

CHAPTER FOUR

RESULTS

4.1. The 1st experiment:

4.1.1. Effect of different levels of some natural phytochemicals on growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings:

Growth performance parameters final weight (FW), weight gain (WG), average daily gain (ADG) and specific growth rate (SGR) of Nile tilapia (*Oreochromis niloticus*) fingerlings fed on the experimental diets treated with different levels of phytochemicals (ginseng extract (GE) at level of 0.2 and 0.4 g/kg diet; tribulus extract (TE) at level of 0.6 and 1.2 g/kg diet and date palm pollen (DPP) at level of 3 and 6 g/kg diet) are presented in table (4). Data of the present study explained that all dietary phytochemicals significantly increase FW, WG, ADG and SGR of Nile tilapia fingerlings. The high values were recorded with the high levels of TE, DPP and GE (26.93%, 22.40% and 19.30% more than control, respectively). In general, different level of phytochemicals had no significant influence on survival rate of experimental fish.

Table (5) shows the data concerning final length, length gain and condition factor during the experimental period. Generally these results indicate that final length, length gain and condition factor increased with the increasing levels of phytochemicals, however the effects were insignificant with all dietary phytochemicals levels.

4.1.2. Effect of different levels of some natural phytochemicals on feed and nutrients utilization of Nile tilapia (*O. niloticus*) fingerlings:

The obtained results, given in Table (6) clearly show that phytochemical supplementations (GE, TE and DPP) exhibited a significant effect on all estimated feed and nutrients utilization traits. Feed intake significantly increase with GE (0.4 g/kg diet) compared to control and surpass control by 15%.

Feed conversion ratio (FCR) improved with all phytochemical supplementations. The significant improvement mentioned with TE (1.2 g/kg diet) and DPP (6 g/kg diet). However, the other treatments insignificantly improved FCR.

Table (4): Effect of different levels of some natural phytochemicals (g/kg diet) on growth performance and survival rate of Nile tilapia, *O. niloticus*, fingerlings.

Items	Initial weight (g/fish)	Final weight (g/fish)	Weight gain (g/fish)	Average daily gain (g/fish/day)	Specific growth rate (%/day)	Survival rate (%)
Control	3.65±.05	38.64±0.58 ^d	34.99±0.63 ^e	0.42±0.01 ^e	2.81±0.03 ^d	95.00±5.00
GE 0.2	3.65±.05	43.89±1.14 ^c	40.24±1.09 ^{cd}	0.48±0.01 ^{cd}	2.96±0.01 ^c	95.00±5.00
GE 0.4	3.60±.10	46.09±1.03 ^{bc}	42.49±0.93 ^{bc}	0.51±0.01 ^{bc}	3.04±0.01 ^{ab}	90.00±0.00
TE 0.6	3.60±.10	43.46±0.79 ^c	39.86±0.69 ^d	0.47±0.01 ^d	2.97±0.01 ^{bc}	90.00±0.00
TE 1.2	3.65±.05	49.05±0.17 ^a	45.40±0.12 ^a	0.54±0.00 ^a	3.09±0.01 ^a	95.00±5.00
DPP 3	3.65±.05	40.82±0.38 ^d	37.17±0.33 ^e	0.44±0.00 ^e	2.87±0.01 ^d	95.00±5.00
DPP 6	3.75±.05	47.30±0.85 ^{ab}	43.55±0.90 ^{ab}	0.52±0.01 ^{ab}	3.02±0.04 ^{bc}	95.00±5.00

Values superscripted by different alphabets within the same column are significantly different ($P<0.05$).
GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

Table (5): Effect of different levels of some natural phytochemicals (g/kg diet) on final length, length gain and condition factor of Nile tilapia, *O. niloticus*, fingerlings.

Treatments	Initial length (cm)	Final length (cm)	Length gain (cm)	Condition factor
Control	6.15±0.10	13.31±.38	7.16±.28	1.65±.16
GE 0.2	6.20±0.04	13.47±.23	7.27±.19	1.80±.14
GE 0.4	6.20±0.11	13.60±.45	7.40±.34	1.84±.14
TE 0.6	6.23±0.11	13.59±.58	7.36±.47	1.75±.19
TE 1.2	6.32±0.03	13.72±.33	7.41±.37	1.91±.15
DPP 3	6.31±0.02	13.36±.75	7.06±.72	1.74±.27
DPP 6	6.32±0.05	13.45±.50	7.13±.45	1.96±.18

Values superscripted by different alphabets within the same column are significantly different ($P<0.05$).
GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

From the nutrients utilization point of view, protein efficiency ratio (PER) and protein productive value (PPV) were increased significantly with TE and DPP in high levels and insignificantly with other phytochemical treatments in comparison with control. The same trend was observed with energy gain, meanwhile, energy utilization increased significantly only with DPP at a level of 6 g/kg diet and increased insignificantly with other phytochemicals. The percents of energy gain increase were 20.82%, 27.46% and 28.18% with GE (0.4 g/kg diet), TE (1.2 g/kg diet) and DPP (6 g/kg diet), respectively, more than control.

4.1.3. Effect of different levels of some natural phytochemicals on whole body chemical composition of Nile tilapia (*O. niloticus*) fingerlings:

Table (7) showed the effect of different phytochemical treatments on body composition and gross energy expressed as percent of wet weight of Nile tilapia, *O. niloticus*, fingerlings at the end of the experiment. The obtained results showed that moisture content of treated fish didn't differ significantly. The ether extract decreased numerically with all phytochemical supplementations in high level and the lowest values recorded with GE (0.4 g/kg diet) followed by TE (1.2 g/kg diet).

The present findings declared that crude protein, ash and gross energy showed significant differences due to supplementation treatments. The highest protein contents gained by fish treated with GE (0.4 g/kg diet), and then DPP (6 g/kg diet). Meanwhile, the lowest protein value showed by fish fed diet supplemented with DPP at level of 6 g/kg diet. Ash decreased significantly only with DPP (3 g/kg diet) compared to other treatments. Also, the gross energy was decreased significantly with DPP (3 g/kg diet) compared to control.

Table (6): Effect of different levels of some natural phytochemicals (g/kg diet) on feed and nutrients utilization of Nile tilapia, *O. niloticus*, fingerlings.

Items	Feed intake (g/fish)	Feed conversion ratio (g)	Protein efficiency ratio (g)	Protein productive value (%)	Energy Gain (kj/g)	Energy Utilization (%)
Control	51.00±1.29 ^b	1.46±.01 ^a	2.12±.02 ^b	34.50±1.43 ^b	2.45±.71 ^c	23.89±.89 ^{ab}
GE 0.2	54.55±0.83 ^{ab}	1.36±.06 ^{ab}	2.28±.10 ^{ab}	37.91±1.78 ^{ab}	2.80±1.65 ^{abc}	25.54±1.02 ^{ab}
GE 0.4	58.87±0.73 ^a	1.39±.05 ^{ab}	2.23±.08 ^{ab}	38.72±1.69 ^{ab}	2.96±2.60 ^{ab}	24.98±1.22 ^{ab}
TE 0.6	56.98±2.13 ^{ab}	1.43±.03 ^{ab}	2.16±.04 ^{ab}	35.94±2.01 ^{ab}	2.78±7.97 ^{abc}	24.21±2.00 ^{ab}
TE 1.2	58.07±0.72 ^{ab}	1.28±.01 ^b	2.41±.02 ^a	40.60±1.60 ^{ab}	3.12±1.11 ^a	26.71±.07 ^{ab}
DPP 3	53.78±3.95 ^{ab}	1.45±.09 ^{ab}	2.14±.14 ^{ab}	34.48±1.28 ^b	2.54±1.84 ^{bc}	23.51±1.01 ^b
DPP 6	55.60±2.82 ^{ab}	1.28±.04 ^b	2.42±.07 ^a	42.15±2.73 ^a	3.14±.39 ^a	23.89±.89 ^{ab}

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$).

GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

¹ Gross energy calculated on the basis of 23.6, 39.4 and 17.2 k joule gross energy/g protein, ether extract and NFE, respectively (NRC, 1993).

Table (7): Effect of different levels of some natural phytochemicals (g/kg diet) on whole body chemical composition and gross energy as percent of wet weight of Nile tilapia, *O. niloticus*, fingerlings.

Items	% on wet weight basis				
	Moisture	Crude protein (%)	Ether extract (%)	Ash (%)	Gross energy (kj/g) ¹
Control	72.81±0.66	15.99±0.11 ^b	7.41±0.01	3.79±0.10 ^a	6.70±0.49 ^b
GE 0.2	72.66±0.11	16.36±0.14 ^{ab}	7.20±0.04	3.80±0.09 ^a	6.70±0.31 ^b
GE 0.4	72.53±0.21	17.05±0.01 ^a	6.82±0.10	3.62±0.11 ^a	6.72±1.00 ^b
TE 0.6	72.61±2.52	16.41±0.36 ^{ab}	7.18±0.31	3.81±0.05 ^a	6.71±0.88 ^b
TE 1.2	72.80±0.04	16.55±0.49 ^{ab}	6.94±0.46	3.72±0.03 ^a	6.65±1.58 ^{bc}
DPP 3	73.68±0.61	15.86±0.04 ^b	7.11±0.07	3.35±0.03 ^b	6.56±0.47 ^c
DPP 6	71.71±0.70	17.05±0.13 ^a	7.38±0.09	3.86±0.04 ^a	6.94±0.16 ^a

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$).

GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

¹ Gross energy calculated on the basis of 23.6, 39.4 and 17.2 k joule gross energy/g protein, ether extract and NFE, respectively (NRC, 1993).

4.1.4. Effect of different levels of some natural phytochemicals on relative organs weight of Nile tilapia (*O. niloticus*) fingerlings:

Data presented in Table (8) showed the Effect of different levels of some natural phytochemicals (GE, TE and DPP) in two levels for each on visceral somatic index, liver somatic index, testes somatic index and spleen somatic index of *O. niloticus* fingerlings. The results showed significant changes in all studied somatic indexes, were phytochemicals treatments decreased in level dependent manner visceral somatic index and increased liver, testes and spleen somatic indexes compared to control.

4.1.5. Effect of different levels of some natural phytochemicals on anabolic steroid hormone of Nile tilapia (*O. niloticus*) fingerlings:

Results presented in Table (9) and illustrated in Figure (1) summarized the effects of dietary phytochemical treatments (GE, TE and DPP) on luteinizing hormone (LH) and testosterone levels in plasma of Nile tilapia, *O. niloticus*, fingerlings. The results indicated that fish received diets containing all phytochemicals showed significant increase in LH hormone level compared to control and the effects were level dependent with each supplementations. The highest LH levels were mentioned with TE (1.2 g/kg diet) followed by DPP (6 g/kg diet) and GE (0.4 g/kg diet), and the percent of increase were 22.9%, 18.5% and 17.6%, respectively.

Plasma testosterone levels were significantly increased with all phytochemicals treatments compared to control. The most effective levels for testosterone level were TE (1.2 g/kg diet) followed by GE (0.4 g/kg diet), TE (0.6 g/kg diet) and DPP (6 g/kg diet), the percent of changes reached 86.23%, 64.49%, 57.40% and 24.62%, respectively, more than control. The increase of testosterone levels were concomitantly to the increase of LH levels with most studied levels.

Table (8): Effect of different levels of some natural phytochemicals (g/kg diet) on relative organs weight of Nile tilapia, *O. niloticus*, fingerlings.

Items	Visceral somatic index (%)	Liver somatic index (%)	Testes somatic index (%)	Spleen somatic index (%)
Control	11.77±0.22 ^a	2.79±0.08 ^b	0.83±0.03 ^c	0.25±.01 ^c
GE 0.2	10.96±0.24 ^{bc}	2.95±0.06 ^{ab}	1.01±0.03 ^{abc}	0.29±.01 ^{ab}
GE 0.4	9.92±0.14 ^{de}	3.05±0.05 ^a	1.12±0.04 ^{ab}	0.30±.01 ^a
TE 0.6	10.43±0.18 ^{cd}	2.96±0.04 ^{ab}	1.02±0.04 ^{abc}	0.27±.01 ^{bc}
TE 1.2	9.41±0.29 ^e	3.02±0.06 ^a	1.18±0.05 ^a	0.29±.01 ^{ab}
DPP 3	11.49±0.27 ^{ab}	2.88±0.06 ^{ab}	0.96±0.10 ^{bc}	0.27±.01 ^{abc}
DPP 6	10.98±0.18 ^{bc}	2.99±0.05 ^a	1.04±0.12 ^{ab}	0.28±.01 ^{ab}

Values superscripted by different alphabets within the same column are significantly different ($P<0.05$). GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

Table (9): Effect of different levels of some natural phytochemicals (g/kg diet) on luteinizing hormone and testosterone levels of Nile tilapia, *O. niloticus*, fingerlings.

Items	Luteinizing hormone (IU/L)	Percent of change	Testosterone (ng/ml)	Percent of change
Control	0.43±0.02 ^b	-	4.15±0.13 ^d	-
GE 0.2	0.48±0.03 ^{ab}	10.44	5.14±0.26 ^c	23.91
GE 0.4	0.50±0.02 ^a	17.56	6.83±0.30 ^b	64.49
TE 0.6	0.47±0.02 ^{ab}	8.66	6.53±0.18 ^b	57.40
TE 1.2	0.53±0.01 ^a	22.85	7.73±0.16 ^a	86.23
DPP 3	0.49±0.01 ^{ab}	13.93	4.83±0.03 ^c	16.30
DPP 6	0.51±0.03 ^a	18.48	5.17±0.28 ^c	24.62

Values superscripted by different alphabets within the same column are significantly different ($P<0.05$). GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

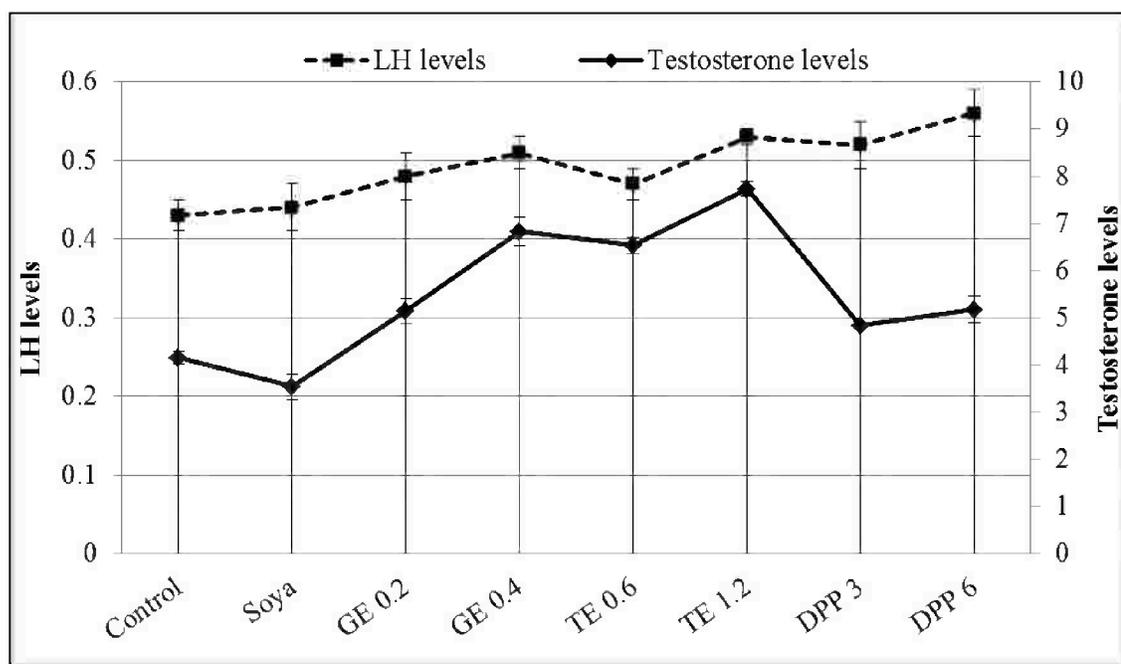


Figure (1): Effect of different phytochemical treatments (g/kg diet) on luteinizing hormone (LH) and testosterone levels of Nile tilapia, *O. niloticus*, fingerlings. GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

