CHAPTER FIVE

DISCUSSION

The 1st experiment:

It is discernible from the present study that all tested phytochemicals (ginseng extract, tribulus extract and date palm pollen) in both levels have clear effect on growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. The results showed a significant increase in terms of body weight, body weight indices and condition factor with supplementation treatments. Also, survival rate didn't differ significantly among all studied phytochemicals and control. The obtained results are supported with Goda, (2008) who found that ginsana® (ginseng extract) increased growth performance of Nile tilapia and didn't show any apparent evidence of fish toxicity of feeding ginseng extract on survival rate. Moreover, dietary ginseng root meal treatment led to a higher significant body weight gain and feed intake of broiler chicken more than control (Yan *et al.*, 2011[a]). Also, Yan *et al.*, (2011[b]) reported that feeding ginseng adventitious root meal increased egg production of laying hens significantly compared to control group.

In the contrary with the present results, Choi *et al.*, (2010) found that dietary ginseng by product decreased weight gain significantly than control group of juvenile olive flounder. This inconsistency may be due to the use of ginseng by product instead of ginseng extract, moreover the difference in animal species and age.

The increase in body weight by treatment with tribulus extract is in agreement with Gauthaman *et al.*, (2002) in rats. Also, Turan and Çek (2007) reported an increase (53%) in body weight of African catfish fry immersed in 9g tribulus/30 l water for 30 days, and improved total length and survival rate. Çek *et al.*, (2007[a&b]) found that treatment with tribulus increased the growth rate of guppy fish, *Poecilia reticulate*, and Convict Cichlid, *Amatitlania nigrofasciata.*

An opposite trend was observed with Şahin and Duru (2010) who found that tribulus extract didn't affect growth performance and muscle building of broiler chicks treated with 180 and 360 ppm tribulus extract. This may be related to the use of low level of tribulus than that used in the current study (0.2 and 0.4 g/kg diet), animal species and its physiological response. Recently, Gültepe *et al.*, (2014) reported that tribulus significantly increase growth performance of Nile tilapia supplemented diets than in the control diet.

Regarding to date palm pollen similar findings with the current study reported by Al-Qarawi *et al.*, (2004) and Bahmanpour *et al.*, (2006) they didn't report any mortality or morbidity in their studies on date palm pollen. Moreover, Iftikhar *et al.*, (2011) reported similar findings with the current study. Who found that date palm

pollen increased rats body weight significantly than control, when treated with 120 mg/kg for 35 days, also there was no mortality observed. Attia *et al.,* (2011[a]) reported an increase in growth rate due to the administration of bee pollen at 100, 200 and 300 mg/kg BW were 24.8%, 45.6% and 25.4%, respectively in treated Nile tilapia.

The acceleration of growth performance of treated Nile tilapia fingerlings via dietary different phytochemicals may interpret by different mechanisms. One of the main possible mechanisms of which tested phytochemicals accelerated growth performance of treated Nile tilapia in the current study is increasing plasma testosterone (anabolic steroid hormone) levels (Table 9) and confirmed via significant regression and positive correlation between testosterone level and growth performance of Nile tilapia (figure 2 and 3).

The obtained results were in agreement with those of Asad *et al.,* (2010), and Norbeck and Sheridan (2011). Both authors reported a positive relation between testosterone supplementation and growth promoting efficacy in fishes.

The mechanisms of testosterone improving growth may be due to the presence of testosterone receptor sites in cells throughout the body, most notably in the brain and heart (Seal, 2009). Moreover, testosterone is highly protein anabolic *in vivo* and enhancing skeletal muscle strength (Mauras *et al.,* 2003). Furthermore, testosterone is the main hormone having nitrogen-retaining (anabolic) properties which increases lean body mass and body weight (Gauthaman *et al.,* 2002). Also, testosterone improves oxygen uptake throughout the body, helps control blood sugar, regulate cholesterol and maintain immune surveillance. The body requires testosterone to maintain youthful cardiac output and neurological function (Seal, 2009).

Moreover, sex steroids are known to modulate growth hormone levels in a variety of fish species (Holloway and Leatherland, 1997). Also, Huggard *et al.,* (1996) reported an increase of growth hormone mRNA production in pituitary gland above basal levels when the fish treated with testosterone (Huggard *et al.,* 1996). Finally, testosterone considers much more than a sex hormone (Seal, 2009).

The second possible mechanism of phytochemicals to increase growth performance is an increase of antioxidant enzymes activities, and decrease of thiobarbituric acid-reactive substances (TBARs) levels (Table 10, 11 and 12) in plasma, liver and testes of treated fish with different tested phytochemicals levels. These antioxidant properties of phytochemicals protect animal body and organs against oxidative material which caused damage (Al-Shagrawi, 1998).

