Introduction

Hypertriglyceridaemia is a disorder of lipid metabolism resulting in accumulation of triacylglycerols in the mammalian blood (Kedzierski *et al.*, 2011). It occurs primarily in pony breed of horses (Schotman and Wagenaar, 1969), and donkeys (Fowler, 1989, and Watson, 1994).

There are three types of hypertriglyceridaemia: (1) Elevations in serum triglycerides (TGC) concentrations up to 5.6 mmol/l without clinical signs or visible lipaemia in blood samples (hyperlipidaemia), (2) Hyperlipaemia, which refers to elevations more than 5.6 mmol/l with evidence of clinical disease, and (3) Severe hypertriglyceridaemia referring to elevations more than 5.6 mmol/l without evidence of clinical disease (Naylor, 1982, Jeffcott and Field 1985a, and Dunkel and McKenzie 2003).

The condition is a response to a negative energy balance (Naylor *et al.*, 1980; Watson *et al.*, 1992a). The negative energy balance is a consequence of food deprivation or starvation, pregnancy, lactation, or disorders of the gastrointestinal and respiratory tracts (Watson *et al.*, 1992a). Food deprivation, either: accidental, intentional or relative to the increased metabolic demands, is a common predisposing factor in primary and secondary hyperlipaemia (Gay *et al.*, 1978, Jeffcott and Field, 1985b, and Mogg and Palmer, 1995).

In donkeys the condition has a higher mortality rate than in ponies (Fowler, 1989) and occurs in middle aged, obese donkeys often secondary to stress (Whitehead *et al.*, 1991). These stressors are diseases, such as laminitis, parasitism, nutritional deprivation, long travels, or psychological stress such as loss of companion (Tarrant *et al.*, 1998). Clinical signs are non-specific and generally result from hepatic and/or renal dysfunction due to lipidosis (Hughes *et al.*, 2004).

Several workers have described the treatment of hyperlipaemia on the basis of nutritional support, identification and treatment of any underlying disease, correction of fluid, electrolyte and acid-base abnormalities, reduction of adipose tissue lipolysis and enhanced clearance of plasma lipids (Naylor, 1982, Jeffcott and Field, 1985a, and Watson and Love, 1994)

Management of hyperlipidaemia in human medicine is divided into: Therapy of lifestyle and lipid-lowering drugs (McPherson *et al.*, 2006; Paul *et al.*, 2008and Lewis, 2009). Lifestyle interventions includes: reduction of body weight, physical exercise, and low lipid diet.

Lipid lowering drugs include: Statins (3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors); Fibrates (Frick *et al.*, 1987, and Barter and Rye, 2006); Azetimibe; and Nicotinic acid (Paul *et al.*, 2008). Nutraceutical supplements have been reported in the management of dyslipidemia (Houston *et al.*, 2009). They are considered as an alternative treatment for patients who are statin intolerant, or those who cannot take other drugs for the treatment of dyslipidemia, or in those who prefer alternative treatments (Houston, 2012).

Generally demand is increased for using natural products in therapy because synthetic drugs are so expensive and have undesirable effects, but natural products are cheap and locally available (Amin and Nagy, 2009).

There is a flow of data supporting the health benefits of dietary fiber. It has been documented that dietary fiber enhances the bowel health, has lipid lowering effect, reduces cardiovascular risks, and improves blood sugar control in diabetics.

Gum Arabic (GA) one of the soluble plant fibers, is an edible, dried, gummy exudate from the stems and branches of *Acacia senegal* and *Acacia seyal* which grow in semi-arid sub-Saharan regions in Africa

(Williams and Phillips, 2000). In Middle Eastern countries GA is employed in the treatment of patients with chronic renal disease and end stage renal failure (Al Majed *et al.*, 2002).

The effects of GA on lipid metabolism are variable. Feeding rats with 5 per cent GA has significantly decreased the absorption of dietary cholesterol; increased cholesterol biosynthesis in rats fed a cholesterol-containing diet, but had no effect in rats on a cholesterol-free diet (Kelly and Tsai, 1978). Ross *et al.,* (1983) reported reduction of total serum cholesterol by 6 per cent when subjects received 25 g/day GA for period of 21 days. Sharma (1986) reported reductions of total serum cholesterol; confined only to low density lipoprotein (LDL) and very low density lipoprotein (VLDL), with no effect on high density lipoprotein (HDL) and triglycerides (TGC).

Topping *et al.* (1985) has shown that plasma cholesterol concentrations were unaffected by feeding GA, but plasma triacylglycerols were significantly lower than in controls.

Gum Arabic is locally produced, available and has less cost and less unwanted effects compared with lipid lowering drugs. The overall objectives of the current research are to:

1. Evaluate the effects of Gum Arabic, if any, on lowering lipid classes in experimentally induced hyperlipidaemia in donkeys.
2. Test the prophylactic effect of Gum Arabic against the experimental induction of hyperlipidaemia in donkeys.

Chapter One

Literature Review

1.1 Gum Arabic (GA)

Gum Arabic (GA) (Figure 1), one of the soluble plant fibers, is an edible, dried, gummy exudates from the stems and branches of *Acacia senegal* and *Acacia seyal* (Figure 2), which grow in semi-arid sub-Saharan regions in Africa. The exudate is a non-viscous liquid, rich in soluble fibers, and its emanation from the stems and branches usually occurs under stress conditions such as drought, poor soil fertility, and injury (Williams and Phillips, 2000). The production of gum Arabic known as "*gummosis*" is a common response to the injury of the bark of the tree (Stephen *et al.*, 1990). In the Sudan, the gum belt lies within the arid and semi-arid zone of mainly 520,000 km^2 in an area that extends across Central Sudan between latitudes 10 and 14 North (IIED and IES, 1990). This belt covers parts of Kordofan, Darfur, Eastern Sudan and Blue Nile (Hamza, 1990).

1.1.1 General taxonomy of *Acacia Senegal*

Kingdom	: Planate
Class	: Magnoliopsida
Order	**:** Fabales
Family	**:** Fabaceae
Genus	**:** *Acacia*
Species	**:** *seyal* - *Senegal* (L) Wild.

1.1.2 Metabolism of GA

Gum Arabic (GA) is an indigestible plant fibre, it passes the small intestine unchanged but it is reported to be fermented and metabolized in the caecum and the colon, the metabolism of GA is mediated by bacteria within the caecum where it is degraded and metabolized to volatile short chain fatty acids such as butyrate, propionate and acetate which may be a

Figure 1.1: Gum Arabic (GA)

Figure 1.2: Acacia tree used for production of Gum Arabic

source of nutrition (Ross *et al*., 1983, and Ross *et al*., 1984). *Bifidobacterium* species which are naturally commensals in the gastro intestinal tract of mammals are capable of fermenting many kinds of complex carbohydrates including gum Arabic and probably use them as important sources of carbon and energy (Wyatt *et al*., 1986) *Bifidobacterium longum*, strain JCM 7052, was found to be able to hydrolyse gum Arabic by unidentified enzymes in the cell surface and was further degraded by α- and β-Galactosidase which were observed at higher levels in extracts from cells grown on gum Arabic as a source of carbon energy (Saishin and Yamamoto, 2009).

