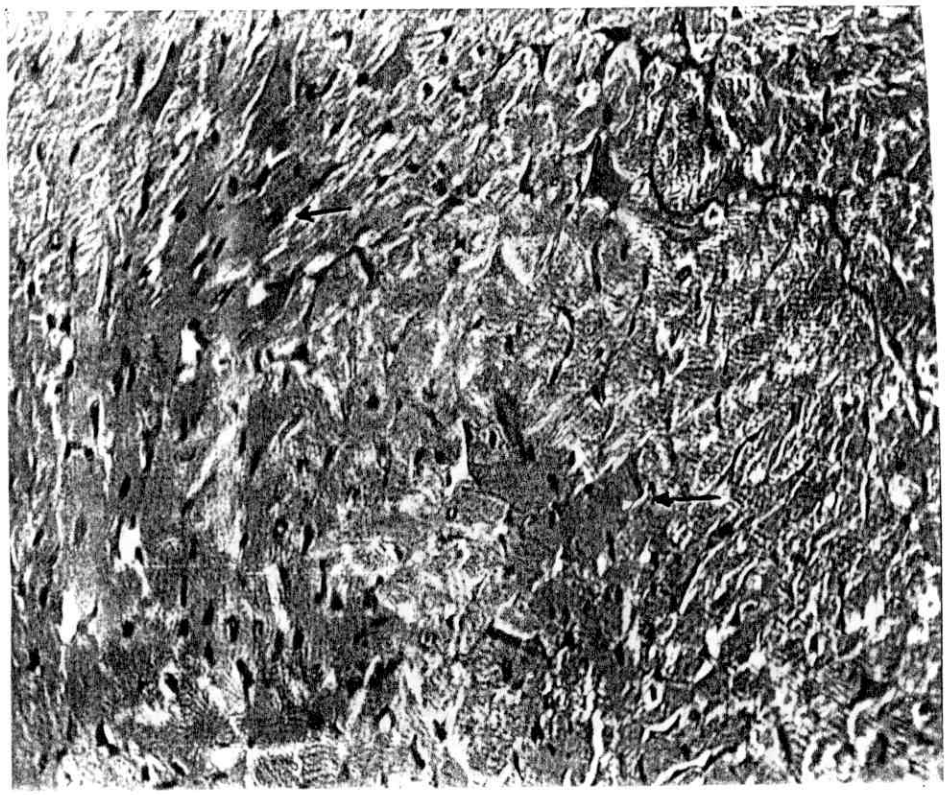
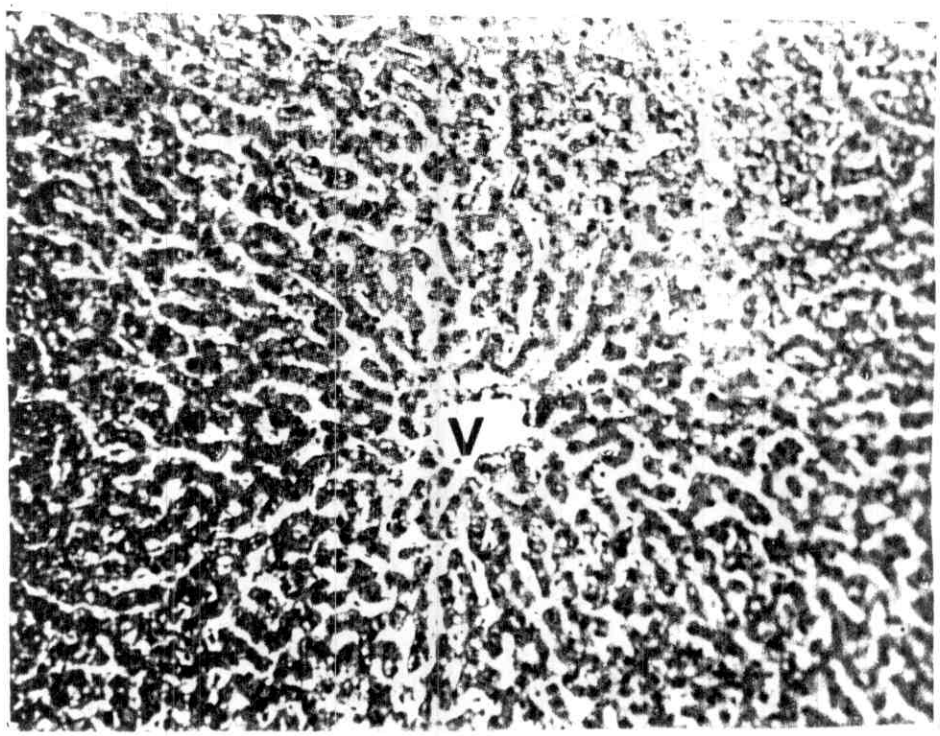
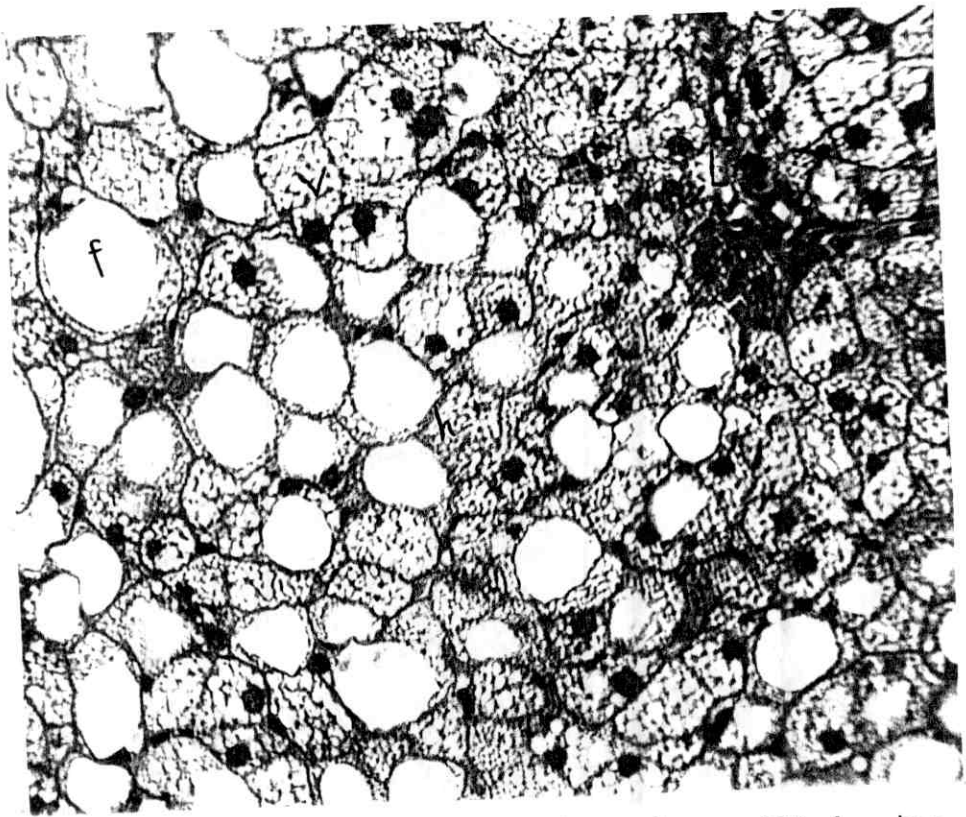