Moreover, the other mechanisms by which phytochemicals improved growth of Nile tilapia in the current study may be due to its role in inhibition the colonization of potential pathogens in the digestive tract, enhancing the population of beneficial microorganism, microbial enzyme activity, improving intestinal microbial balance, and consequently improving feed digestibility and nutrient absorption. Hu *et al.,* (2003)

reported that the interactions among intestinal microflora, gut morphology, the immune system, and nutrient uptake have a major influence of ginseng improving effects.

The obtained results of feed and nutrients utilization indicate that supplemented diets with all phytochemicals increased feed intake, and improves feed conversion ratio (FCR), protein and energy utilization (Table 6). The best treatments of feed and nutrients utilization were tribulus extract, followed by date palm pollen and ginseng extract in high levels for each. These results were in consistence with the results of growth performance (Table 4). These phytochemicals may be useful for optimizing protein and energy use for the tilapia growth, these improvements could be attributed to the enhancement effect of the tested supplementation on diet utilization and digestibility. The present findings were in agreement with Goda (2008) who found that dietary ginseng extract improved protein efficiency ratio, protein productive value and energy utilization values compared to fish fed the control diet

However, Choi *et al.,* (2010) found that dietary ginseng by product reduced feed utilization significantly than control group of juvenile olive flounder. Also, Gültepe *et al.,* (2014) found that tribulus extract significantly improve feed utilization of Nile tilapia than in the control diet. The effect of tribulus extract may be due to decreasing the injuries of epithelial tissue of intestine. (Şahin and Duru, 2010). Similar results were observed with Attia *et al.,* (2011[a]) who found that feed intake, FCR significantly improved with administration of bee pollen. Moreover, Abbass *et al.,* (2012) reported that dietary honey bee pollen significantly improved feed efficiency ratio of *O. niloticus*. This may be due to the effect of pollen grains in increasing length and thickness of villi from the duodenum, jejunum, and ileum in treated broiler chickens (Wang *et al.,* 2007).

The carcass chemical analysis of treated fish with tested phytochemicals showed significant increase in protein content and insignificant decrease in ether extract due to treatment with phytochemicals. These results are supported by Yan *et al.,* (2011[a]) who found that ginseng induced a linear decrease in abdominal fat in broilers chicken. However, Goda, (2008) didn't find any statistical differences for dietary Ginsana® levels whole-body proximate analysis. The increase in protein content and decrease in ether extract in the present study may be attributed to the increase in testosterone levels (Table 9). The Testosterone hormone found to be highly protein anabolic hormone (Mauras *et al.,* 2003) and increase nitrogen retention capacity (Gauthaman *et al.,* 2002). Moreover, testosterone induced lipid degradation (Higgs *et al.,* 1982) to use as energy source, subsequently play a role in protein sparing effect (Bromley, 1980).

The present results showed decrease in visceral somatic index and increase liver, testes spleen somatic indexes. In agreement with the obtained results, Yan *et al.,* (2011[a]) reported an increased in the relative weight of spleen in broiler chicken treated with ginseng root meal compared with control. The measurement of immune organ weight is a common method to evaluate the immune status of animals (Heckert *et al.,* 2002).

Furthermore, the good development of these organs is also considered to be crucial for optimal site of immunoglobulin synthesis (Glick, 1977).

Moreover, the decrease in visceral somatic index which concomitant to increase liver, spleen relative weight, may be due to the decrease in body lipid content (Table 7). In agreement with the present findings Yan *et al.*, (2011[a]) observed a decrease in abdominal fat in ginseng root meal treated chicken. However, the same authors didn't showed significant changes in liver relative weight.

Furthermore, the increase in testes somatic index in the current experiment are supported with Sharma *et al.*, (2013) who reported that tribulus extract increased testes and epididymis weight in treated rat. Also, Bahmanpour *et al.*, (2006) showed an increase in the weight of testis and seminal vesicle in the rats that consumed date palm pollen suspension. The protective effect of phytochemicals upon some organs such as liver, spleen and testes may be due to its contents of some flavonoids, which play a role as antioxidant against oxidative material which caused damage to such organs (Al-shagrawi, 1998).