1.1.3 Molecular weight and solubility

Gum Arabic has a very high molecular weight approximately 850.000 daltons, and dissolves in aqueous solution to the extent of approximately 400 g/l (Ross *et al.*, 1984).

1.1.4 Chemical structure of Gum Arabic

Gums are a complex group of highly branched polysaccharides containing glucuronic and galacturonic acid as well as xylose, arabinose, and mannose (Anderson and Chen, 1979).

Gum Arabic is a polysaccharide based on branched chains of (1-3) linked β-D-galactopyranosyl units containing α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl and 4-O-methyl-β-D-glucuronopyranosyl units (Deckwer *et al*., 2006).

The molecule of Gum Arabic consists of three fractions: (1) Arabinogalactan fraction representing 88.4 per cent of the total molecule with molecular mass of 2.79×10^5, and is deficient in protein, (2) Arabinogalactan-protein complex, representing 10.4 per cent of the total molecule, with a molecular mass of 1.45×10^6, and (3) GA glycoprotein representing only 1.24 per cent of the total gum molecule, but contains 5 per cent of the total protein. The protein components of arabinogalactan

and arabinogalactan-protein complex contain hydroxyproline and serine (Randall *et al*., 1989)

Gum Arabic arabinogalactan is the major fraction. It is a highly branched polysaccharide consisting of β-galactose backbone with linked branches of arabinose and rhamnose which terminate in glucuronic acid and found in nature as magnesium, potassium, and calcium salts. The GA arabinogalactan-protein complex is arabinogalactan chains linked to a protein chain through serine and hydroxyproline groups. The smallest fraction which has the highest protein content is a glycoprotein and it is different in its amino acid composition from the GA arabinogalactan-protein complex (Idris *et al.*, 1998 and Randall *et al*., 1989). Osman *et al*., (1993) characterized different commercial samples of Gum Arabic and found that each consist of three molecular mass fractions classified as arabinogalactan, arabinogalactan-protein complex and glycoprotein and the proportion of each varied in individual samples.

1.1.5 Uses of gum Arabic

Gum Arabic is being used in a wide range of industrial sectors such as textiles, ceramics, lithography, cosmetics and mainly in the food and pharmaceuticals industry. It is used as a stabilizer, a thickener and/or an emulsifying agent in soft drink syrup, gummy candies and creams (Verbeken *et al*., 2003).

1.1.6 Pharmacological properties of GA

1.1.6.1 Effect on the gastrointestinal tract

The small intestine is the main site for absorption of nutrients and electrolytes in the gastrointestinal tract, and plants fibers are reported to play the role of modifiers of this function, a well-known of these fibers is Gum Arabic (Rehman *et al*., 2004). Rehman *et al*., (2004) hypothesized that GA has the ability to remove nitrous oxide (NO) diffused into the intestinal lumen and may also partially inhibit intestinal NO Synthase and

thus modulate intestinal absorption through these mechanisms, and since NO metabolism is linked to gastrointestinal physiology, this means that gum Arabic affects the function of the gastrointestinal tract.

Probiotics refer to the live microorganisms found in the gastrointestinal tract and survive the passage through this tract and have beneficial effect on the health such as prevention from diarrheal diseases and cancer. Consumption of GA by men was reported to enhance probiotic efficacy by increasing the number of *Bifidobacteria* and *lactobacilli* which are considered as beneficial bacteria (Calame *et al*., 2009).

1.1.6.2 Effect of GA on renal function

In Middle Eastern countries GA is employed in the treatment of patients with chronic renal disease and end stage renal failure. In chronic renal failure, plasma concentrations of the products of protein metabolism, including urea, are increased; retention of these nitrogen metabolites is associated with adverse clinical symptoms. Interventions that restrict protein intake are by offering low-protein diets that lower the serum urea nitrogen concentration and alleviate adverse clinical symptoms (Berlyne *et al.*, 1965, Franklin *et al.*, 1967, and Johnson *et al.*, 1972). Another approach is by increasing nitrogen excretion via faeces, the ingestion of dietary fibers increases faecal nitrogen excretion in animals and in normal human subjects (Weber *et al*., 1985, and Bird *et al*., 1990), and lower serum concentrations of urea in chronic renal failure patients (Bliss and Settle 1991, and Little and Trafford 1991). Supplementing with a highly fermentable fiber, gum Arabic in a dose 50 g/day in low protein diet consuming patients, increases fecal nitrogen excretion and faecal bacteria mass and results in lower serum urea nitrogen concentrations (Bliss *et al*., 1996). Ali and co-workers (2004) reported that GA alleviates adverse effects of chronic renal failure in

human patients consuming GA at doses of 50 g/ day for 3 months. Serum urea, creatinine and uric acid levels have significantly decreased, while serum calcium levels increased and serum phosphorus levels significantly decreased in the groups of gum users. Gum Arabic appears to have nephron-protection effect in adenine-induced renal failure and attenuating renal dysfunctions by uncertain mechanism (Ali *et al*., 2010). According to the authors, the mechanism may be due to the anti-oxidant or/and the anti-inflammatory effects. In renal diseases the prognosis depends on renal interstitial fibrosis which is mediated by pro-fibrotic cytokine, transforming growth factor-beta. Increasing blood butyrate and propionate resulting from fermentation of gum Arabic, has a beneficial effect by inhibiting renal pro-fibrotic cytokine (Matsumoto *et al*., 2006).

1.1.6.3 Effects of GA on lipid metabolism

The effects of GA on lipid metabolism are variable. Feeding rats with 5 per cent GA has significantly decreased the absorption of dietary cholesterol by 17 per cent. It increased cholesterol biosynthesis in rats fed a cholesterol-containing diet, but had no effect in rats on a cholesterol-free diet (Kelly and Tsai, 1978). Ross *et al.,* (1983) reported reduction of total serum cholesterol by 6 per cent when subjects received 25 g/day GA for a period of 21 days, and Sharma (1986) reported reduction of total serum cholesterol by 10.4 per cent, when subjects received 30 g/day of GA for a period of 30 days. The reduction was confined to LDL and VLDL cholesterol only, with no effect on HDL and triglycerides. In a study conducted to explore the lipid lowering effect of water soluble plants fibers, consumption of GA at a dose of 15 g/day for 4 weeks by healthy men had no significant effect on plasma lipids (Haskell *et al*., 1992). In a comparison between the medium viscosity mixture of psyllium, pectin, guar gum and locust bean gum and a similar amount of acacia gum, which has a lower viscosity, provided to