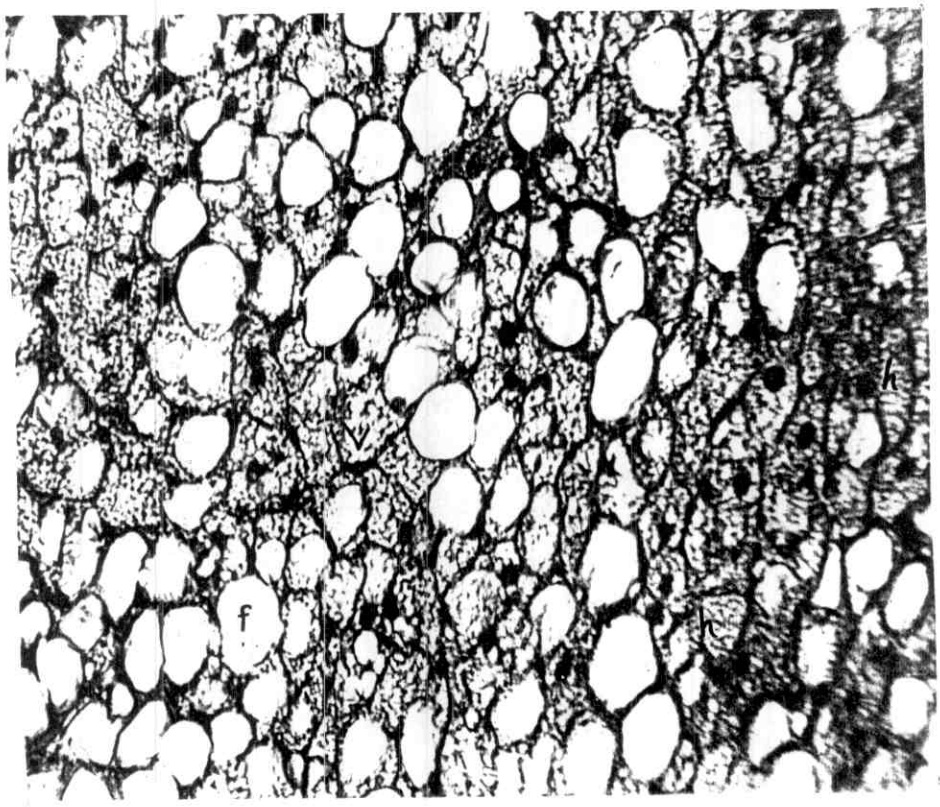
Fig (8) Photomicrograph in the wall of the heart of the group (E) showing hyaline degeneration in the cardiac muscle (arrows) HX&E.....X100.