Regarding to the effect of dietary phytochemicals on luteinizing hormone (LH) and testosterone. The obtained results showed an increase in luteinizing hormone (LH) and testosterone due to all studied phytochemicals in both low and high levels for each. These results were in agreement Tsai *et al.*, (2003) reported that ginseng extract (ginsenoside- Rb1) increases secretion of LH by acting directly on rat anterior pituitary gland cells. This might be interpreted the increase of testosterone level (Antonio *et al.*, 2000). Moreover, the effect of ginseng on testosterone levels could be attributed to its gene regulations effects on testes. Kim *et al.*, (2011[c]) found dietary ginseng for aged rats changed significantly 33 genes expressions, where 13 genes were up-regulated and 20 were down-regulated in the older rats compared to the 2 month old young rats. The most up-regulated genes were that responsible for steroid hormone metabolism and the top molecular and cellular functions.

In respect of elevation of testosterone level in groups treated with tribulus, the current results were supported by the findings of Adimoelja, (2000) who reported that the use of tribulus resulted in an increase of LH levels by 72% and free testosterone levels by 41%. Also, El-Tantawy *et al.*, (2007) found a significant increase in the level of free serum testosterone when rat treated with *T. alatus* extracts. Gauthaman and Adaikan (2008) recorded increase in testosterone, dihydrotestosterone and dehydroepiandrosterone sulphate levels to reached 52%, 31% and 29%, respectively in primate treated with tribulus extract. Moreover, in castrated rats tribulus increased testosterone by 25% compared to control. The effect of tribulus on testosterone levels may be directly attributed to the presence of protodioscine which consider testosterone precursors and enhance testosterone production because protodioscine conversion to dehydroepiandrosterone (DHEA) (Adimoelja, 2000). Moreover, indirectly tribulus is

believed to affect testosterone levels by stimulating the release of LH, which serves to stimulate the natural production of testosterone (Antonio *et al.*, 2000).

Regarding to the improving effect of date palm pollen on LH and testosterone where supported by Marbeen *et al.*, (2005) who revealed that serum levels of LH and testosterone significantly increased compared to control, the percentage of hormonal changes were (112.98%) and (64.25 %) respectively, on human treated with 500 mg date palm pollen twice daily for 3 months. Moreover, Iftikhar *et al.*, (2011) reported that date palm pollen suspension given orally resulted in increased serum testosterone levels of rats. Also, Abbass *et al.*, (2012) reported that honey bee pollen significantly increased testicular weight, gonadosomatic index and improved the semen quality of male Nile tilapia, *O. niloticus.*

Date palm pollen improvement of LH and testosterone levels could be attributed to the presence of gonadotropically active substance in date palm pollen (Mahran *et al.*, 1976). In addition to gonadotropically active substance, date palm pollen contain steroid precursor (Rymond *et al.*, 1966), which may enhances testosterone synthesis. This will elucidate the increment of testosterone levels.

The histological sections of experimental groups testes showed increase of the abundance of spermatids in the lumen of the testicular lobules with phytochemicals dietary supplementation. The best treatment was *T. trestriss* (1.2 g/kg diet) which showed highly abundance of spermatids. This results are supported by the findings of Çek *et al.*, (2007[a]) who showed that spermatogenesis was accelerated among the tribulus treated group of *Cichlasoma nigrofasciatum* compared to the control group; further, it was observed that the histological response of the testes in tribulus treated groups invariably included an increased number of spermatogenetic cysts and an excess of late stages of spermatogenesis. Also, showed that males in the control group had smaller gonads than the experimental group. Moreover, Esfandiari and Dehghani (2010) concluded that TT may cause early puberty and it may increase testosterone levels hence it can increase compactness of spermatogenic cells and sperms. Abbass *et al.*, (2012) found that dietary bee pollen showed increase in sperms accumulation and size of the interstitial cells when compared to control in Nile tilapia treated fish.

Furthermore, Hwang *et al.*, (2010) reported that ginseng produced a distinct testicular histological improvement in old rats, where markedly improved the number of germ cells, seminiferous tubular size. The histological improvement in the present study may be due to herbal flavonoid drug contains saponin, steroid and different essence. It has a lot of effects such as increasing testosterone and dihydrotestosterone levels (El-tantawy, 2007; Gauthaman and Adikan, 2008), preventing cell death and destruction of mitochondrial membrane (Liu, 2008).

Oxidative stress occurs as a consequence of excessive production of reactive oxygen species and reactive nitrogen species and it is ameliorated by endogenous antioxidant enzyme activity and exogenous dietary antioxidants (Sugino, 2007).

Antioxidants present in the diet can delay lipid peroxidation by inhibiting the initiation or propagation phase of oxidizing chain reactions by scavenging free radical (Shahidi and Zhong, 2010).