hypercholesterolemic subjects, the medium viscosity mixture decreased the total and low density lipoprotein cholesterol while acacia gum appeared to have no change in lipid parameters (Jensen *et al*., 1993). Mee and Gee (1997) studied the effects of a mixture of apple fibre and acacia gum (10 g/day, approximately half apple fibre and half acacia gum) in a crossover study in 27 men and found a significant 10 per cent reduction in serum cholesterol and a significant 14 per cent reduction in LDL-cholesterol concentrations with the fibre mixture as compared to the non-fibre control. Topping *et al*., (1985) has shown that plasma cholesterol concentrations were unaffected by feeding GA, but plasma triacylglycerols were significantly lower than in controls. Various mechanisms have been proposed to explain the hypocholesterolemic effect of GA (Kelly and Tsai, 1978; Moundras *et al*., 1994; Tiss *et al*., 2001). Some studies have suggested that the viscosity of fermentable dietary fibers contribute to the lipid lowering effects in animals and humans (Gallaher *et al*., 1993 and Moundras *et al*., 1994), while Evans *et al*., (1992) suggested that this property does not relate to plasma lipids. Some authors related this mechanism to the increase of bile acid and neutral sterol excretion or a modification of lipid digestion and absorption (Eastwood, 1992 and Moundras *et al*., 1994). Dietary fibers are bound or sequester bile acids, diminishing their active reabsorption in the ileum and leading to their excretion in the faeces. This promotes diversion of cholesterol to bile acid synthesis, in addition to inducing increased numbers of lipoprotein receptors in the liver and decreased plasma cholesterol concentration (Trustwell and Beynen, 1992).

1.1.6.4 Anti-oxidant effect of GA

Gum Arabic protects against doxorubicin-induced cardiotoxicity (Abd-Allah *et al*., 2002) and gentamycin-induced nephrotoxicity (Al-Majed *et al*., 2002, and Ali *et al*., 2003), and acetaminophen-induced

hepatotoxicity (Gamal el-din *et al.*, 2003) where oxidative stress is a common denominator of these models of toxicity. The protection is due to the reduction of oxidative stress. Pre-treatment of mice with gum Arabic is found to produce less nitric oxide (nitrate + nitrite) than acetaminophen-treated mice without pre-treatment with Gum Arabic and there was significant decrease in serum ALT and AST and hepatic lipid peroxides. It has been reported that gum Arabic has nitric oxide scavenging properties (Rehman *et al.*, 2001) and macrophage inhibitory functions by reducing its ability to produce superoxide anions (Mochida *et al.*, 1990 and Fujiwara *et al.*, 1995). These observations suggest that Gum Arabic may find clinical application in a variety of conditions where cellular damage is a consequence of oxidative stress.

1.1.6.5 Effect of GA on glucose concentration

Wadood *et al.* (1989) found that the powdered seeds of *Acacia arabica* given orally at doses of 2, 3 and 4 mg/kg showed hypoglycaemic effect in normal rabbits but not diabetic ones.

The dietary fiber GA, 100g/l in drinking water provided to mice for four weeks, inhibited intestinal glucose absorption via interaction with the membrane abundance of intestinal sodium-coupled glucose transporter (SGLT1) which determines the rate of glucose transport, influences glucose-induced insulin release, and development of obesity. This effect could prevent obesity and the development of metabolic syndrome (Nasir *et al.*, 2010).

1.1.5.6 Anti-malarial Effect of GA

GA influenced different courses of malaria; treatment with GA increased the survival of mice infected with *Plasmodium berghei* and delayed the lethal course of malaria (Ballal *et al.*, 2011).

1.1.6 Adverse effects and toxicity of GA

The American food and drug administration committee regarded Gum Arabic as safe when used in food (FDA, 1973). In Europe gum Arabic is recognized scientifically as a food additive (Phillips *et al*., 2008).

The safety of GA (5, 10, 20 or 40 g in water for 4 weeks) has recently been confirmed in healthy men (Calame *et al*., 2009). Doi *et al*., (2006) studied the sub-chronic toxicity of a new type of GA (SUPER GUM [*Acacia senegal* SUPER GUM]). It was concluded that 5.0 per cent, SUPER GUM (equivalent to 3117 mg/kg body weight/day for males, and 3296 mg/kg body weight/day for females) caused no adverse effect. This confirmed earlier reports that documented the safety of GA (Anderson, 1986).

Sander *et al*., (2006) found that sensitization to GA carbohydrate structures occurs in atopic patients with pollen sensitization without obvious exposure to GA, and that allergy to GA is mediated preferentially by Immunoglobulin E antibodies directed to polypeptide chains of GA. GA (10 per cent in the drinking water) for either 3 or 14 days decreased urinary excretion of inorganic phosphate and increased urinary excretion of calcium (Ca^{2+}) and magnesium (Mg^{2+}), and also decreased plasma concentrations of 1,25-dihydroxy vitamin D in healthy mice (Nasir *et al*., 2008), These metabolic disturbances in mice with renal insufficiency are expected to be more pronounced.

1.2 Hyperlipaemia

Hyperlipaemia is a disorder of lipid metabolism, which occurs primarily in pony breeds of horse (Schotman and Wagenaar, 1969), and donkeys (Fowler, 1989, and Watson, 1994). The condition is a response to a negative energy balance due to food withholding (but not water) leading to lipid mobilization and initial release of free fatty acids and

glycerol from adipose tissue followed by arise in serum triglycerides, fatty infiltration of body tissues leading to organs dysfunction (Naylor *et al*., 1980). The negative energy balance is a consequence of food deprivation or starvation, pregnancy, lactation, or disorders of the gastrointestinal and respiratory tracts (Watson *et al*., 1992a).

There are three types of hypertriglyceridaemia: (1) Elevations in serum TGC concentrations up to 5.6 mmol/l without clinical signs or visible lipaemia in blood samples termed "hyperlipidaemia". (2) Hyperlipaemia, which referring to elevations more than 5.6mmol/l with evidence of clinical disease. (3) Severe hypertriglyceridaemia referring to elevations more than 5.6mmol/l without evidence of clinical disease (Naylor, 1982, and Dunkel and McKenzie, 2003). The condition has a high mortality rate of between 60-100 per cent giving it a considerable concern to veterinarians (Watson *et al*., 1992a, and Jeffcott and Field, 1985a).

1.2.1 Epidemiology

There are many risk factors for hyperlipaemia. Hyperlipaemia occurs commonly in ponies, fat ponies in late pregnancy are most susceptible (Jeffcott and Field, 1985a) and donkeys (Watson, 1994, and Mair, 1995) and miniature breeds (Moore *et al.,* 1994; Mogg and Palmer, 1995) and it is uncommon in light and draft horse breeds (Naylor *et al.*, 1980, and Jeffcott and Field, 1985a).

The disease is most common in mares of all breeds (Gay *et al*., 1978, and Watson, 1994). The reproductive activities appear to be predisposing factors of the disease (Gay *et al*, 1978, and Jeffcott and Field, 1985b), and this leads to seasonality of the incidence (Watson, 1994). Hyperlipaemia in ponies usually affect mature animals, the prevalence increases with age and this may be due to the increased

reproductive activities and increased incidence of obesity in mature animals (Hughes *et al.*, 2004).