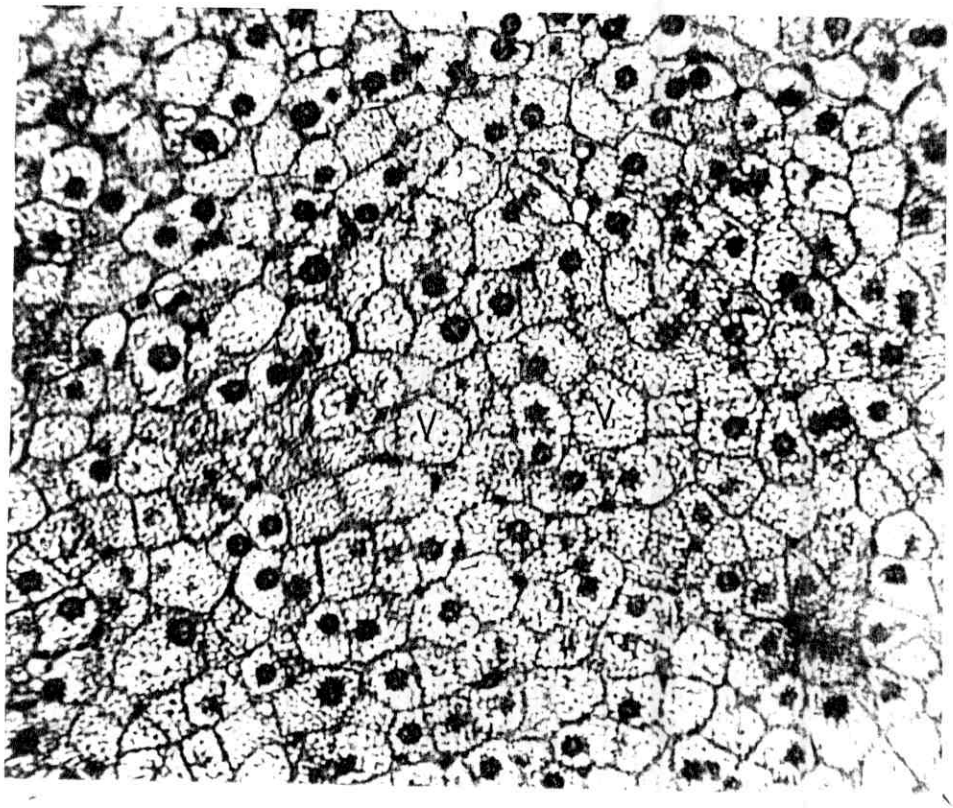
Fig(9) Cross section in the liver of the group (A) showing central vein (V)surrounded by hepatic cords consist of hepatocytes contain central nucleus . H & E .X100



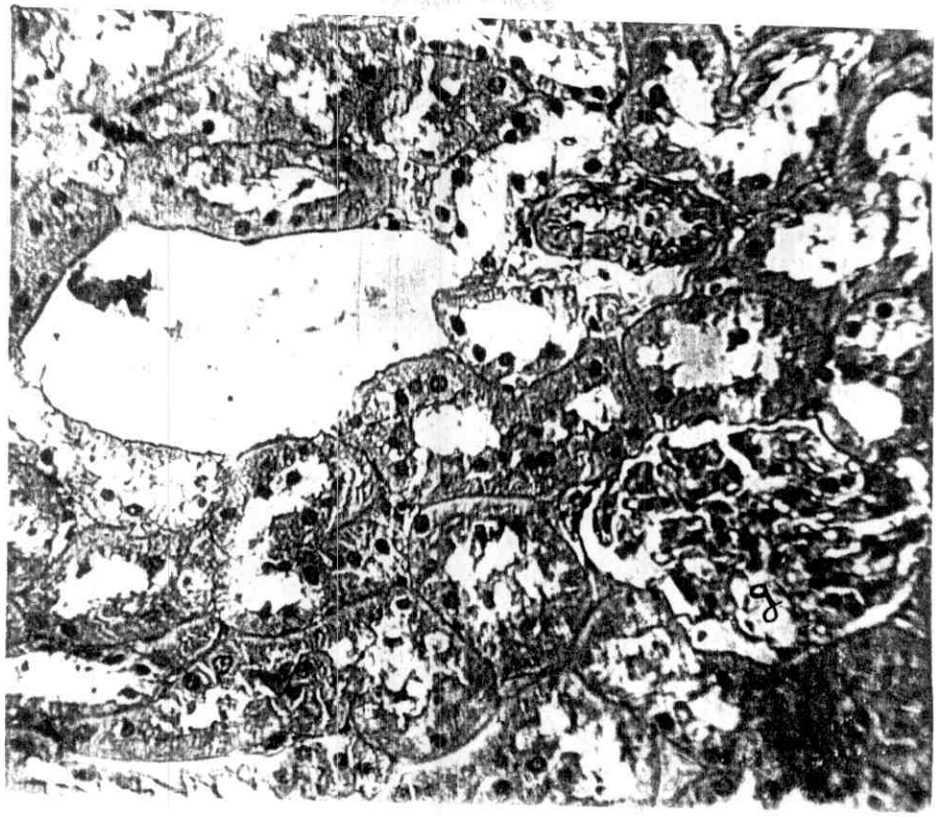
Fig(10) Cross section in the liver of group(B) showing vacuolar degeneration(v), Hydrobic degeneration (h)and lymphocytic infiltration (L),notice fatty changes (f) .H & E ..... X400 .