Moreover, there are number of protective antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione *S*-transferase (GST), glutathione peroxidase (GPX) and reduced glutathione (GSH)) for dealing with these toxic substances. The delicate balance between the production and catabolism of oxidants is critical for maintenance of the biological function (Sridevi *et al.*, 1998).

The peroxidases are enzymic antioxidants widely distributed in all animal tissues which decompose hydrogen peroxide and protect the tissue from highly reactive hydroxyl radicals. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Murugesh *et al.*, 2005). Glutathione is one of the most abundant tripeptide, nonenzymatic biological antioxidants present in the liver. It removes free radical species such as hydrogen peroxide and superoxide radicals and maintains membrane protein thiols. Also, it is substrate for GPx (Prakash *et al.*, 2001).

The thiobarbituric acid-reactive substances are a biomarker of lipid peroxidation, and the reduction of its levels protects the body from oxidative stress (Scandalios, 2005). The obtained results revealed a significant decrease in TBARs in plasma, liver and testes with all phytochemicals supplementation treatments compared to control (Table 10, 11 and 12). Furthermore, the current study showed that the selected phytochemicals (ginseng extract, tribulus extract and date palm pollen) have been reported to increase antioxidant enzymes activities in plasma, liver and testes. The elevated antioxidants clearly observed with increasing the activities of SOD, GST, CAT and GPx enzymes in the high level of each tested phytochemicals. Moreover, increased GSH content, this improvement of antioxidant defense systems may be an attempt to neutralize the impact of TBARs.

In accordance with the obtained results, Voces *et al.*, (2004) and Kim *et al.*, (2007) found that ginseng protect the cell from oxidative stress and maintain glutathione. El-Khayat *et al.*, (2011) found that liver antioxidants were increased in diabetic rats treated with ginseng. Also, Sawiress *et al.*, (2011) found that ginseng resulted in a significant improvement in testicular antioxidants with a decrease in malondialdehyde in diabetic rats. Also, Ramesh *et al.*, (2012) found that administration of the fermented ginseng to aged rats resulted in increased activities of SOD, CAT, GPX, GSH and GST as well as reduced the level of malondialdehyde.

The improvement of tribulus extract on the antioxidant status were in agreement with Kadry *et al.*, (2010) who found that GPx and SOD increased by 68% and 109% respectively in rat blood via *T. alatus* extract. Also, the *in vivo* study of Kavitha *et al.*, (2011) and the *in vitro* study of Zheleva-Dimitrova *et al.*, (2012) proved the antioxidant

properties of tribulus. Moreover, tribulus extract ameliorate the effects of cypermethrin in treated rats and increasing GSH, CAT, SOD, GST, GSH, GPx and reduced lipid peroxidation (Sharma *et al.*, 2013).

In respect of date palm pollen, the present results were in agreement with the finding of Hassan *et al.*, (2012) who mentioned that treatment with date palm pollen ameliorate antioxidant status of rats via counteracted the increases in antioxidant systems in rat testis induced by cadmium chloride as assessed by restoration of GSH, SOD and CAT. Moreover, El-Neweshy *et al.*, (2013) found that rats treated with date palm pollen restored the toxic effects of cadmium on the antioxidant systems (decreased malondialdehyde and increased reduced glutathione levels) in treated animals. Therefore, it is evident that dietary ginseng extract, tribulus extract and date palm pollen can increase antioxidant enzymes activities in fish. However, fish studies on the effect of these phytochemicals on antioxidant are limited, and no comparison could be made with the results of this study.

The most relevant biological property of phytochemicals is their ability to act as antioxidants. The antioxidant capacity in general, is conferred by a high number of hydroxyl substitutions which has a direct effect on the donating ability of hydrogen (Kim, 2001). The antioxidant activity of ginseng extract, tribulus extract and date palm pollen could be attributed to its flavonoidal content. Flavonoids act as scavengers of various oxidizing species i.e. super oxide anion ($O_2^{-\bullet}$), hydroxyl radical or peroxy radicals, they also act as quenchers of singlet oxygen (Das *et al.*, 1990). It was reported that carbonyl group at C-4 and the double bond between C-2 and C-3 in the chain between the two aromatic rings are important features for high antioxidant activity in flavonoids (Temraz *et al.*, 2006).

In addition, flavonoids may regenerate other antioxidants such as tocopherol by donating a hydrogen atom to the tocopheryl radical (Boyle *et al.*, 2000). In the public health domain, consumption of phytochemicals is associated with decreased risk of cardiovascular disease by protecting against oxidative cell damage (Reiterer *et al.*, 2004).