An underlying disease is present in only one third of hyperlipaemic ponies studied by Gay *et al.*, (1978) and Watson *et al.*, (1992a). In miniature breeds an underlying disease process is present in most of the cases studied by Moore *et al.*, (1994) and Mogg and Palmer (1995). In horses it is almost induced by concurrent disease with azotaemia and pituitary pars intermedia dysfunction (Naylor, 1982, and Field, 1988)

Hyperlipaemia is a common disorder of the donkeys with mortality rates higher than ponies (Fowler, 1989). Donkeys develop hyperlipaemia, in response to stress or primary disease (Burden *et al.*, 2011). In donkeys the condition occurs in middle aged, obese animals (Whitehead *et al.*, 1991) in response to stress, these stressors are diseases, such as laminitis, parasitism, nutritional deprivation, long travels, or psychological stress such as loss of companion (Tarrant *et al.*, 1998).

Recently in Sudan, a controlled experiment was conducted to induce hyperlipidaemia in donkeys. Animals in two treatment groups each of six were subjected to four or five days fasting, respectively. Feed withholding significantly increased mean plasma urea, AST, bilirubin and creatinine level. Total protein and albumin, decreased significantly in animals subjected to five days fasting, compared with baseline values. Mean plasma TG and total cholesterol concentrations significantly increased with time in feed-deprived donkeys. In conclusion, withholding feed for four and five days significantly increases blood lipid concentrations in donkeys, but individual donkeys respond differently (Bulldan *et al.*, 2013).

Also in another study Bulldan (2013), demonstrated that feed withholding in combination with helminths infestation induced severe

hyperlipidaemia in donkeys with varying degrees that may end with death.

1.2.2 Pathogenesis

In normal metabolism, triglycerides (TGC) in adipose tissue continually undergo lipolysis due to the action of hormone sensitive lipase (HSL), producing glycerol and free fatty acids (FFA). Lipolysis is balanced by the esterification of FFA with glycerol to reform TGC in adipocytes, so that the net release of FFA does not occur (Watson, 1994). The activity of HSL is inhibited by insulin and glucose and increased by glucagon (Watson, 1994, Breidenbach *et al.,* 1999, and Hammond, 2004). The majority of FFA are transported to the liver where they are either oxidized completely to provide energy, partially oxidized for ketone production or re-esterified to form TGC (Naylor, 1982, Jeffcott and Field, 1985a, and Watson *et al.*, 1992a). The TGC is then released into circulation in the form of very low density lipoproteins VLDL, and in the peripheral tissues FFAs are released from VLDL under the action of lipoprotein lipase (LPL) and are used as energy source. Once this metabolic response to fasting has met the energy requirement, the activity of HSL is normally suppressed by insulin and no further release of FFA (Jeffcott and Field, 1985a, and Watson, 1994).

Lipoprotein lipase (LPL) is an enzyme active in the capillary endothelium. It acts to clear TGC (carried in VLDL) from the circulation into adipocytes and skeletal and cardiac muscles. The enzyme is inhibited by glucocorticoids, growth hormone and azotaemia, and its action is promoted by insulin and heparin. Any decrease in the enzyme activity will increase TGC level in the circulation (Hammond, 2004).

Hyperlipaemia occur when animals experience negative energy balance with increases in HSL activity resulting in marked lipolysis, and FFA are released in the circulation at a rate that exceeds the liver ability

to process them via oxidative pathways (Watson, 1994). Most FFA are re-esterified to form TGC increasing the rate of TGC enriched VLDL production and release from the liver (Naylor *et al*., 1980, Watson *et al*., 1992a, and Watson, 1994). Hyperlipaemia results in fatty infiltration of the body organs primarily, the liver and kidneys (Gay *et al*., 1978, Naylor *et al*., 1980, and Watson, 1994).

In pony breeds tissue insensitivity to insulin (insulin resistance) is an important predisposing factor for the development of hyperlipaemia (Jeffcott and Field, 1985a, Jeffcott *et al*., 1986, and Watson *et al*., 1992a). Because insulin is an important regulator of HSL activity; insulin resistance may result in an inability to regulate adipose tissue lipolysis. In donkeys, insulin resistance may contribute to the pathogenesis of hyperlipaemia (Forhead *et al*., 1994). The mechanism of tissue insensitivity to insulin is incompletely understood (Hughes *et al*., 2004), however, several causes including a decrease in the concentration or affinity of insulin receptors or receptors defect results in the alteration of signal transduction (Jeffcott and Field, 1985a, andVan der Kolk *et al*., 1995). Changes in hormone homeostasis during pregnancy and lactation (Fowden *et al*., 1984, Jeffcott and Field, 1985a, and Watson *et al*., 1993), and increased plasma concentrations of cortisol and adrenocorticotrophic hormone (Van der Kolk *et al*., 1995) or stress (Jeffcott and Field, 1985a), are all factors that exacerbate tissue insulin resistance through the antagonism of the action of insulin. Other predisposing factors include: fasting (Naylor *et al*., 1980, and Freestone *et al*., 1992) increasing age (Jeffcott and Field, 1985a) and obesity (Watson *et al*., 1990, and Watson *et al*., 1992b). Additional factors leading to the development of hyperlipaemia including: endotoxin which can induce changes in lipid metabolism with stimulation of hepatic synthesis and secretion of VLDL, blood endotoxin may inhibit lipolysis of TGC in peripheral tissue

(Feingold *et al*., 1992), and azotaemia is an essential factor by interfering with the action of LPL (Naylor *et al*., 1980).

1.2.3 Clinical signs

Clinical signs are non-specific and generally result from hepatic and/or renal dysfunction due to lipidosis (Hughes *et al*., 2004). In the initial stage of the disease clinical signs include: depression, lethargy, inappetence, adipsia, weakness and reduced gastrointestinal motility and faecal output. In the mid-stage of the disease the animal is reluctant to move, showing intermittent pain, diarrhoea, central nervous system dysfunction, ataxia, sham drinking, dysphagia, head pressing and circling. In the late stage of the disease the animal becomes recumbent showing champing, mania and abortion occurs (Gay *et al*., 1978, Jeffcott and Field, 1985a, Field, 1988, Watson *et al*., 1992a, Watson, 1994, and Hughes *et al*., 2002). In many animals, abdominal pain occurs as a result of stretching of the liver capsule secondary to TGC accumulation (Watson and Love, 1994), icterus resulting from a combination of hepatic dysfunction and inappetance (Naylor, 1982), and ventral subcutaneous oedema (Gay *et al*., 1978, and Naylor, 1982). Hyperlipaemia is rapidly progressive and associated with a high mortality rate (Hughes *et al*., 2004).

1.2.4 Diagnosis

Diagnosis of hyperlipaemia cannot be made from the non-specific clinical signs alone; it is made from both clinical signs and history (Hughes *et al.,* 2004). A preliminary diagnosis can be made if the plasma/serum is opalescent (Jeffcott and Field, 1985a), and diagnostic confirmation is achieved by quantification of serum TGC concentrations (Jeffcott and Field, 1985a, and Watson and Love, 1994).