4.1.6. Regression and correlation between plasma testosterone levels and growth performance of Nile tilapia (*O. niloticus*) fingerlings:

Data are presented in figure (2 and 3) illustrated a simple regression, linear regression equation and correlation coefficients of final body weight, SGR and plasma testosterone levels of Nile tilapia, *O. niloticus*, fingerlings. The results showed significant simple regression between final weight, SGR and plasma testosterone level. The prediction variable or regression coefficient was 59% and 73% with final weight and SGR respectively. Moreover, there was a significant positive correlation between plasma testosterone level, final weight and SGR of Nile tilapia.

4.1.7. Effect of different levels of some natural phytochemicals on histological examination of testes of Nile tilapia (*O. niloticus*) fingerlings:

The histological examination of testis showed nearly normal histological appearance in control group. All dietary phytochemicals treatment showed slightly to highly improvement of spermatids abundance. Where, the treatment with GE in low level showed normal histological appearance with slightly abundance of spermatids. Meanwhile, the abundance of spermatids improved to be moderate to high with the high level of GE.

However, the treatment with TE showed moderate to high abundance of spermatids in the lumen of the testicular lobules in level dependent manner. However, treatment with DPP in both levels showed slight abundance of spermatids in the lumen of the testicular tubules.

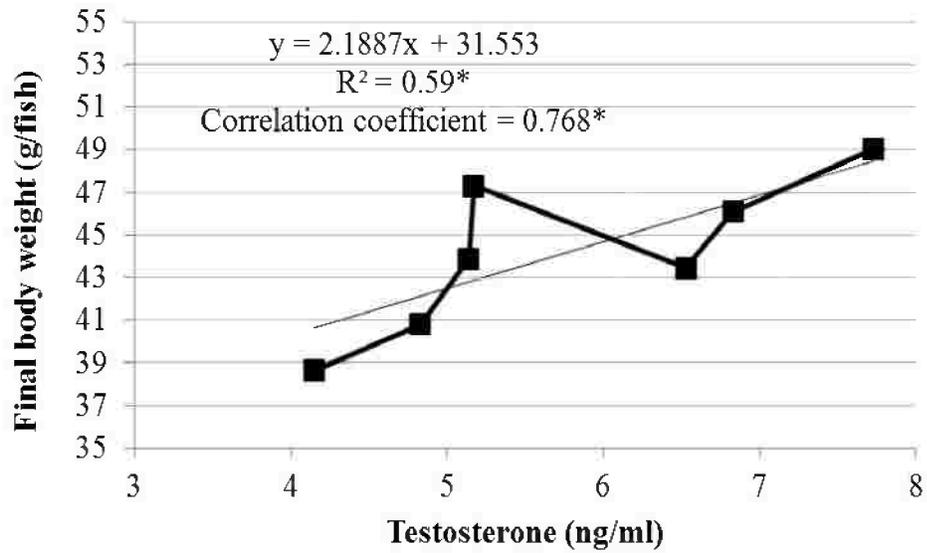


Figure (2): Relationship between the plasma testosterone levels and final body weights (g/fish) of Nile tilapia, *O. niloticus*, fingerlings.

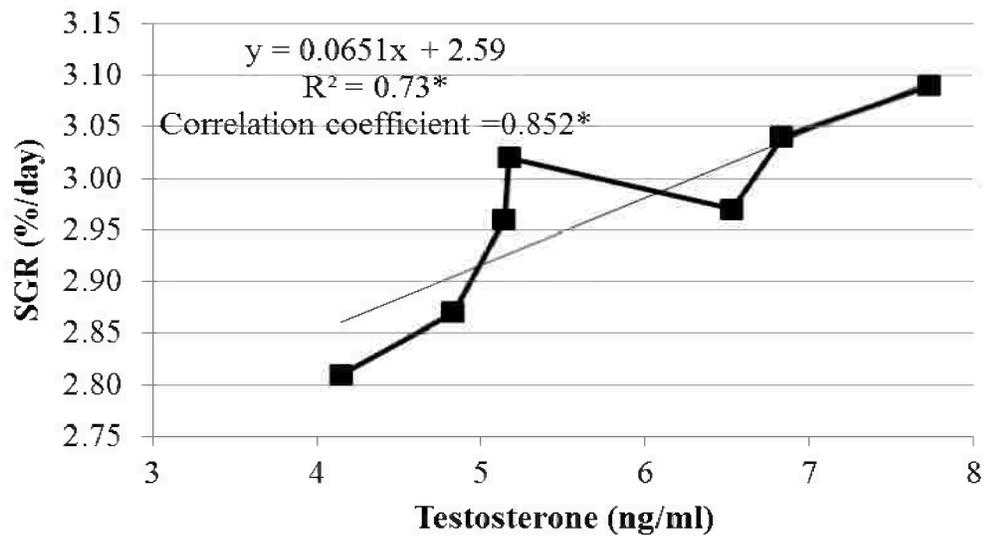


Figure (3): Relationship between the plasma testosterone levels and specific growth rate (SGR; g/fish) of Nile tilapia, *O. niloticus*, fingerlings.

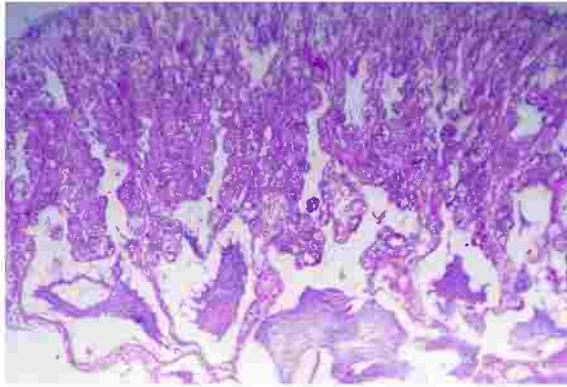


Figure (4): Testis of a Nile tilapia, *O. niloticus*, fingerlings of control group showed normal histological appearance. H &E. (X. 250).

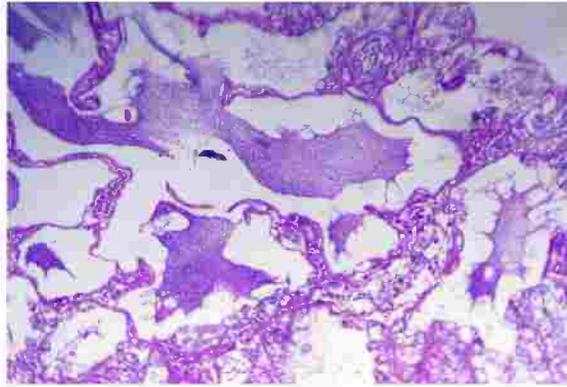


Figure (5): Testis of a Nile tilapia, *O. niloticus*, fingerlings exposed to 0.2 g/kg ginseng extract showed normal histological appearance with slightly abundance of spermatozoa in the lumen of the testicular lobules. H &E. (X. 250).