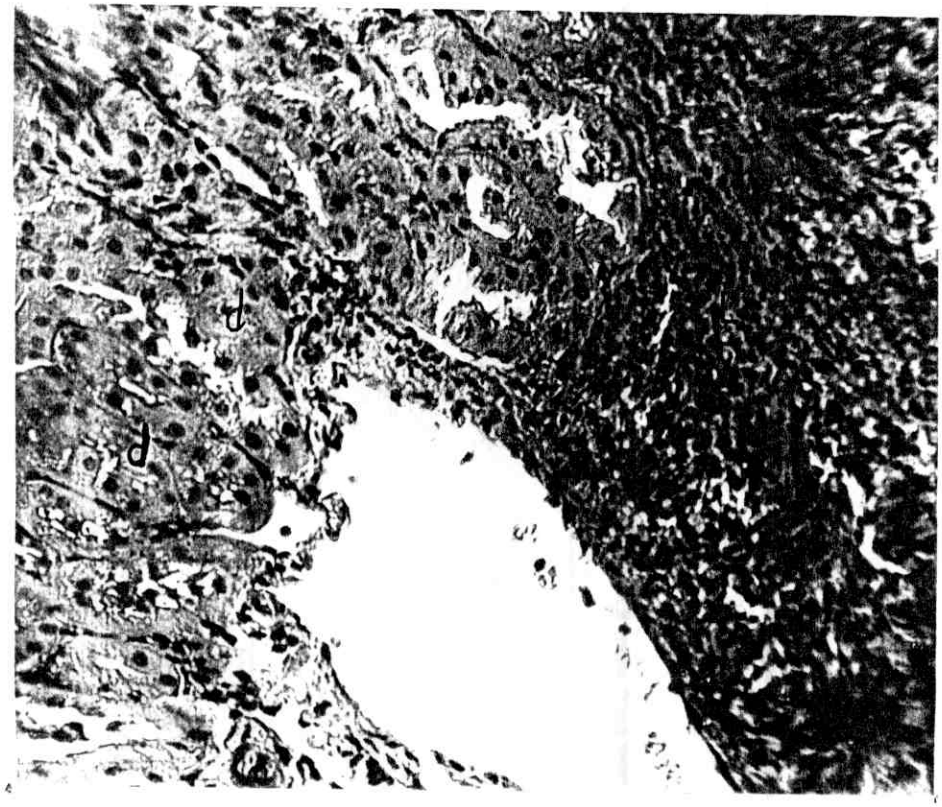
Fig(11) Cross section in the liver of group(E) showing vacuolar degeneration(v) Hydrobic degeneration (h),notice fatty changes (f) . H & E ..... X400 .



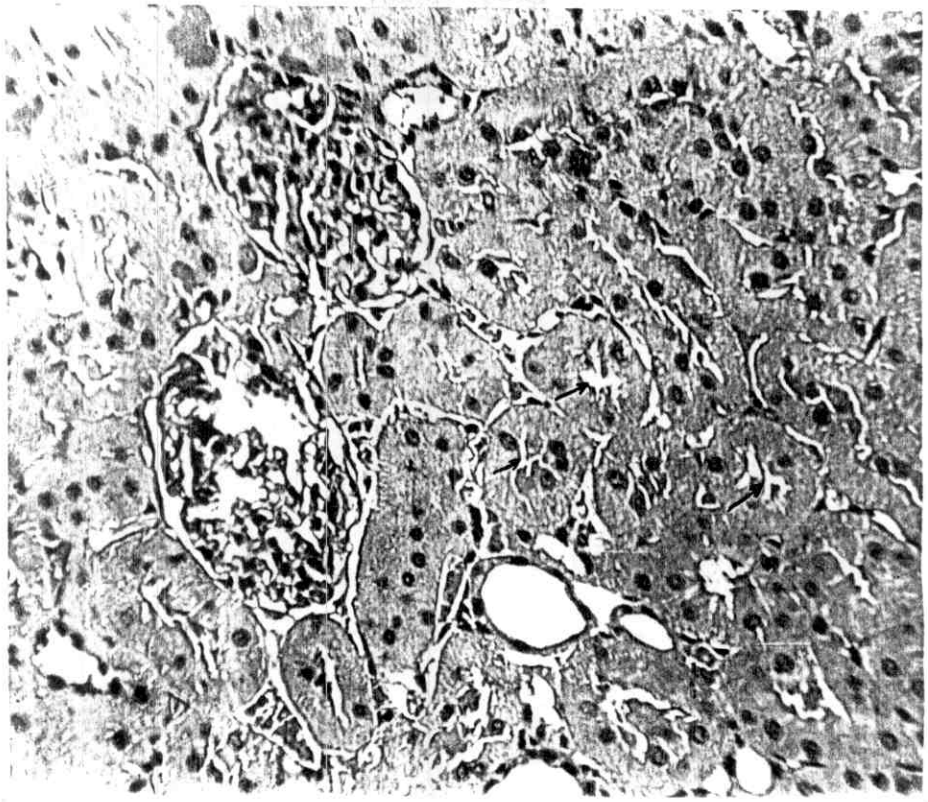
Fig(12) Cross section in the liver of group(F) showing vacuolar degeneration(v)
H & E ..... X400 .



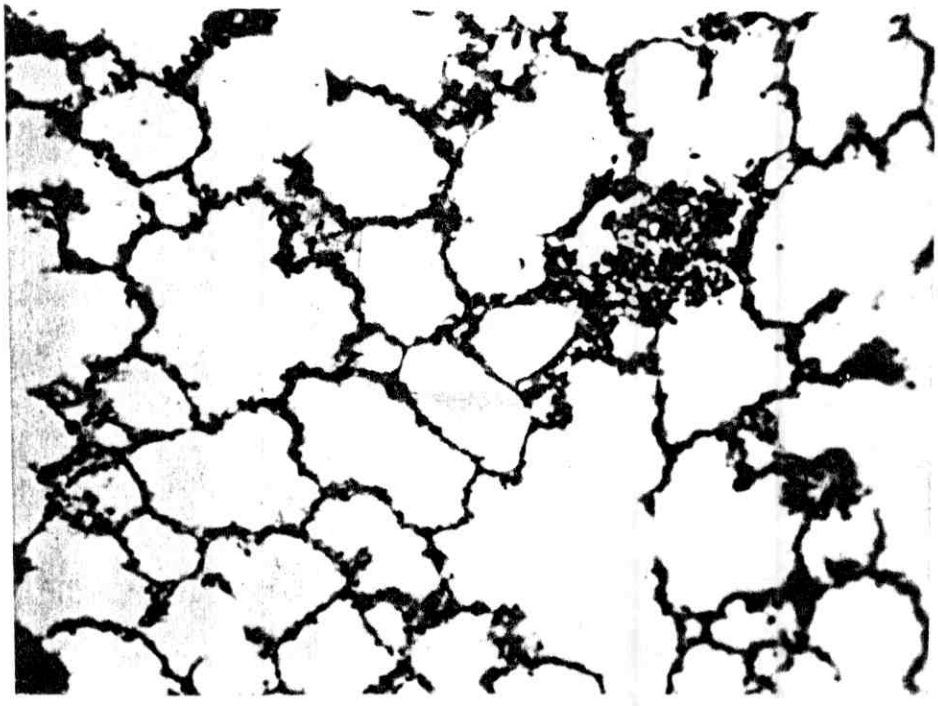
Fig(13)Cross section in the kidney of group (A) showing glomeruli(g),Proximal convoluted tubules & D.C.T . H & E X100