In healthy horses and non-pregnant ponies TGC concentrations are usually less than 1.00 mmol/L (Freestone *et al*., 1991, and Watson *et al*.,

1991), while in ponies during the last trimester of pregnancy concentrations reaching 2.83 mmol/l have been reported (Watson *et al.*, 1993). In healthy donkeys concentrations of up to 2.94 mmol/l have been reported (Watson *et al.*, 1991, and Forhead *et al.*, 1994) with a positive relation between body condition and serum TGC concentration (Watson *et al.*, 1991). The values reported for fasted healthy horses serum TGC concentration more than 2.26 mmol/l (Naylor *et al.,* 1980) is lower compared to fasted healthy ponies, mean value 8.27 mmol/l (Bauer 1983), and fasted healthy donkeys, 4.24 ± 0.56 mmol/l (Forhead *et al.,* 1994). In naturally occurring hyperlipaemia, the elevation of Serum TGC exceeds the values obtained from fasting normal animals. Levels reaching 25.4 ± 18.1 mmol/l in ponies (Watson *et al.* 1992a), and 16.6 ± 1.9 mmol/l in donkeys (Forhead *et al.,* 1994).

Mild elevations of serum TGC concentrations (up to 5.6 mmol/L are often sub-clinical, detected only by laboratory means, and are not associated with lactescent plasma or fatty infiltration of the liver (Naylor *et al.*, 1980, and Naylor, 1982). Such elevations are termed hyperlipidaemia, and are usually indicative of insufficient feed intake (Naylor, 1980, and Freestone *et al.*, 1991), and requirement for nutritional support (Naylor *et al.*, 1980, and Naylor, 1982). The condition rapidly corrects when feed intake and energy balance, improves (Naylor *et al.*, 1980). Hyperlipaemia involves elevation of serum TGC concentrations to concentrations more than 5.6 mmol/l, opalescent plasma, hepatic lipidosis and severe clinical signs (Naylor, 1982; Watson *et al.*, 1992a). Plasma concentrations of FFA, glycerol, cholesterol and phospholipids are elevated in hyperlipaemia (Naylor *et al.*, 1980, and Watson *et al.*, 1992b). Ketonaemia and ketonuria are not features of hyperlipaemia (Gay *et al.*, 1978, and Naylor *et al.*, 1980). Haematological changes are non-specific and may include haemoconcentration (Gilbert, 1986) a stress leukogram

(Field, 1988) or changes associated with underlying disease including neutrophilia, neutropenia (Gilbert, 1986), left shift (Gilbert, 1986, and Mair, 1995), and hyperfibrinogenaemia (Mair, 1995). Serum biochemical evaluation is useful in determining the presence of organ dysfunction and the metabolic status of hyperlipaemic patients. Hepatic dysfunction is common (Watson *et al.*, 1992a), hepatic dysfunction is reflected by increases in serum enzymes activities of hepatocellular and biliary origin, and an elevated serum bilirubin concentration (Naylor *et al.*, 1980, Watson *et al.*, 1992a, and Moore *et al.*, 1994).

Hyperlipaemic animals are frequently azotaemic, which is generally an indication of renal dysfunction (Watson *et al.*, 1992a), often supported by post-mortem findings of severe renal lipid infiltration (Gay *et al.*, 1978). Hypoglycaemia is common in hyperlipaemic ponies and donkeys (Gay *et al.*, 1978, Naylor *et al.*, 1980, and Mair, 1995). Metabolic acidosis is common, and is associated with marked base deficits (Watson and Love, 1994, and Hughes *et al.*, 2002), and decreased arterial partial pressures of carbon dioxide may be found due to respiratory compensation (Hughes *et al.*, 2002).

Serum biochemical data may be complicated by interference of biochemical assays from lipids present in the blood. Lipaemic serum should be cleared by either ultracentrifugation or chemically by the use of polyethylene glycol 6000 prior to analysis (Duncan, 1994).

1.2.5 Necropsy findings

Gross and histopathological findings in hyperlipaemia are associated with the deposition of lipids in body tissues (Gay *et al.*, 1978, Naylor *et al.*, 1980, Gilbert, 1986, and Watson and love, 1994). The liver and kidneys are most severely affected with lipid infiltration characterized grossly by swelling and development of a greasy texture (Gay *et al.*, 1978, Naylor *et al.*, 1980, and Field, 1988).

Histopathological examination presents tissue lipidosis. Other lesions include lipid infiltration of skeletal muscle, adrenal cortex and the myocardium (Gay *et al.*, 1978), pancreatitis (Mogg and Palmer, 1995) and a variety of vascular lesions including focal haemorrhages, pulmonary oedema, left ventricular myocardial infarction, renal infarction, and venous thrombosis (Gay *et al.*, 1978). In cases of secondary hyperlipaemia, changes associated with underlying disease may also be found (Naylor *et al.*, 1980, and Hughes *et al.*, 2002).

1.2.6 Treatment

Dyslipidaemia in human is one of the major risk factors for cardiovascular disease including atherosclerosis which is implicated in 75 per cent of all cardiovascular- related death in The USA (Lewis, 2009).

Management of hyperlipidaemia in human medicine is divided into: Therapeutic of lifestyle and lipid-lowering drugs (McPherson *et al.*, 2006, Paul *et al.*, 2008, and Lewis, 2009). Lifestyle interventions including: weight loss and physical exercise additional to low lipid diet.

Lipid lowering drugs including: Statins (the 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors): which are considered as agents of choice for reducing the level of LDL, and have modest HDL rising effect and triglyceride lowering effect (Paul *et al.,* 2008). All statins are competitive inhibitors of HMG-CoA reductase and act by blocking the HMG-CoA reductase enzyme (Istvan and Deisenhofer, 2001).

Depending on the statin and the dose that decreases triglycerides by up to 30 per cent, and LDL cholesterol by up to 55 per cent and raised HDL cholesterol by up to 15 per cent. According to Lewis, (2009) the current available statins are: Rosuvastatin in a dose of 5-10 mg/day reduces TG concentrations in a range between 10-35 per cent. Atorvastatin in a dose of 10 mg/day reduces TG concentrations up to 19

per cent. Simvastatin in a dose of 20-40 mg/day reduces TG concentrations by up to 18-19 per cent. Pravastatin in a dose of 40 mg/day reduces TG concentrations by up to 24 per cent. Lovastatin in a dose of 40 mg/day reduce TG concentrations by up to 8 per cent. Fluvastatin in a dose of 40-80 mg/day reduces TG levels by up to 14-19 per cent. Statins are well tolerated by most individuals, a significant increase in alanine aminotransferase levels more than three times the upper limit of normal, occurs in 0.3 to 2.0 per cent of the patients and are generally dose-related. Although underlying liver disease is considered a contraindication to statin therapy, Statin-induced myopathy is reported but is a rare side effect. The incidence of myalgia (muscle discomfort without significant increases in creatine phosphokinase) is approximately 3 to 4 per cent in statin-treated patients (McPherson *et al.,* 2006). Myositis may occur with statin therapy, but this is common with the administration of other drugs besides statin, including cyclosporine, gemfibrozil, certain antifungal drugs and macrolide antibiotics (Pasternak *et al.*, 2002).