Conclusively, phytochemicals supplementation causes both reactivation of antioxidant enzymes and scavenging of free radicals, which in turn, results in decreased TBARS synthesis and its accumulation, and improve the positive balance between oxidant and antioxidant systems (Seung and Park, 2003).

Hematology is an important factor that could be considered in fish diet quality assessment. Svobodová *et al.*, (1991) reported that ichthohematology would be useful in the assessment of suitability of diets and feed mixtures, evaluation of fish conditions, determination of toxic effect of substances, as well as diagnosis of disease.

The obtained results of improving blood hematological assays via ginseng extract, tribulus extract and date palm pollen were supported by Goda (2008) who found

that mean red blood cells (RBCs), heamatocrit (Hct) and heamoglobin (Hb) of Nile tilapia significantly increased with increasing dietary ginseng extract (Ginsana®) levels compared to the fish fed the control diet. Also, the author agreed with the obtained results which showed insignificant changes in blood indices compared to control. Moreover, Yan *et al.*, (2011[a]) reported that a significant increase of lymphocyte count was observed in ginseng root meal treatments compared with non-treated broiler chickens for five weeks. Yan *et al.*, (2011[b]) found that inclusion of ginseng adventitious root meal in laying hens diet increased RBCs, WBCs and lymphocytes count in level dependent manner.

The improving effect of tribulus extract on blood hematology supported by Wilkins *et al.*, (2009) who reported an increase in PCV and hemoglobin of tribulus treated horses. In the contrary with the obtained results, Wilkins *et al.*, (2009) didn't found changes in WBCs and neutrophil:lymphocyte ratio due to tribulus treatment. Also, Gültepe *et al.*, (2014) found that hematocrit, hemoglobin, MCH and MCHC were not significantly affected by tribulus extract.

Moreover, date palm pollen found to be increased PCV, Hb and WBCs and these results were in agreement with (Marbeen *et al.*, 2005). Also, nearly similar study was reported with Attia *et al.*, (2011[b]) who reported an increase in RBCs and WBCs, however, Hb and PCV didn't affected with bee pollen treatment of treated rabbit. This contrast with the present results may be attributed to the differences in animal species and pollen grains sources.

The improvement of hematological parameter in the present results may be due to the improvement in the nutritional status (Table 6). Moreover, the optimization of heamatobiotic organs (liver and kidney) functions (Table 16, 17 and 18). Also, Table (4) proved the nontoxicity properties of the tested phytochemicals on treated animals and maintains the normal survival rate.

Hematological indices have been used as a useful indicator to identify diseased fish (Harikrishnan *et al.*, 2012). Furthermore, Hematological and biochemical variables are the most significant physiological indicators of fish health, stress, and welfare (Campbell, 2004).

The increase in plasma proteins (Table 14) due to different tested phytochemicals were in agreement with Goda, (2008) who found that fish fed Ginsana® (ginseng extract) supplemented diet had significantly higher levels of total protein, albumin, and globulin compared to fish in the control. Also, Sharma *et al.*, (2013) reported an increase in rat plasma total protein in rat treated with tribulus, or toxicated with Cypermethrin. However, Gültepe *et al.*, (2014) found that serum albumin and total protein of Nile tilapia were not affected by dietary tribulus extract. Meanwhile, globulin was increased significantly than control.

Regarding to the obtained results of date palm pollen are agreed with the findings of Marbeen *et al.,* (2005) who reported an increase in total protein in men treated with date palm pollen. Moreover, Attia *et al.,* (2011[a]) found that, there was significant beneficial effect of bee pollen on plasma total protein, albumin and globulin in comparison to the control diet.

The significant changes in total proteins in the current study could be attributed to the changes in plasma globulin, this results supported with Helmy *et al.,* (1974) who reported that the cyclic nature of the total serum protein is an indicator of the changes taking place in the serum globulin fraction. Moreover, the increase in serum protein would result when anabolic processes exceeded catabolic ones, and reserved protein is being produced in greater quantity to meet increased metabolic requirements of the fish.

The increase in plasma globulin (Table 14) may attribute to the increase in WBCs (Table 13) and this could be proving the immune stimulation activity of Ginseng extract, tribulus extract and date palm pollen. In accordance, Tizard, (1992) reported that most techniques employed to investigate the immune state of an animal are those that depend on detection and measurement of antibody in blood serum and other body fluids. However, the source of antibody in blood serum is globulin. Therefore, the total serum globulin level probably reflects the level of specific immunoglobulin (antibody) (Blazer and Wolke, 1984).