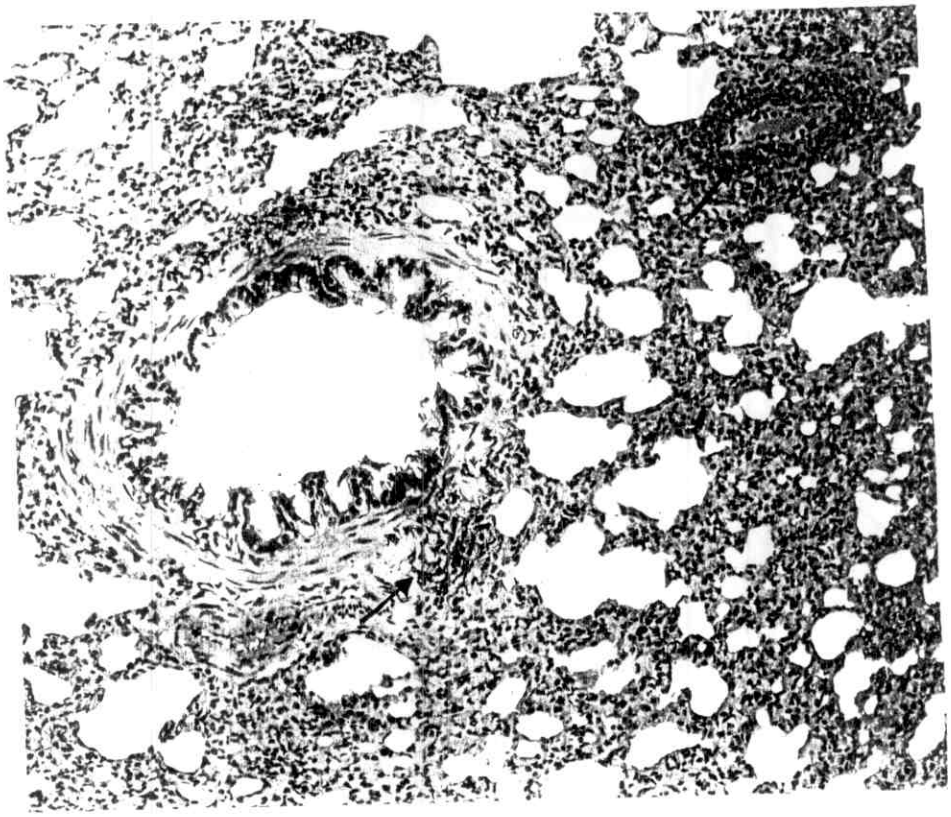
Fig(14) Cross section in the kidney of group (B) showing destructive changes (d) and lymphocytic infiltration (L) H & E .....X100 .



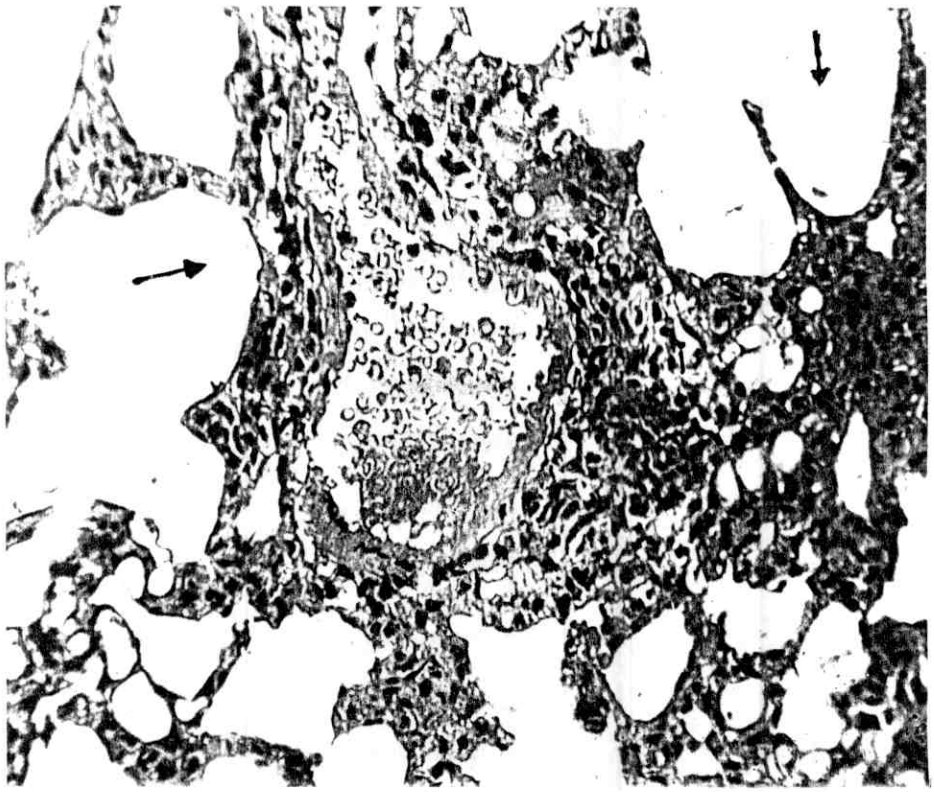
Fig(15)Cross section in the kidney of group (E) showing cloudy swelling in the cells of the kidney (arrows)H & E .....X100 .