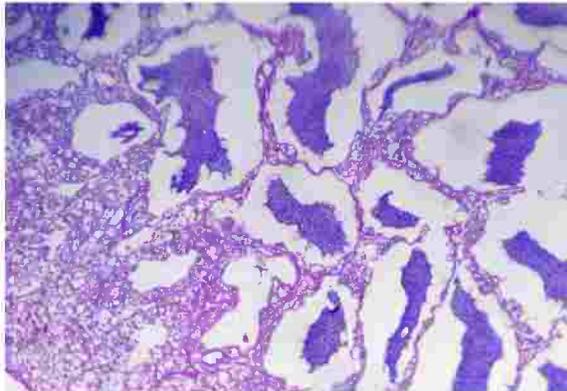


Figure (6): Testis of a Nile tilapia, *O. niloticus*, fingerlings exposed to 0.4 g/kg ginseng extract showed normal histological appearance with moderate to high abundance of spermatozoa in the lumen of the testicular lobules. H &E. (X. 250).

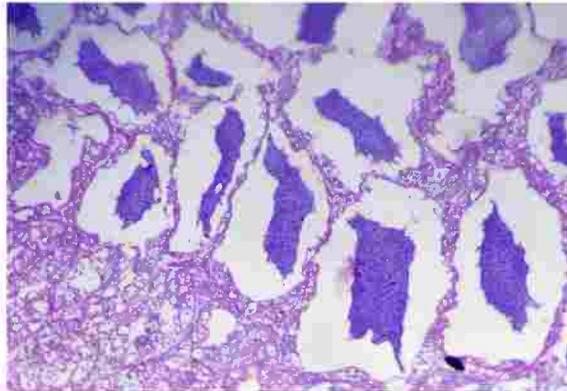


Figure (7): Testis of a Nile tilapia, *O. niloticus*, fingerlings exposed to 0.6 g/kg tribulus extract showed normal histological appearance with moderate abundance of spermatozoa in the lumen of the testicular lobules. H &E. (X. 250).

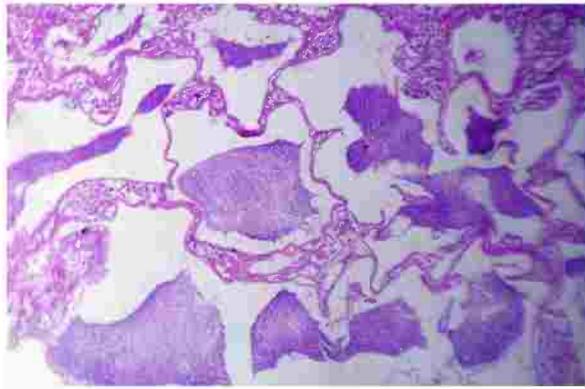


Figure (8): Testis of a Nile tilapia, *O. niloticus*, fingerlings exposed to 1.2 g/kg tribulus extract showed normal histological appearance with highly abundance of spermatids in the lumen of the testicular lobules. H&E. (X. 250).

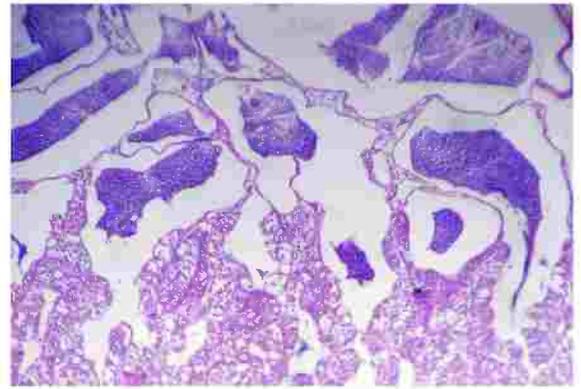


Figure (9): Testis of a Nile tilapia, *O. niloticus*, fingerlings exposed to 3 g/kg date palm pollen showed normal histological appearance with slightly abundance of spermatids in the lumen of the testicular lobules. H &E. (X. 250).

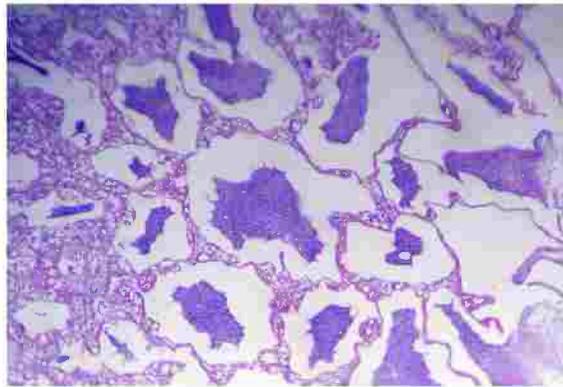


Figure (10): Testis of a Nile tilapia, *O. niloticus*, fingerlings exposed to 6 g/kg date palm pollen showed normal histological appearance with slightly abundance of spermatids in the lumen of the testicular lobules. H &E. (X. 250).

4.1.8. Effect of different levels of some natural phytochemicals on thiobarbituric acid-reactive substances and antioxidant enzymes of Nile tilapia (*O. niloticus*) fingerlings:

Table (10) represented the changes in the levels of thiobarbituric acid-reactive substances (TBARs), reduced glutathione (GSH), and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione *S*-transferase (GST), glutathione peroxidase (GPx) in plasma of Nile tilapia, *O. niloticus*, fingerlings fed diet supplemented with different phytochemicals at two levels.

Results indicated that treatment with all levels of phytochemicals significantly reduced plasma TBARs levels than control and the lowest values were recorded with GE (0.4 g/kg diet) followed by TE (1.2 g/kg diet). However GSH content and SOD, CAT, GST and GPx activities increased significantly than control with each studied levels.

The effect of different phytochemicals on TBARs, GSH, SOD, CAT, GST and GPx in liver and testes homogenate of *O. niloticus* fingerlings are shown in Table (11 and 12). The results illustrated that TBARs levels in liver decreased significantly with each phytochemicals levels than control. The GSH content, CAT, SOD, GST and GPx activities increased significantly compared to control. It is noteworthy that, the levels of lipid peroxidation (TBARs) in liver are higher than plasma and testes.

It is noticeable that, the Effect of different levels of some natural phytochemicals on the oxidant and antioxidant balance in plasma, liver and kidney was level dependently. Moreover, GE and TE exhibit a strong antioxidant more than DPP.

Table (10): Effect of different levels of some natural phytochemicals (g/kg diet) on the levels of thiobarbituric acid-reactive substances, reduced glutathione, and the activities of antioxidant enzymes in plasma of Nile tilapia, *O. niloticus*, fingerlings.

Items	Thiobarbituric acid-reactive substances (nmol/ml)	Reduced glutathione ($\mu\text{mol/ml}$)	Superoxide dismutase (U/ml)	Catalase (U/ml)	Glutathione S-transferase ($\mu\text{mol/h}$)	Glutathione peroxidase (U/ml)
Control	2.69 \pm 0.07 ^a	1.17 \pm 0.07 ^c	1.11 \pm 0.05 ^d	7.08 \pm 0.35 ^c	1.30 \pm 0.08 ^d	13.81 \pm 0.56 ^c
GE 0.2	1.94 \pm 0.05 ^c	1.37 \pm 0.07 ^b	1.33 \pm 0.03 ^{bc}	7.86 \pm 0.34 ^{abc}	1.40 \pm 0.04 ^{cd}	14.25 \pm 0.36 ^c
GE 0.4	1.71 \pm 0.04 ^d	1.57 \pm 0.05 ^a	1.47 \pm 0.04 ^{ab}	8.67 \pm 0.15 ^a	1.59 \pm 0.07 ^{ab}	16.42 \pm 0.26 ^a
TE 0.6	1.99 \pm 0.06 ^c	1.38 \pm 0.02 ^b	1.37 \pm 0.08 ^{abc}	7.58 \pm 0.30 ^{bc}	1.48 \pm 0.04 ^{bc}	15.05 \pm 0.27 ^{ab} _c
TE 1.2	1.80 \pm 0.06 ^{cd}	1.44 \pm 0.04 ^{ab}	1.49 \pm 0.05 ^a	8.46 \pm 0.19 ^{ab}	1.66 \pm 0.06 ^a	15.94 \pm 0.64 ^{ab}
DPP 3	2.33 \pm 0.04 ^b	1.27 \pm 0.08 ^{bc}	1.26 \pm 0.03 ^{cd}	7.92 \pm 0.07 ^{abc}	1.40 \pm 0.03 ^{cd}	14.50 \pm 0.72 ^{bc}
DPP 6	1.93 \pm 0.09 ^c	1.40 \pm 0.05 ^{ab}	1.33 \pm 0.06 ^{bc}	8.13 \pm 0.40 ^{ab}	1.57 \pm 0.03 ^{ab}	15.38 \pm 0.44 ^{ab} _c

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$).

GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

Table (11): Effect of different levels of some natural phytochemicals (g/kg diet) on the levels of thiobarbituric acid-reactive substances, reduced glutathione, and the activities of antioxidant enzymes in liver homogenate of Nile tilapia, *O. niloticus*, fingerlings.

Items	Thiobarbituric acid-reactive substances (nmol/ml)	Reduced glutathione (μ mol/ml)	Superoxide dismutase (U/ml)	Catalase (U/ml)	Glutathione S-transferase (μ mol/h)	Glutathione peroxidase (U/ml)
Control	89.41 \pm 0.81 ^a	6.14 \pm 0.26 ^b	10.33 \pm 0.63 ^d	53.07 \pm 0.54 ^c	5.88 \pm 0.13 ^d	46.63 \pm 2.98 ^c
GE 0.2	76.51 \pm 0.87 ^{bc}	7.12 \pm 0.35 ^a	11.67 \pm 0.30 ^{bc}	59.53 \pm 1.30 ^b	7.24 \pm 0.23 ^{bc}	59.95 \pm 1.87 ^a _b
GE 0.4	69.42 \pm 0.86 ^d	7.53 \pm 0.33 ^a	12.67 \pm 0.31 ^{ab}	67.08 \pm 1.93 ^a	7.77 \pm 0.09 ^a	64.48 \pm 1.28 ^a
TE 0.6	75.26 \pm 1.38 ^{bc}	7.02 \pm 0.16 ^a	11.42 \pm 0.26 ^c	66.67 \pm 1.56 ^a	7.14 \pm 0.17 ^{bc}	58.57 \pm 1.97 ^a _b
TE 1.2	67.53 \pm 1.46 ^d	7.39 \pm 0.30 ^a	12.78 \pm 0.22 ^a	70.00 \pm 0.72 ^a	7.48 \pm 0.23 ^{ab}	63.08 \pm 1.95 ^a _b
DPP 3	78.64 \pm 1.22 ^b	6.77 \pm 0.18 ^{ab}	11.37 \pm 0.34 ^c	58.75 \pm 2.94 ^b	6.92 \pm 0.15 ^c	57.00 \pm 1.79 ^b
DPP 6	74.90 \pm 1.39 ^c	7.06 \pm 0.18 ^a	12.25 \pm 0.26 ^{abc}	61.08 \pm 1.41 ^b	7.42 \pm 0.14 ^{abc}	58.02 \pm 1.51 ^b

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$).

GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

Table (12): Effect of different levels of some natural phytochemicals (g/kg diet) on the levels of thiobarbituric acid-reactive substances, reduced glutathione, and the activities of antioxidant enzymes in testes homogenate of Nile tilapia, *O. niloticus*, fingerlings.

Items	Thiobarbituric acid-reactive substances (nmol/ml)	Reduced glutathione ($\mu\text{mol/ml}$)	Superoxide dismutase (U/ml)	Catalase (U/ml)	Glutathione S-transferase ($\mu\text{mol/h}$)	Glutathione peroxidase (U/ml)
Control	49.97 \pm 1.05 ^a	3.10 \pm 0.09 ^d	8.92 \pm 0.33 ^b	20.22 \pm 0.79 ^d	3.50 \pm 0.18 ^c	5.96 \pm 0.14 ^c
GE 0.2	45.44 \pm 1.50 ^b	3.48 \pm 0.06 ^{bc}	10.83 \pm 0.32 ^{ab}	24.50 \pm 0.84 ^b	4.21 \pm 0.12 ^{ab}	8.23 \pm 0.20 ^a
GE 0.4	42.56 \pm 0.47 ^{bc}	3.92 \pm 0.08 ^a	12.44 \pm 1.37 ^a	29.00 \pm 0.68 ^a	4.55 \pm 0.16 ^{ab}	8.70 \pm 0.19 ^a
TE 0.6	43.94 \pm 0.74 ^b	3.41 \pm 0.10 ^c	11.59 \pm 0.37 ^a	24.00 \pm 0.26 ^{bc}	4.26 \pm 0.16 ^{ab}	7.97 \pm 0.39 ^{ab}
TE 1.2	40.34 \pm 0.59 ^c	3.76 \pm 0.07 ^a	12.80 \pm 0.74 ^a	24.67 \pm 0.15 ^b	4.67 \pm 0.13 ^a	8.71 \pm 0.25 ^a
DPP 3	45.29 \pm 0.82 ^b	3.45 \pm 0.09 ^c	10.69 \pm 0.41 ^{ab}	22.11 \pm 0.37 ^{cd}	4.17 \pm 0.18 ^b	7.33 \pm 0.15 ^b
DPP 6	42.75 \pm 0.86 ^{bc}	3.70 \pm 0.08 ^{ab}	12.00 \pm 0.24 ^a	23.40 \pm 1.36 ^{bc}	4.21 \pm 0.07 ^{ab}	7.95 \pm 0.31 ^{ab}

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$).

GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

4.1.9. Effect of different levels of some natural on hematological parameters of Nile tilapia (*O. niloticus*) fingerlings:

Data presented in table (13) showed the effect of different phytochemicals treatments on red blood cells (RBCs), haemoglobin (Hb), hematocrit (Hct), white blood cells (WBCs), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The obtained results showed that all phytochemicals significantly increased RBCs, Hb, Hct and WBCs with high levels. The treatment with GE (0.4 g/kg diet) recorded the highest values of WBCs, Hb and PCV. However, tribulus (1.2 g/kg diet) recorded the highest value of RBCs. All tested blood indices (MCH, MCV and MCHC) of studied groups didn't show any significant differences compared to control.

4.1.10. Effect of different levels of some natural phytochemicals on protein profile of Nile tilapia (*O. niloticus*) fingerlings:

Effect of different phytochemicals levels on total protein, albumin, globulin and albumin/globulin ratio (A/G ratio) demonstrated in table (14). The results showed a significant increase in total protein and globulin with all tested phytochemicals levels. The percent of total protein increase reached 25% with GE and 24% with TE in high levels than control. While, globulin increased by 64% and 59% with GE and TE in high levels than control, respectively. However, albumin didn't show any significant differences among different treatments. Moreover, A/g ratio decreased significantly only with GE in high level and TE in both levels.

Table (13): Effect of different levels of some natural phytochemicals (g/kg diet) on blood hematological parameters of Nile tilapia, *O. niloticus*, fingerlings.