The current experiment demonstrated a decrease of total lipids (TL), cholesterol, triglycerides (TG), low density lipoprotein-cholesterol (LDL-c) and very low density lipoprotein-cholesterol (VLDL-c) and increased high density lipoprotein-cholesterol (HDL-c) in plasma of animals fed diet supplemented with different phytochemicals. These results are supported by Kim, (2001) and, Seung and Park (2003), both authors found a reduction of cholesterol levels, and increase of HDL-c due administration of ginseng extract. Moreover, Choi *et al.,* (2010) found a significant decrease in triglycerides due to dietary ginseng by product of juvenile olive flounder. Yan *et al.,* (2011[a]) found that broiler chicken fed diet supplemented with ginseng showed decrease in the total cholesterol and triglycerides compared with the control group. Ginsenoside Rg3 significantly reduced hepatic cholesterol and triglyceride levels in rats (Lee *et al.,* 2012). Also, ginseng extract reduces the levels of cholesterol, LDL-C and TG (Song *et al.,* 2012).

The improvement of lipid profile due to dietary ginseng extract in the present study may be attributed to the antioxidant potential of ginseng extracts which involved in hypolipidemic effect as one of action mechanism; also, ginsenoside-Rb2 is one of the active components of ginseng saponins which may accelerate serum cholesterol turnover by increased cholesterol degradation and excretion in the feces (Seung and Park, 2003). Moreover, Song *et al.,* (2012) found that ginseng down-regulated genes associated with lipid and cholesterol metabolism, which were up-regulated by high fat diet. Also, levels of leptin, adiponectin and insulin, which regulate glucose and lipid

metabolism, were impaired profoundly by high fat diet. However, ginseng treatment brought these levels back to normal, and reduces of intestinal absorption of dietary fat via the inhibition of pancreatic lipase activity.

Moreover, El-Tantawy and Hassanin (2007) mentioned that the alcoholic extract of tribulus possess a hypolipidemic effect on treated rats, where decreased significantly total cholesterol, triglucerides and LDL-c and increased HDL-c. It is known that HDL plays a key role in the protection against oxidative damage of membranes and lipid metabolism by transporting cholesterol from peripheral tissues to the liver through a process known as reverse cholesterol transport (Farias *et al.*, 1996).

Regarding to the improvement of lipid profile due to dietary date palm pollen, the obtained results were in accordance with Al-Shagrawi, (1998), who found that date palm pollen grains reduced plasma TL, cholesterol, TG and LDL and elevate HDL of treated rats. Also, Abo-El-Soaud *et al.*, (2004) and Attia *et al.*, (2011[b]) reported that date palm pollen decreased the total lipids and total cholesterol.

Liver is an important organ for metabolism and detoxification. The determination of the pathophysiological enzymes like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) is a common mean of detecting.

The present results revealed a decrease in plasma and increase in liver activities of AST, ALT, ALP and ACP of treated fish with different phytochemicals levels. These results revealed the hepatoprotective effect of the tested phytochemicals, where the leakage of cellular enzymes into plasma indicates a hallmark sign of hepatic injury or damage (Kumar *et al.*, 2004; Ramaiah, 2007). In addition, the extent and type of liver injury or damage can be assessed based on the presence or absence of specific enzymes in the bloodstream (Kumar *et al.*, 2004). An increase in the levels of ALP and AST has been shown to reflect liver damage, whilst a rise in the ALP level may be indicative of renal and liver damage (Gill *et al.*, 1990; Bhattacharya *et al.*, 2005).

The present results were in accordance with (Huh *et al.*, 1988) who reported that Korean ginseng has the activity of prompting the detoxification of toxic substances, protecting the liver from damage, and generating and promoting the regeneration and recovery of the liver. In the same trend Choi *et al.*, (2010) reported decrease of ALT level significantly due to dietary ginseng by product of juvenile olive flounder. Moreover, Kavitha *et al.*, (2011) showed a remarkable hepatoprotective activity of tribulus against acetaminophen-induced hepatotoxicity. This improvement showed clearly in reducing the levels of AST, ALT, ALP and ACP in Tilapia plasma.

In accordance with the obtained results of date palm pollen, Al-Shagrawi, (1998) found that liver function enzyme activities (AST, ALT and ALP) were significantly reduced in treated rats with date palm pollen grains in level dependent

manner. Also, Abo-El-soaud *et al.*, (2004) reported a decrease of AST, ALT and ACP in hypoglycemic rats with date palm pollen treatment. Marbeen *et al.*, (2005) found that date palm pollen decreased serum ALT, AST and ALP in treated men with 500 mg date palm pollen twice daily for 3 months. Moreover, Abbass *et al.*, (2012) concluded that honeybee pollen reduced ALT in Nile tilapia, *Oreochromis niloticus*, but AST didn't differ.