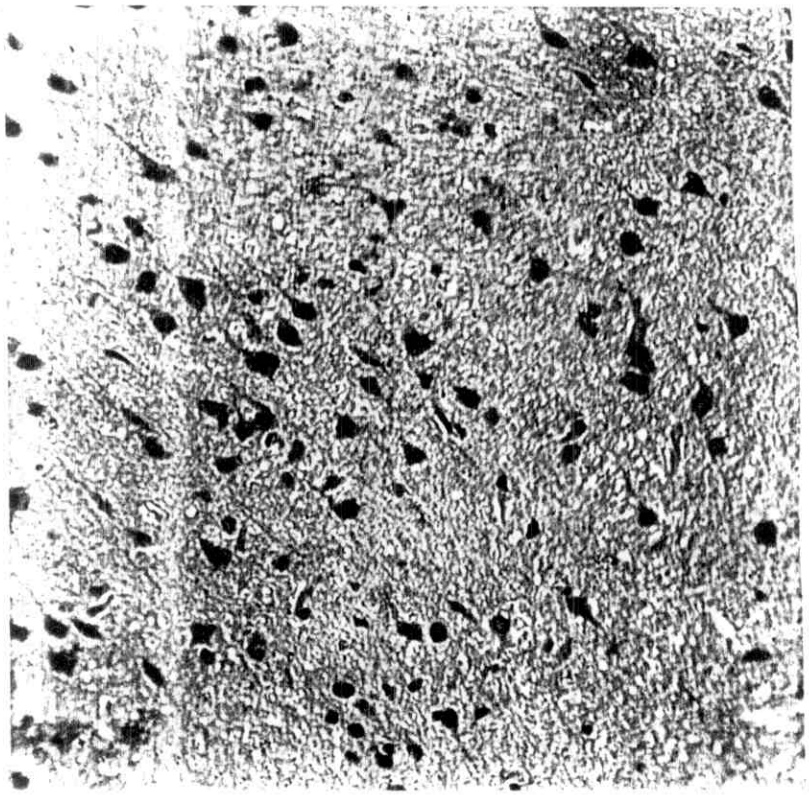
Fig(16)Cross section in the lung of group (A) showing normal alveoli covered with endothelial cells.
H & E .....X100 .



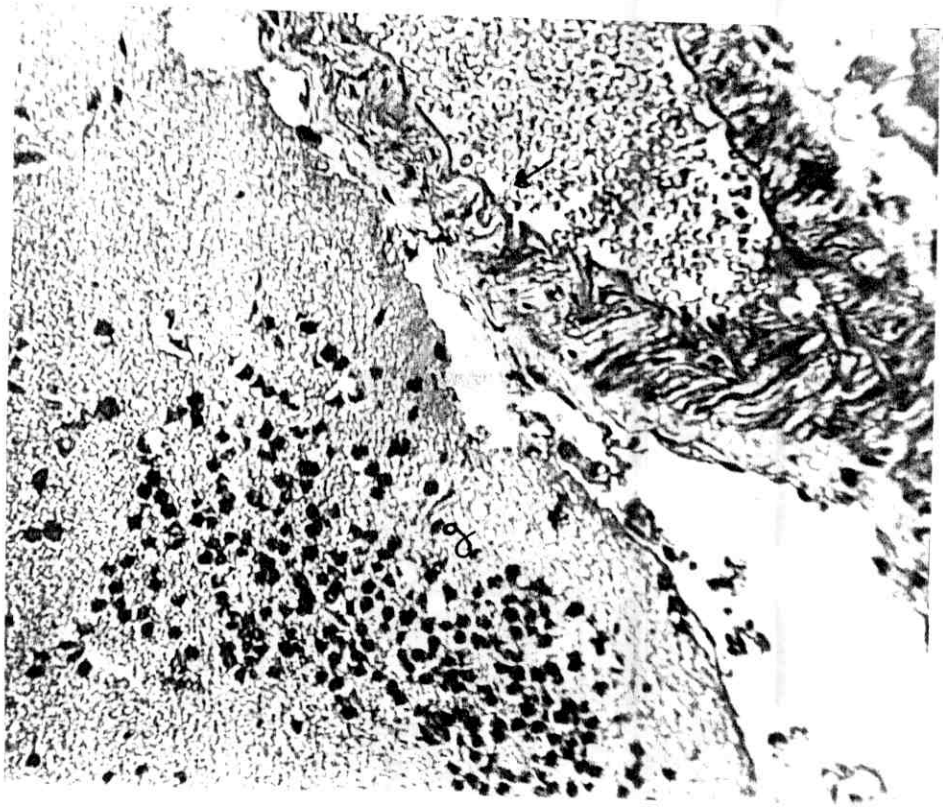
Fig(17)Cross section in the lung of group (B) showing active hypermia (arrow).
H & E .....X100.