Items	Red blood cells x10 ⁶	Hematocrit (%)	Haemoglobin (g/dl)	White blood cells x10 ³	Mean corpuscular volume (µm ³)	Mean corpuscular haemoglobin (pg)	Mean corpuscular haemoglobin concentration (%)
Control	2.01±0.04 ^c	26.63±.24 ^c	7.68±.13 ^c	89.49±3.70 ^c	133.08±2.42	38.36±.85	28.83±.43
GE 0.2	2.10±0.02 ^{bc}	28.63±.38 ^{ab}	8.34±.17 ^a	93.69±1.86 ^c	136.41±1.08	39.76±.76	29.14±.37
GE 0.4	2.17±0.04 ^{ab}	29.50±.29 ^a	8.42±.10 ^a	109.48±4.64 ^a	135.88±2.06	38.75±.29	28.53±.27
TE 0.6	2.12±0.03 ^{bc}	27.25±.32 ^{bc}	8.11±.05 ^{ab}	97.85±2.44 ^{bc}	128.43±1.69	38.20±.42	29.75±.25
TE 1.2	2.26±0.04 ^a	28.50±.65 ^{ab}	8.16±.10 ^{ab}	107.38±3.47 ^a	126.28±4.30	36.11±.33	28.70±1.01
DPP 3	2.09±0.04 ^{bc}	27.75±.66 ^{bc}	7.91±.12 ^{bc}	93.29±.93 ^c	132.73±2.72	37.89±1.23	28.59±1.12
DPP 6	2.15±0.05 ^{ab}	28.50±.79 ^{ab}	8.32±.10 ^a	103.18±2.40 ^{ab}	132.79±4.55	38.81±1.32	29.26±.74

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$).

GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

Table (14): Effect of different levels of some natural phytochemicals (g/kg diet) on proteins profile in plasma of Nile tilapia, *O. niloticus*, fingerlings.

Items	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control	4.06±0.05 ^d	3.05±0.11	1.01±0.05 ^e	3.07±0.27 ^a
GE 0.2	4.59±0.13 ^{bc}	3.37±0.07	1.22±0.05 ^{de}	2.78±0.07 ^{ab}
GE 0.4	5.03±0.03 ^a	3.48±0.07	1.55±0.05 ^{ab}	2.26±0.11 ^{bc}
TE 0.6	4.75±0.08 ^b	3.31±0.07	1.44±0.03 ^{bc}	2.29±0.07 ^{bc}
TE 1.2	5.07±0.08 ^a	3.36±0.22	1.71±0.14 ^a	2.05±0.32 ^c
DPP 3	4.45±0.04 ^c	3.30±0.09	1.15±0.05 ^{de}	2.89±0.19 ^a
DPP 6	4.69±0.06 ^b	3.37±0.05	1.32±0.05 ^{cd}	2.56±0.11 ^{abc}

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$).
GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

4.1.11. Effect of different levels of some natural phytochemicals on plasma lipid profile of Nile tilapia (*O. niloticus*) fingerlings:

Data in Table (15) showed the changes in plasma concentrations of total lipids (TL), cholesterol, triglycerides (TG), high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c) and very low density lipoprotein-cholesterol (VLDL-c) due to the treatment with different phytochemicals levels. Results indicated that treatment with all studied phytochemicals levels significantly decreased TL, cholesterol, TG, LDL-c and VLDL-c. The lowest values of the above mentioned parameters were recorded with TE (1.2 g/kg diet). However plasma HDL-c significantly increased with all tested phytochemicals in comparison with control.

4.1.12. Effect of different levels of some natural phytochemicals on liver function enzymes of Nile tilapia (*O. niloticus*) fingerlings:

Tables 16 and 17 showed the mean values of plasma and liver activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) of fish treated with different phytochemicals levels. Treatment with all phytochemicals levels resulted in a significant decrease in AST, ALT, ALP and ACP activities in plasma. The lowered AST, ALT and ACP values were recorded with TE, the percent of decrease were 24, 28, 29% lowered than control, respectively. Meanwhile, in the liver homogenate of treated fish with different phytochemicals, the activities of AST, ALT, ALP and ACP were significantly increased in level dependent manner with different phytochemicals.

4.1.13. Effect of different levels of some natural phytochemicals on kidney function of Nile tilapia (*O. niloticus*) fingerlings:

Data presented in Table (18) showed the Effect of different levels of some natural phytochemicals (GE, TE and DPP) in two levels for each on urea, creatinine and bilirubin of *O. niloticus*. The results showed that plasma urea, creatinine and bilirubin concentration significantly decreased with all phytochemicals supplementation treatments than control. Moreover, the lowest values of urea, creatinine and bilirubin were mentioned with high levels of all tested phytochemicals.

Table (15): Effect of different levels of some natural phytochemicals (g/kg diet) on lipids profile in plasma of Nile tilapia, *O. niloticus*, fingerlings.

Items	Total lipids (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	high density lipoprotein (mg/dl)	low density lipoprotein (mg/dl)	very low density lipoprotein (mg/dl)
Control	936.18±7.86 ^a	233.85±1.73 ^a	224.31±1.25 ^a	34.34±0.68 ^d	154.65±2.34 ^a	44.86±0.25 ^a
GE 0.2	879.16±9.84 ^b	214.92±3.46 ^b	197.48±10.14 ^b	38.28±0.86 ^{bc}	137.14±4.02 ^b	39.50±2.03 ^b
GE 0.4	871.87±19.32 ^{bc}	183.93±2.63 ^d	144.27±.28 ^d	42.73±1.76 ^a	112.35±1.34 ^d	28.85±0.06 ^d
TE 0.6	894.77±6.28 ^b	204.96±5.73 ^{bc}	193.92±2.48 ^b	37.27±0.45 ^{cd}	128.91±5.80 ^{bc}	38.78±0.50 ^b
TE 1.2	834.35±9.33 ^c	172.95±2.73 ^e	162.39±8.50 ^c	41.90±1.46 ^{ab}	98.57±3.92 ^e	32.48±1.70 ^c
DPP 3	884.93±9.64 ^b	211.75±1.92 ^b	195.07±4.07 ^b	39.39±1.82 ^{abc}	133.35±3.39 ^b	39.01±0.81 ^b
DPP 6	863.39±21.27 ^{bc}	198.07±2.96 ^c	184.09±6.01 ^b	40.41±0.54 ^{abc}	120.85±2.25 ^{cd}	36.82±1.20 ^b

Values superscripted by different alphabets within the same column are significantly different (P< 0.05).
GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

Table (16): Effect of different levels of some natural phytochemicals (g/kg diet) on some liver function enzymes in plasma of Nile tilapia, *O. niloticus*, fingerlings.

Items	Aspartate transaminase (U/ml)	Alanine transaminase (U/ml)	Alkaline phosphatase (IU/L)	Acid phosphatase (U/L)
Control	48.13±0.13 ^a	25.11±0.88 ^a	46.00±0.41 ^a	12.50±0.20 ^a
GE 0.2	40.49±0.78 ^c	24.05±0.48 ^{ab}	39.57±0.44 ^{cd}	8.97±0.44 ^{bc}
GE 0.4	37.61±0.63 ^d	20.53±0.62 ^d	37.59±0.61 ^{de}	8.93±0.33 ^{bc}
TE 0.6	40.82±0.81 ^{bc}	22.93±0.42 ^{bc}	42.21±0.94 ^b	9.42±0.70 ^{bc}
TE 1.2	36.65±0.35 ^d	18.01±0.39 ^e	38.69±0.91 ^{cde}	8.76±0.54 ^c
DPP 3	42.46±0.56 ^b	24.92±0.88 ^a	40.68±0.81 ^{bc}	10.31±0.36 ^b
DPP 6	38.01±0.71 ^d	21.34±0.28 ^{cd}	36.28±1.15 ^e	9.78±0.50 ^{bc}

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$). GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

Table (17): Effect of different levels of some natural phytochemicals (g/kg diet) on some liver function enzymes in liver homogenate of Nile tilapia, *O. niloticus*, fingerlings.