Fibrates: Fibric acid derivatives such as gemfibrozil, bezafibrate and fenofibrate are used as main treatments of hypertriglyceridemia (Barter and Rye, 2006); they can reduce plasma triglyceride levels by up to 50 per cent. The mechanism of action of fibrates includes modulation of the activity of peroxisome proliferator–activated receptor-α in the liver, with reduced hepatic secretion of VLDL, and increased lipolysis of plasma triglycerides (Rubins *et al.*, 2002). Fibrates reduced LDL particles and increase HDL-C (Frick *et al.,* 1987).

Patients with elevated triglycerides and/or low HDL may benefit from fibrate therapy alone, or in combination with a statin, especially when LDL is also raised (Paul *et al.,* 2008).). Fibrate therapy is generally very well tolerated, with very rare reports of leading to hepatitis or

myositis (Barter and Rye, 2006); their main side effects include gastrointestinal problems and a possible increase in gallstones (Lewis, 2009). Increases in plasma creatinine of 15 to 20 per cent are common in fibrate-treated patients with a high increase in patients with underlying renal disease (Ellen and McPherson, 1998).

Azetimibe is a member of a new class of drugs that inhibit the absorption of cholesterol by the intestine, and has a synergistic effect when combined with statins.

Nicotinic acid is an effective drug in raising HDL-c, lowering triglycerides, and reducing LDL (Paul *et al.,* 2008). One mechanism responsible for the lipid-modifying effects of nicotinic acid is the activation of the G-protein-coupled receptor GPR109A expressed on adipocytes (Soga *et al*., 2003). Activation of GPR109A on fat cells has an anti-lipolytic effect leading to a decreased release of free fatty acids from adipocytes. The reduced supply of free fatty acids to the liver leads to reduced triglyceride synthesis, VLDL production, and LDL cholesterol levels (Carlson, 1963). The most common side effect of nicotinic acid is flushing or redness of the skin, gastrointestinal symptoms and high doses may worsen glucose control in patients with type2 diabetes (Lewis, 2009).

Fish oils contain highly polyunsaturated long chain n-3 fatty acids which can lower TG concentrations. A 50 per cent reduction in TG concentrations has been shown with a daily intake of 2-5 g of n-3 fatty acids (equivalent to 6-15 g of fish oil).

In veterinary medicine many therapeutic approaches have been proposed for the treatment of hypertriglyceridaemic animals. Several workers have described the treatment of hyperlipaemia on the basis of nutritional support, identification and treatment of any underlying disease, correction of fluid, electrolyte and acid-base abnormalities,

reduction of adipose tissue lipolysis and enhanced clearance of plasma lipids (Naylor, 1982, Jeffcott and Field, 1985a, and Watson and Love, 1994), correcting inappetence or anorexia, supplying nutrients to correct the negative energy balance (Watson, 1994). Hammond (2004) reported that care should be taken to minimize additional stress in hyperlipidaemic animals because they are at risk of developing hyperlipaemia.

Nutritional support has been suggested by many workers including high palate food, such as green grass (Jeffcott and Field, 1985a, and Watson and Love, 1994), enteral feeding using glucose and electrolytes solutions (Jeffcott and Field, 1985a, Watson *et al* 1992a, and Mair, 1995), and commercial enteric feeding preparations (Moore *et al.,* 1994, and Mair, 1995). Naylor *et al.*, (1984) attempted a home mixed diet containing: electrolytes, water, dextrose, cheese and alfalfa. Patients should be observed during enteral feeding to avoid occurrence of abdominal pain or gastric reflux through the nasogastric tube (Moore *et al.*, 1994). Parenteral feeding provide calculated amounts of nutrients without stress as the hyperlipaemic animal is inappetant and unable to eat, but there are side effects such as hyperglycaemia and sepsis (Durham *et al.,* 2004, and Hughes *et al.,* 2004). Durham, (2006) treated hyperlipaemic animals with a balanced electrolyte solution administered intravenously at rates calculated to rehydrate and maintain normohydration in addition to subcutaneous sodium heparin of a dose 40-80iu/kg and 0.15iu/kg insulin zinc suspension both twice a day. This treatment reduced serum triglycerides concentrations. Parentral nutrition in the form of 50 per cent dextrose infused intravenously was described by Watson and Love, (1994) and Mogg and Palmer, (1995). Laura and Cebra, (2009) found that Equids may benefit from the administration of exogenous insulin although this is questionable according to Watson and Love, (1994) and Durham, (2004) because of the peripheral insensitivity

to insulin in these animals. Hammond, (2004) described a parenteral nutrition administered using a 5:2 ratio of 50 per cent glucose and 8.5 per cent amino acid solution with vitamin B supplement in a balanced polyionic fluid.

Successful treatment of concurrent disease increases the survival chance in hyperlipaemic patients (Durham, 2006, and Conwell, 2010). Gastrointestinal parasitism is a common predisposing disease in hyperlipaemia (Gay *et al.*, 1978, and Jeffcot and Field, 1985b), and Moore *et al.*, (1994) reported that 33 per cent of hyperlipaemic animals had a gastro intestinal tract nematode infection. Watson and Love (1994) suggested that anthelmintics should be included in the treatment of hyperlipaemia. Additional research is needed to identify effective pharmacological agents to reduce the mobilization of adipose tissue triglycerides in equidae (Hughes *et al.,* 2004).

Nutraceutical supplements have been reported in the management of dyslipidemia (Houston *et al.*, 2009). They are considered as an alternative treatment for patients who are statin intolerant, or those who cannot take other drugs for the treatment of dyslipidemia, or in those who prefer alternative treatments (Houston, 2012). Houston (2012) reported that nutritional supplement act as hypolipidaemic by different mechanisms such as inhibition of HMG CoA reductase by red yeast rice, sesame, green tea extract and green tea, Omega 3 fatty acids, citrus, garlic, curcumin, and plant sterols, or lowering of triglycerides by niacin, red yeast rice, Omega 3 fatty acids, pantethine, citrus, bergamot, flax seed, monounsaturated fats and orange juice.

Generally demand is increased for using natural products in therapy because synthetic drugs are so expensive and have undesirable effects, these products are cheap and locally available (Amin and Nagy, 2009).

1.2.7 Prevention

Care should be taken of susceptible animals and good management strategies should be followed (Hughes *et al*., 2004). Provision of nutrients and avoidance of stress such as transport and parasitic infections, close monitoring and periodic blood samples analysis is recommended in case of breed risk factor (Jeffcott and Field, 1985, and Watson and Love, 1994)

Chapter two

Materials and Methods

2.1 Site of the study

This study was conducted in Khartoum state, which is located between 15° 47′ 0″ North latitudes and 32° 43′ 0″ East longitudes (Figure 2.1), at the farm of the College of Veterinary Medicine, Sudan University of Science and Technology.

2.2 Ethical approval

The study protocol was approved by the College of Veterinary Medicine Research Board as well as the Scientific Research Deanship, Sudan University of Science and Technology.

2.3 Experimental animals

The donkeys were purchased from a local animal market and kept in pens in the farm of the college of Veterinary Medicine, Sudan University of Science and Technology. The donkeys were provided with free access of water and *Abu Sabeen ad libitum* and a calculated amount of Dura maize.