The improvement of ginseng extract, tribulus extract and date palm pollen on liver function enzymes may be due to the increase of antioxidant enzymes activities in plasma and liver Table (8, 9 and 10) which may help to protect the hepatocyte integrity and preserve the enzymes inside the cells (Al-shagrawi, 1998).

In consistent with these explanations, kidney functions were improved via reducing plasma urea and creatinine levels (Table 18). The obtained results are in accordance with Ramesh *et al.*, (2012) who found that administration of *P. ginseng* extract to aged rats resulted in reduced urea and creatinine. Also, tribulus significantly decreased the levels of blood urea nitrogen, uric acid and creatinine in serum (Kamboj *et al.*, 2011). Moreover, Abo-El-soaud *et al.*, (2004) reported a decrease of urea and creatinine in hypoglycemic rats with date palm pollen treatment. However, Marbeen *et al.*, (2005) didn't find any effect of date palm pollen on total bilirubin, urea and creatinine. Also, Abbass *et al.*, (2012) concluded that honeybee pollen didn't differ urea and creatinine in *O. niloticus*.

The 2nd experiment:

The target of the second experiment was to find an alternative masculinization agent to induce all male tilapia fry and improve growth performance, for the method currently used to be more effective, easy and environmental friendly. Toward this goal, some natural phytochemicals were incorporated in diet of *O. niloticus* fry in a comparison with 17 α–methyl testosterone (MT) treatment. The use of phytochemicals as masculinizing agents in fish is a recent research area. Mirza and Shelton, (1988) reported that the manipulation of biological synthesis of steroids have a similar effect to the administration of exogenous steroid hormones. During the specific critical periods of early gonad development, changes in sex hormone levels can affect the final sex independently of the genetic sex (Andersen *et al.*, 2003). Consequently, embryonic differentiation of the fetus into a male and its subsequent growth along this line is essentially due to the presence of physiological amounts of androgens (especially testosterone and its metabolite dihydrotestosterone) in the body (Baskin *et al.*, 1997).

From the current findings, the MT treatment was the most effective treatment on masculinization of *O. niloticus*. However, synthetic hormones and hormone metabolites persistence and their fate in fish, water and sediment will provide information on the potential risks of using hormonal sex control technology (Contreras-Sanchez *et al.*, 2001). Fish offered to the consumer will not be treated with synthetic hormones and producers may have an alternative method for producing of monosex populations based on natural products (Yılmaz *et al.*, 2009).

Among studied phytochemicals, tribulus extract (1.2 g/kg diet) treatment was the most effective treatment on sex differentiation of *O. niloticus* fry and increasing male ratio in the offspring with the maximum ratio 64.48%. Meanwhile the other phytochemicals treatments didn't have obvious effect on sex ratio skewness.

The present findings were supported by Turan and Çek (2007) they reported an increase in male sex ratio of *Clarias gariepinus*, as a level dependent manner with tribulus extract treatment and the highest male ratio (80.42%) recorded with 9g tribulus/30 liter water in immersion methods. Moreover, the male percent reached to 87.23% via immersion of newly hatched Convict Cichlid, *Cichlasoma nigrofasciatum*, fry once a week for two months on tribulus extract (Çek *et al.*, 2007[a]). The increase in the sex ratio than the present study may be attributed to the alteration of the tribulus extractapplication methods, levels and species. Also, Omitoyin *et al.*, (2013) found that percentage of males in treated fish increased as concentration of tribulus extract increased up to reach 85.7% with 2.5 g/kg diet. This findings mount our results by 20% and this could be the used of double level of tribulus extract than used in our study.

The effect of tribulus extract to increase male percent may be due to the increase of androgens production, where tribulus extract contains a number of different substances including steroidal saponins. Protodioscin, the most dominant saponin in

tribulus extract, is thought to be the main substance responsible for increasing testosterone production (Ganzera *et al.,* 2001), dehydroepiandrosterone (Adimoelja and Adaikan, 1997), dihydrotestosterone, and dehydroepiandrosterone sulfate (Gauthaman *et al.,* 2002).

However, the other studied phytochemicals ginseng and date palm pollen showed improvement effects on testosterone secretion (Tsai *et al.*, 2003; Abedi *et al.*, 2012). its effect on sex reversal is weak. This can be interpreted to its mechanism to promote testosterone secretion is different than tribulus extract, where each ginseng extract and date palm pollen act on the pituitary gland level and promote luteinizing hormone secretion, hence increase testosterone production (El-Desoky, 1995; Salvati *et al.,* 1996). Meanwhile, tribulus extract reported to increase luteinizing hormone secretion (pituitary level) and act as testosterone precursor (testes level) (Bucci, 2000; Gauthaman *et al.,* 2002).