Items	Aspartate transaminase (U/g wet tissue)	Alanine transaminase (U/ g wet tissue)	Alkaline phosphatase (IU/ g wet tissue)	Acid phosphatase (U/ g wet tissue)
Control	190.12±3.96 ^c	109.44±2.82 ^b	99.75±0.91 ^e	56.45±0.78 ^d
GE 0.2	208.15±3.25 ^b	123.08±2.37 ^a	115.74±1.29 ^d	61.68±0.98 ^c
GE 0.4	213.75±1.44 ^{ab}	129.20±4.32 ^a	127.86±1.10 ^a	67.62±1.06 ^a
TE 0.6	210.49±2.94 ^b	120.52±2.61 ^a	116.79±0.90 ^{cd}	63.17±0.74 ^{bc}
TE 1.2	220.26±.99 ^a	128.87±1.15 ^a	120.20±0.68 ^b	68.72±1.70 ^a
DPP 3	209.67±2.40 ^b	119.22±3.37 ^a	113.81±0.92 ^d	61.66±1.79 ^c
DPP 6	218.50±1.12 ^a	127.29±3.93 ^a	119.82±1.51 ^{bc}	66.03±0.75 ^{ab}

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$). GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

Table (18): Effect of different levels of some natural phytochemicals (g/kg diet) on urea, creatinine and bilirubin. of Nile tilapia, *O. niloticus*, fingerlings.

Items	Urea (mg/dl)	Creatinine (mg/dl)	Bilirubin (mg/dl)
Control	23.00±0.41 ^a	0.79±0.06 ^a	2.65±0.19 ^a
GE 0.2	19.64±1.06 ^b	0.64±0.02 ^b	2.34±0.14 ^{abc}
GE 0.4	18.72±1.68 ^b	0.57±0.02 ^b	2.00±0.11 ^{bc}
TE 0.6	18.91±.61 ^b	0.65±0.01 ^b	2.37±0.12 ^{ab}
TE 1.2	18.01±1.41 ^b	0.64±0.04 ^b	1.62±0.09 ^d
DPP 3	20.29±0.52 ^{ab}	0.65±0.00 ^b	2.16±0.08 ^{bc}
DPP 6	19.30±0.70 ^b	0.62±0.04 ^b	1.97±0.03 ^c

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$). GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

4.2. The 2nd experiment:

4.2.1. Effect of different levels of some natural phytochemicals compared with 17 α -methyl testosterone on sex-reversal of Nile tilapia (*O. niloticus*) fry:

Results in Table (19) and Figure (11) showed the effect of different 17 α -methyl testosterone (MT), GE, TE and DPP on sex ratio of Nile tilapia, *O. niloticus*, fry. The highest significant male percent (92.30%) was observed in MT treatment; however control group recorded the lowest male ratio (48.36 %).

The male percent skewed via dietary TE in high level to reach two third of populations (64.48%). The other phytochemicals levels didn't statistically affect the male percent compared to control, and were nearly expected ratio of 1:1 (male: female). The inter sex has shown only with MT and TE (1.2 g/kg) treatments, concomitantly to rise of sex reversal process.

The wet squash technique and coupled histological section used in sex determination for sex reversed fry at age 84 days are illustrated in Figures (12 to 14).

Table (19): Effect of different levels of some natural phytochemicals (g/kg diet) compared with 17 α -methyl testosterone (MT) on sex-reversal of Nile tilapia, *O. niloticus*, fry.

Items	Sex ratio (%)		
	Male	Female	Intersex
Control	48.36±0.53 ^d	51.64±0.53 ^a	0.0
MT	92.30±1.19 ^a	5.48±1.04 ^d	2.22±2.22
GE 0.2	51.11±0.0 ^d	48.89±0.0 ^a	0.0
GE 0.4	51.14±0.02 ^d	48.87±0.02 ^a	0.0
TE 0.6	57.76±0.94 ^c	42.24±0.94 ^b	0.0
TE 1.2	64.48±1.44 ^b	31.13±0.70 ^c	4.40±2.13
DPP 3	50.58±1.69 ^d	49.42±1.69 ^a	0.0
DPP 6	56.67±1.11 ^c	43.33±1.11 ^b	0.0

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$). GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

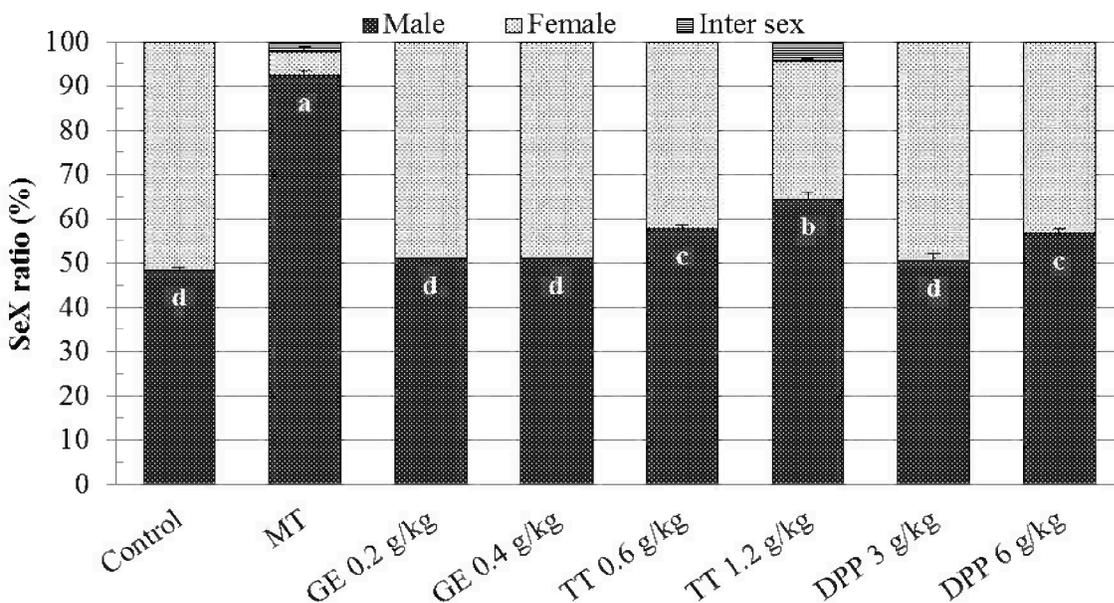


Figure (11): Effect of different phytochemicals (g/kg diet) compared with 17 α -methyl testosterone (MT) on sex ratio of Nile tilapia, *O. niloticus*, fry. GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

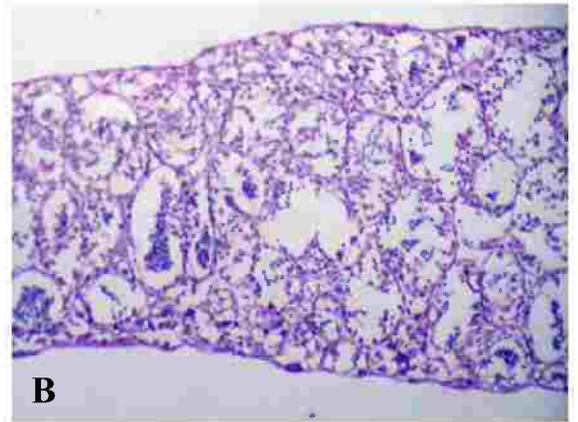


Figure (12): Male thread like gonadal of Nile Tilapia, *O. niloticus*, (A) wet-squash technique; (B) histological section (H&E; 250) showed normal histological appearance.

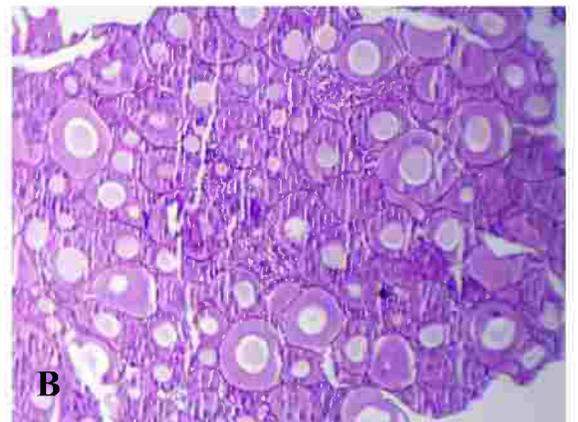
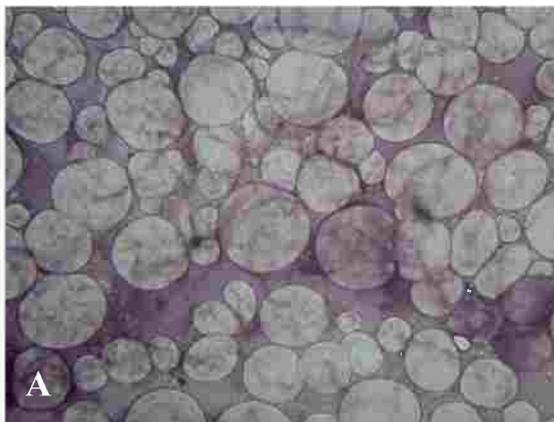


Figure (13): Female thread like gonadal of Nile Tilapia, *O. niloticus*, (A) wet-squash technique; (B) histological section (H&E; 250) showed normal histological appearance.