The donkeys were left to acclimatize for two weeks. During this period each donkey received Penstrept 400 (Interchemie Werken, Holland - 200,000 procaine penicillin G + 200mg dihydrostreptomycin/ml) at dose rate of 1ml/20 kg/bwt, by the I/M route for three successive days, and a single dose of Paramectin (Pharma Swede, Egypt-10 mg/ml ivermectin) at dose rate of 0.2 mg/kg/bwt by the S/C route.

2.4 Gum Arabic

A fine pure powder of Gum Arabic (Active-Acacia®) was obtained from the Sudanese Limited Company of the Gum Arabic and was used as received. Two different doses (50 mg and 25 mg) of Gum Arabic were measured using sensitive balance (AND GR. 200-EC from A and D

Figure 2.1: Map of the Sudan showing Khartoum State (site of study)

Figure 2.2: Experimental animals in the College of Veterinary Medicine farm

2.5 Experimental design

2.5.1 First experiment

Three groups of donkeys each of six animals were used in this experiment. All animals were subjected to four days fasting to induce hyperlipaemia as described by Bulldan *et al*., (2013). After such period, the 1st group (TG1) was treated with Gum Arabic at dose rate of 25gm/day for seven successive days, the 2nd group (TG2) was treated with Gum Arabic at dose rate of 50 gm/day as in the 1st group, and the 3rd group was kept untreated as control.

2.5.2 Second experiment

Three groups of donkeys each of six animals were used in this experiment. The 1st group (PG1) was treated with Gum Arabic at dose rate of 25gm/day gum Arabic for seven successive days, the 2nd group (PG2) was treated with Gum Arabic at dose rate of 50 gm/day as in the 1st group, and the 3rd group was kept untreated as control. All animals in all groups were then subjected to five days fasting to induce hyperlipidaemia as described by Bulldan *et al*., (2013).

2.6 Blood Sampling

2.6.1 Sampling schedule

First experiment:

Blood samples were withdrawn from the jugular vein of the donkeys on four occasions: before induction of hyperlipidaemia (baseline), during fasting period (F1, F2, and F3), during treatment period (T1 "2nd day", T2 "4th day" and T3 "6th day" during treatment) and at the 10th (PostT1) and 14th (postT2) day from the beginning of the treatment.

Second experiment:

Blood samples were withdrawn from the jugular vein of the donkeys on four occasions: before induction of hyperlipidaemia (baseline), during gum Arabic supplementation (T1 and T2), during fasting period (F1, F2,

F3, and F4), and after re-feeding at the 10th (PostF1) and 14th (postF2) day from the beginning of the treatment.

2.6.2 Blood collection

Blood samples were collected using 10 ml syringes and transferred into tubes coated with fluoride oxalate as anti-coagulant (AFCO- DISPO, Jordan). The blood in the tubes was immediately and thoroughly mixed with the anticoagulant by gently inverting the tube several times, placed in ice, transported to the laboratory and centrifuged for five minutes at 5×1000 round/minute using tube centrifuge (EBA20- Hettich zentrifugen, Germany). The separated plasma in each tube was harvested in labelled Eppendorf tubes and was kept at -20 ºC until analysed.

2.7 Biochemical methods

2.7.1 Plasma total protein

The most suitable method used for routine analysis of samples in the current investigation is the Biuret Colorimetric Endpoint Method described by Doumas *et al.*, (1981). In this method the divalent copper reacts with the peptide bonds of protein under alkaline conditions to form the characteristic pink to purple biuret complex. Total protein was measured using a commercial kit (Vitro Scient-Egypt). The intensity of colour is determined by measuring the increase in absorbance at 530 nm using spectrophotometer (Jenway 6305 U. V./vis. Spectrophotometer, U.K.).

2.7.2 Plasma albumin

Albumin was measured using commercial kit (Vitro Scient-Egypt). The method used was the Colorimetric Endpoint Method according to modified bromcresol green binding described by Doumas *et al.*, (1971). Albumin at pH 4.2 bind to bromcresol green dye to form a blue green coloured complex the intensity of the blue green colour was determined

by measuring the increase in absorbance at 630 nm using spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer, U. K.).

2.7.3 Plasma Triglycerides

Triglycerides were measured using commercial kit (Vitro Scient-Egypt). Generally triglycerides are determined by a combination of hydrolysis to glycerol and free fatty acids and measurement of the amount of released glycerol. Triglycerides were analyzed by enzymatic colorimetric method according to Bucolo and David (1973) using a combination of lipase and at least one proteolytic enzyme. Vitro triglycerides reagent combines the use of lipoprotein lipase, glycerol kinase and glycerol phosphate oxidase with the peroxidase, phenol and 4-aminoantipyrine Trinder (1969) system. In this assay system triglycerides are hydrolyzed to glycerol followed by oxidation to dihdroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminoantipyrine and 4-chlorophenol under the catalytic action of peroxidase to form quinoneimine, a red dyestuff. The optical density of the developing colour was measured at 500 nm using Spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer, U. K.).

2.7.4 Plasma cholesterol

Cholesterol was analyzed with enzymatic colorimetric method according to Allain *et al.,* (1974) using commercial kit (Vitro Scient-Egypt). This method uses a combination of cholesterol esterase and cholesterol oxidase into a single enzymatic reagent for the determination of total cholesterol. Vitro cholesterol reagent which used in this analysis combine these enzymes with peroxidase, phenol and 4- aminoantipyrine according to Trinder (1969) where cholesterol esters are hydrolyzed to cholesterol and then the free cholesterol oxidized to form hydrogen peroxide which coupling the phenol and 4- aminoantipyrine to form quinoneimine, a red dyestuff. The optical density of the developing

colour was measured at 500 nm using Spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer, U. K.).

2.7.5 Plasma glucose

Glucose was determined using enzymatic colorimetric method using glucose oxidase and 4-aminoantipyrine according to Trinder (1969) method modified from Keston (1956). In this method glucose was oxidized by glucose oxidase to gluconic acid and hydrogen peroxide which coupling the phenol and 4- aminoantipyrine to form quinoneimine, a red dyestuff. The optical density of the developing colour was measured at 500 nm using Spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer, U. K.).

2.7.6 Plasma urea

Urea was measured using commercial kit (Vitro Scient-Egypt) according to enzymatic colorimetric method described firstly by Marshall (1913) who added urease enzyme and measured the liberated ammonia with titration with an acid, this method has been used and modified and later modified by Fawcett and Scott (1960). In this modified method urea is hydrolyzed by urease to form ammonium and carbonate, the ammonium ions react with the salicylate and hypochlorite to form a green coloured indophenol. The optical density of the developing colour was measured at 578 nm using Spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer, U. K.).

2.7.7 Plasma creatinine

Creatinine was analyzed using commercial kit (Vitro Scient-Egypt).Vitro creatinine reagent is based on modified Jaffe reaction which was firstly described by Jaffe (1886) in a method involved precipitation of protein and still widely used today. In this reaction creatinine forms a yellow-red complex with alkaline picrate. The optical density of the

developing colour was measured at 578 nm using Spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer, U. K.).