Intersex fingerlings were recorded with MT and tribulus extract (1.2 g/kg) treatments. As sexing of fish was performed 84 days after the treatmental period, it might have been speculated that a 100% sex reversal was induced by MT treatment and the fish reversed back to their original sex when the effects of MT diminished (Piferrer, 2001). Meanwhile, in the case of tribulus extract the treatment continue for last of the experiment, so the intersex occurrence didn't due to the back reverse of sex, it may be caused by incomplete differentiation process.

The survival rate after 28 and 84 days of experiment wasn't reduced by any studied treatments compared to control. These findings revealed that the used treatments didn't have potent toxic effect on studied fish. This result in accordance with the studied of Adimoelja, (2000) and Adaikan *et al.,* (2000) they have presented evidence that tribulus extract is not toxic to humans and rabbits, respectively. Also, from the previous literature, ginseng (Goda, 2008) and date palm pollen (Iftikhar *et al.,* 2011) didn't report any toxicity cases.

In agreement of MT effect on improving growth performance, considerable information are available on the growth promoting efficiency of anabolic steroid hormones in fishes (Tveiten *et al.,* 1998; Asad *et al.,* 2010; Norbeck and Sheridan 2011). The acceleration of growth due to MT treatment may due to the increase of growth hormone mRNA production in pituitary gland above basal levels when the fish treated with MT (Huggard *et al.,* 1996).

The use of phytochemicals treatments increased growth performance (FBW, SGR and length) rather than control. Meanwhile, the effects on condition factor didn't clearly appear. Furthermore, the phytochemicals inclusion to fish diets increase feed and nutrients utilization compared to control in the case of FCR and PER, without significant effects on feed intake and consequently protein intake. In accordance, ginseng helps regulate body functions, specifically improve adaptability and is believed to help build muscle and endurance (Francis *et al.,* 2002). Moreover, Goda, (2008)

found a significant increase in growth performance and feed utilization of Nile tilapia, *O. niloticus*, fed diets supplemented with ginseng extract. Also, Yan *et al.*, (2011[a]) found that the addition of wild-ginseng adventitious root to the diet increased growth performance of broilers chicken.

Parallel results to our findings reported by Gauthaman *et al.*, (2002) who found an increase in body weight in rats. New-born guppies (*Poecilia reticulata*) treated with tribulus extract exhibit successful growth acceleration comparing to control group, which reached to 4.5 time more than control for the two concentration (Çek *et al.*, 2007[b]). Moreover, Çek *et al.*, (2007[a]) found that tribulus extract improved growth rate by 1.6 times more than control, the survival rate was uniformly high in control and treated group ranging from 88.57 to 90%.

In the case of date palm pollen, the current findings in accordance with Iftikhar *et al.*, (2011) who found that rat treated with 120 mg/kg daily had normal feed intake and didn't have any negative effect on the survival of experimental animals. Attia *et al.*, (2011[a]) reported that rabbit offspring received a water solution containing 0, 100, 200 and 300 mg bee pollen/kg body weight, twice per week for 12 week, showed improvement in growth performance in bee pollen groups than control. The Bee pollen at 200 mg/kg BW reveal increase in weight gain and survival rate and reduced feed intake and feed conversion ratio of offspring. Furthermore, Abbass *et al.*, (2012) investigate the supplementation of Nile tilapia, *O. niloticus*, diet with 2.5% of honeybee pollen for 21 days. The results showed that dietary honey bee pollen significantly improved length, specific growth rate, average daily gain and feed efficiency ratio. On the other hand, Hassan *et al.*, (2012) study the effect of 240 mg date palm pollen/kg body weight of adult male albino rats daily for 30 days, the weight of treated adult male rat didn't differ compared to control.

From a chemical composition point of view, the MT treatment increased moisture and decreased ether extract content than control and other treatments, meanwhile other treatments didn't affect the moisture level. The application of phytochemicals slightly increased protein content than control. Moreover, ether extract decreased with the MT treatment. The effect of phytochemicals didn't clearly appear on the body composition in our study. The same trend was observed by Goda (2008), where whole body proximate analysis didn't present any statistical differences for dietary Ginsana® levels. In contrast, Yan *et al.*, (2011[a]) found that ginseng induced a linear decrease in abdominal fat in broilers chicken.