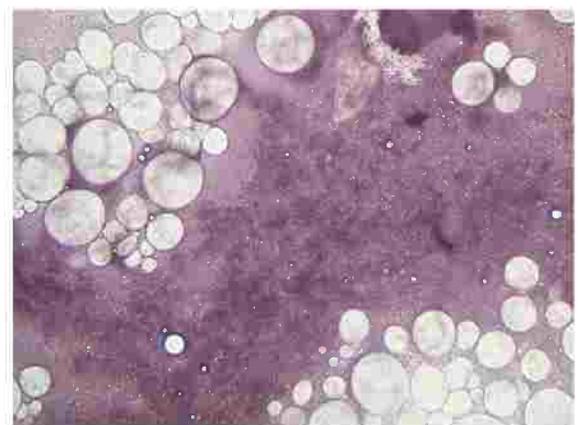


Figure (14): Intersex thread like gonadal of Nile Tilapia, *O. niloticus*, wet-squash technique showed ovary and testes tissue in the same gonads.

4.2.2. Effect of different levels of some natural phytochemicals compared with 17 α -methyl testosterone on growth performance of Nile tilapia (*O. niloticus*) fry:

The effect of different phytochemicals compared with 17 α -methyl testosterone on growth performance of *O. niloticus* after 28 of treatments is showed in Table (20). The FW, weight gain and SGR of all studied treatments increased compared than the control group. Furthermore, all investigated phytochemical levels showed acceleration of growth performance than MT treated fish except with TE (0.6 g/kg diet). The highest FW observed with TE (1.2 g/kg) diet followed by GE (0.4 g/kg diet), DPP 3 (g/kg diet), GE (0.2 g/kg diet) and MT. The percent of final weight increase were 45.32%, 38.85%, 29.50%, 28.06% and 18.71%, respectively more than control.

The overall survival rate ranged from 94% to 96.5% after the first 28 days of treatment without any significant drastic effect of all studied treatments. The noticed mortality couldn't be attributed to any obvious cases and considered acceptable during this period of *O. niloticus* fry live span.

Results presented in Table (21) summarized the effects of sex-reversal agents on total length and condition factor of *O. niloticus*. The results revealed that fish total length didn't differ significantly after 28 days of treatment; however, all treatments represent more length than control. The condition factor didn't differ significantly after 28 days of treatment.

4.2.3. Effect of different levels of some natural phytochemicals compared with 17 α -methyl testosterone on feed and nutrients utilization of Nile tilapia (*O. niloticus*) fry:

Data in Table (22) showed the effect of different phytochemicals treatment compared with 17 α -methyl testosterone on feed and protein utilization of treated fry. All diets were acceptable for all treatments, which appeared in insignificant feed intake after 28 days of feeding treatment, the same pattern was reflexed in protein intake. The FCR was improved in all supplementation treatments significantly than the control group except with TE (0.6 g/kg diet) and DPP (6 g/kg diet). The best FCR was observed with TE (1.2 g/kg diet) followed by GE (0.4 g/kg diet) after 28 days.

Table (20): Effect of different levels of some natural phytochemicals (g/kg diet) compared with 17 α -methyl testosterone (MT) on growth performance and survival rate of Nile tilapia, *O. niloticus*, fry after 28 days of age.

Items	Initial weight	Final weight (g/fish)	Weight gain (g/fish)	Specific growth rate (%/day)	Survival rate (%)
Control	0.02	0.70±0.01 ^d	0.68±0.00 ^d	13.20±0.05 ^d	94.50±0.50
MT	0.02	0.83±0.01 ^{bcd}	0.81±0.00 ^{bcd}	13.81±0.06 ^{bc}	96.50±1.50
GE 0.2	0.02	0.89±0.02 ^{abc}	0.87±0.02 ^{abc}	14.08±0.16 ^{abc}	96.50±1.50
GE 0.4	0.02	0.97±0.08 ^{ab}	0.95±0.08 ^{ab}	14.36±0.36 ^{ab}	94.50±0.50
TE 0.6	0.02	0.80±0.02 ^{cd}	0.78±0.02 ^{cd}	13.70±0.17 ^{cd}	95.00±5.00
TE 1.2	0.02	1.01±0.07 ^a	0.99±0.07 ^a	14.53±0.17 ^a	94.00±2.00
DPP 3	0.02	0.90±0.01 ^{abc}	0.88±0.01 ^{abc}	14.12±0.04 ^{abc}	95.00±3.00
DPP 6	0.02	0.84±0.04 ^{bc}	0.82±0.04 ^{bc}	13.88±0.09 ^{bc}	96.50±1.50

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$). GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

Table (21): Effect of different levels of some natural phytochemicals (g/kg diet) compared with 17 α -methyl testosterone (MT) on length, length gain and condition factor of Nile tilapia, *O. niloticus*, fry after 28 days of age.

Items	Initial length (cm)	Length (cm)	Length gain (cm)	Condition factor
Control	1.13	3.54±0.05	2.41±0.05	1.5±0.07
MT	1.13	3.70±0.04	2.58±0.04	1.63±0.05
GE 0.2	1.13	3.58±0.06	2.46±0.06	1.94±0.06
GE 0.4	1.13	3.69±0.07	2.57±0.07	1.93±0.26
TE 0.6	1.13	3.58±0.02	2.46±0.03	1.74±0.01
TE 1.2	1.13	3.71±0.05	2.59±0.05	1.97±0.06
DPP 3	1.13	3.64±0.04	2.51±0.05	1.88±0.09
DPP 6	1.13	3.63±0.04	2.51±0.04	1.76±0.15

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$). GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen.

Table (22): Effect of different levels of some natural phytochemicals (g/kg diet) compared with 17 α -methyl testosterone (MT) on feed and nutrients utilization of Nile tilapia, *O. niloticus*, fry after 28 days of age.

Items	Feed intake (g/fish)	feed conversion ratio (g)	Protein intake (g/fish)	protein efficiency ratio (g)
Control	0.94±0.03	1.38±0.03 ^a	0.41±0.01	1.64±0.04 ^d
MT	0.95±0.05	1.18±0.05 ^{bcd}	0.42±0.02	1.93±0.09 ^{abcd}
GE 0.2	1.01±0.01	1.16±0.04 ^{bcd}	0.45±0.00	1.96±0.06 ^{abc}
GE 0.4	1.03±0.03	1.09±0.05 ^{cd}	0.46±0.01	2.08±0.10 ^{ab}
TE 0.6	1.00±0.00	1.28±0.03 ^{ab}	0.44±0.00	1.77±0.05 ^{cd}
TE 1.2	1.04±0.00	1.05±0.07 ^d	0.46±0.00	2.16±0.15 ^a
DPP 3	1.05±0.03	1.19±0.02 ^{bcd}	0.47±0.01	1.90±0.04 ^{abcd}
DPP 6	1.01±0.01	1.23±0.05 ^{abc}	0.45±0.00	1.84±0.07 ^{bcd}

Values superscripted by different alphabets within the same column are significantly different ($P < 0.05$). GE, ginseng extract; TE, tribulus extract; DPP, date palm pollen