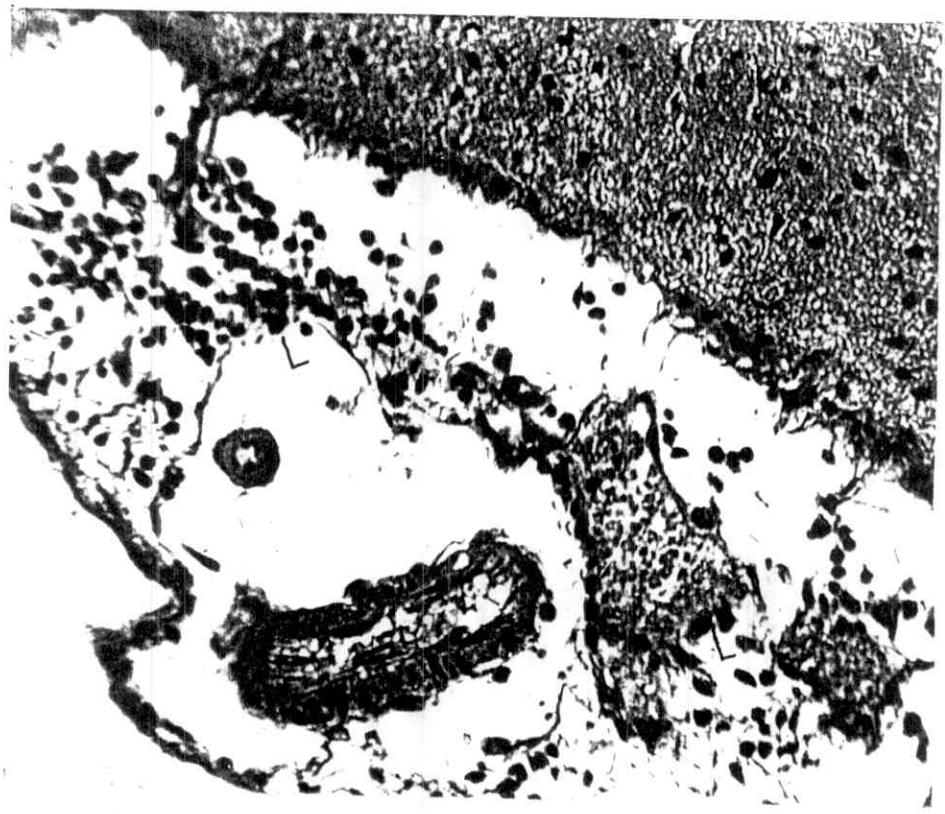
Fig(18)Cross section in the lung of group (E) showing emphysema (arrow) & inflammatory cells (i).
H & E .....X100 .



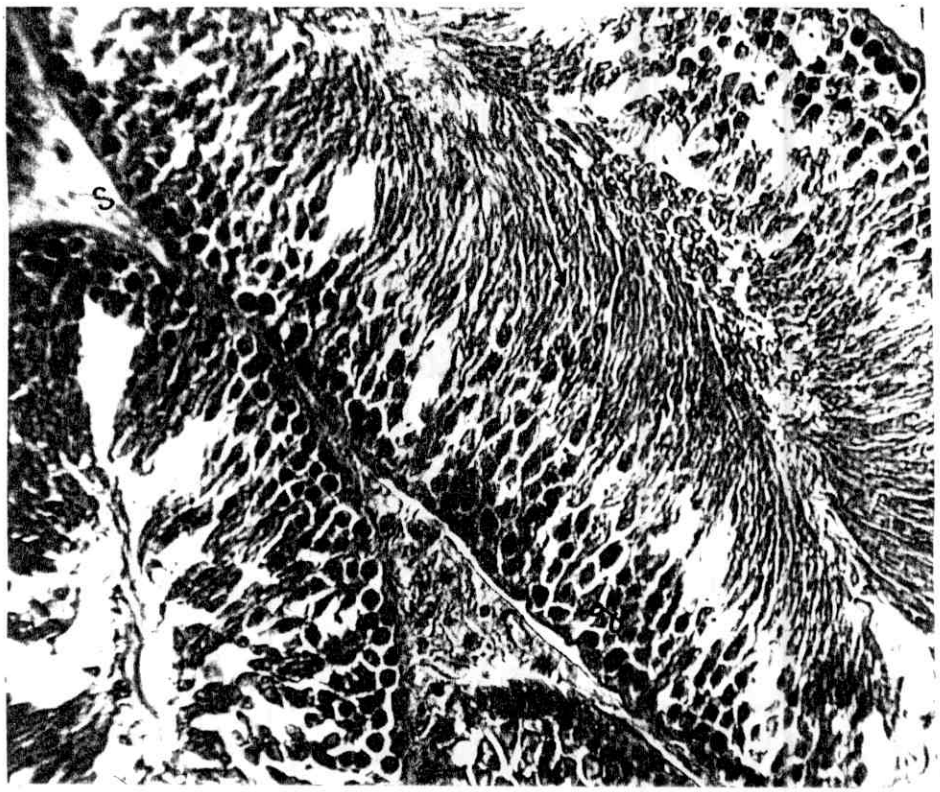
Fig(19) Cross section in the brain of group (A) showing the pyramidal cells and Nerve cells and fibres.
H & E .....X100 .



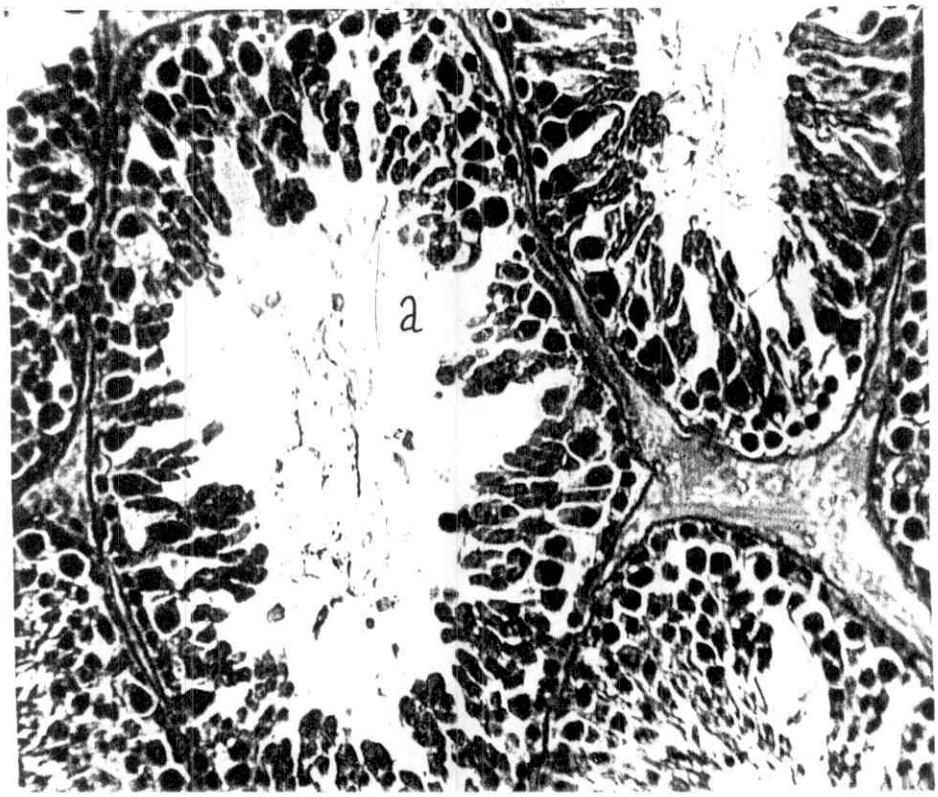
Fig(20) Cross section in the brain of group (B) showing hemorrhage in the brain artery (arrows) and geliosis (g) .
H & E .....X100.



Fig(21) Cross section in the brain of group (E) showing lymphocytic infiltration (L) in the meninges.
H & E .....X100.



Fig(22) Cross section in the testes of group (A) showing seminephrous tubules (s) spermatogonium (g) and spermatozoa (arrows).
H & E .....X400.



Fig(23) Cross section in the testes of group (E) showing azospermia(a).
H & E .....X100.

4.4. Organoleptic evaluation of biscuits:

Table (25) showed the physical properties of biscuits including diameter (cm), thickness (mm) and density (g/cm^3) and sensory characteristics included colour, taste, odour , crust appearance and overall acceptability as a result of different substitutions.

From the obtained data. Non significant difference between biscuits made from corn oil and control for sensory characteristics. Was found the statistical analysis indicated that no significant difference between the biscuits made from wheat flour substituted with 27.5% defatted lupine meal and control sample for colour, taste, texture and crust appearance. The results showed that addition of the ethanolic extract of propolis resulted in improving the biscuit Organoleptic properties, these results agree with **Abd El-Salam (1999).**

From the previous results. It could be noticed that defatted lupine meal, corn oil and propolis ethanol extract were suitable for the production of hard biscuits with high acceptability.

Table (29): physical properties and sensory evaluations of biscuits contained defatted lupine meal, corn oil and propolis ethanol extract.

Biscuits substitution	Sensory characteristics						Physical properties		
	Crust appearance (30)	Color (10)	Texture (30)	Odor (15)	Taste (15)	Overall acceptability (100)	Density (g/cm^3)	Thickness (mm)	Diameter (cm)
Biscuits contents: 100% shortening (control)	27.80^{A}	8.65^{A}	27.90^{A}	13.70A	13.70^{A}	91.5^{A}	0.62	0.68	4.02
100% corn oil	27.60^{AB}	9.10^{A}	27.00^{AB}	13.30^{AB}	12.90^{AB}	89.90^{AB}	0.76	0.675	5.23
27.5% D.F. lupine meal + 100% shortening	26.45^{AB}	8.75^{A}	26.30^{AB}	12.25^{B}	12.95^{AB}	86.70^{B}	0.83	0.62	5.16
27.5% D.F. lupine meal + 100% corn oil	26.20^{B}	8.75^{A}	25.55^{B}	12.35^{B}	12.45^{B}	85.30^{B}	0.81	0.63	5.22
27.5% D.F. lupine meal + 100% corn oil + 0.5% P.E.E.	27.15^{AB}	8.60^{A}	25.80^{B}	12.50^{AB}	12.65^{AB}	86.70^{B}	0.75	0.57	5.40
L.S.D	1.5334	0.8178	1.9316	1.2039	1.1032	4.9189			

D.F= defatted **P.E.E**= propolis ethanol extract