2.7.8 Aspartate aminotransferase (EC 2.6.1.1) (AST) (Glutamic – Oxaloacetic transaminase (GOT)

AST was analyzed in accordance with kinetic ultraviolet method. Karmen *et al.*, (1955) described the first kinetic method using a couple reaction of MDH and NADH in 1960 this method modified by Henry *et al.,* (1960). AST was analyzed using commercial kit (Vitro Scient-Egypt). Vitro reagent is based on the recommendation of the IFCC (IFCC, 1986) where the amino group is enzymatically transferred by AST present in the specimen from aspartate to the carbon atom of 2-oxoglutarate yielding oxaloacetate and L-glutamate. Oxaloacetate is reduced to malate by LDH present in the reagent with the simultaneous oxidation of NADH to NAD. The rate of oxidation of the coenzyme NADH is proportional to the AST activity in the specimen and it was determined by measuring the decreases in absorbance at 340 nm using Spectrophotometer (Jenway 6305 U. V. /vis. Spectrophotometer, U. K.).

2.7.9 Alanine aminotransferase (EC 2.6.1.2) (ALT) (Glutamic – Pyruvic Transaminase (GPT)

Alanine aminotransferase (ALT) is a soluble cytosolic enzyme that catalyzes the reversible transamination of L-alanine and 2-oxoglutarate to pyruvate and glutamate. Hepatic ALT activity in horses, cattle, sheep, goats, and pigs is very low, which precludes its use for detecting liver disease in these species (Duncan, 1994).

Glutamic – Pyruvic Transaminase (GPT) was measured using kits (Randox laboratories Ltd., United Kingdom, BT29 4QY) by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine according to Reitman and Frankel (1957). The

formed colour was measured at 530 nm using Jenway spectrophotometer (Jenway 6105 U. V. /vis. Spectrophotometer, U. K).

2.8 Statistical analysis:

Data obtained are presented as mean ±SD.

1. In the first experiment fasting values were compared with baseline values, treatment values were compared with the values obtained at the end of fasting period (F3), and post treatment values were compared with baseline values.
2. In the second experiment treatment values were compared with baseline values, while that of fasting period were compared with T2 value, and post fasting values were compared with baseline value.

T-test was used to compare between means and $P<0.05$ was considered significant.

Chapter three

Results

3.1 First experiment

In the first experiment where donkeys in the two treatment groups were subjected to four days fasting and then treated with gum Arabic at dose rate of 25 and 50 g per day, respectively for seven successive days, analysis of selected biochemical parameters revealed the following results:

At the start of the study, the baseline level of triglycerides in donkeys in the three groups was within the reference normal values (Table 3.1). Following fasting, triglycerides level gradually increased in all experimental groups. Statistically significant (P<0.05) increased values were obtained in the second and the third day following fasting. In the control, group triglycerides concentration increased to reach 4.51mmol/l by the end of fasting period (*p value* 0.032). Following re-feeding the concentration dropped to 0.58 ± 0.37 mmol/l by the end of the experiment (*p value* 0.027). The decrease in triglycerides concentrations was statistically significant.

In treatment group 1 (TG1) there was significant increase (P<0.05) in triglycerides concentration at the end of fasting period (*p value* 0.033). After two days re-feeding accompanied with GA supplementation the concentration dropped to 0.37 mmol/l (*p value* 0.038). In the second treatment group (TG2) there was slight decrease in triglycerides concentration that is considered statistically (P>0.05) insignificant.

Cholesterol baseline level was within the reference normal values in the three different groups. Following fasting there was statistically insignificant slight increase in cholesterol level in the control and TG2 group. In TG1 group the increase in cholesterol level is statistically (*p value* 0.01) significant by the end of the fasting period. Following

treatment for seven successive days cholesterol level in TG1 group dropped to statistically significant level (*p value* 0.001) and the level in the control group also decreased to statistically significant level (*p value* 0.01) when compared with the level reached at the last fasting day. In TG2 there was slight insignificant decrease of cholesterol level (*p value* 0.20) at the end of treatment period (Table 3.2).

Plasma creatinine increased significantly only in TG1 following fasting while the other two groups exhibited no significant increase. Following treatment, statistically significant decrease in TG1 group was observed during and after the end of the treatment period, in TG2 group this significant decrease appeared after the end of dosing. In the control group the decrease didn't reach a significance level (Table 3.3).

Plasma urea level increased significantly following induction of hyperlipidaemia in the control and TG1 group and there was significant increase ($P<0.05$) in TG2 group (Table 3.4) only in the third day of fasting. Following treatment with gum Arabic the urea level in TG1 group decreased significantly below the level of the baseline value. The level of urea in TG2 group showed no significant ($P>0.05$) decrease at the end of the experiment when compared with baseline value (*p value* 0.06).

As shown in Figure (3.1), following induction of hyperlipidaemia there was no significant change in glucose concentration in all groups ($P>0.05$). Treatment with the two doses of gum Arabic did not affect glucose level significantly, after the end of treatment period there was significant decrease in glucose level in TG1 treated group (*p value* 0.004).

Four days fasting caused no significant ($P>0.05$) increase in total protein concentration in all groups. During and post treatment period total protein concentration decreased significantly ($P<0.05$), but in the control group the decrease didn't reach significant level (Figure 3.2).

Albumin concentration increased significantly ($P<0.05$) by the end of the fasting period in all treatment groups, during re-feeding albumin decreased gradually in all groups but the decrease wasn't significant ($P>0.05$) in the group treated with 50 mg/day GA (TG2 group) as shown in Figure (3.3).

As illustrated in Figures (3.4 and 3.5), there was a slight increase during fasting followed by slight decrease after treatment in AST and ALT activities but these changes didn't reach the significance level.

Table 3.1: Plasma triglycerides concentration (mmol/l) in donkeys at baseline, during four days fasting, one -week treatment with 0, 25, and 50 g/day gum Arabic, and after gum Arabic drenching

Days	Control		TG1 (25g/day)		TG2 (50g/day)	
	Mean ±SD	P value	Mean ±SD	p value	Mean ±SD	p value
Baseline	0.33 ±0.13		0.38 ± 0.23		0.37 ± 0.30	
F_1	0.96 ±0.67	0.180	0.38 ± 0.26	0.40	1.70 ± 1.91	0.17
F_2	2.58 ± 1.41*	0.029	1.91 ± 1.61*	0.042	2.16 ± 1.85*	0.04
F_3	4.51 ± 2.85*	0.032	2.97 ± 2.38*	0.033	2.57 ± 2.58*	0.03
T_1	1.06 ± 0.61a	0.034	0.37 ± 0.22a	0.038	2.44 ± 2.71	0.54
T_2	0.81 ± 0.57a	0.033	0.72 ± 0.82a	0.044	2.16 ± 2.50	0.54
T_3	0.51 ± 0.19a	0.042	0.47 ± 0.12	0.052	1.13 ± 1.73	0.15
Post T_1	0.49 ± 0.16*	0.040	0.53 ± 0.17	0.057	0.64 ± 0.53	0.15
Post T_2	0.58 ± 0.37*	0.027	0.52 ± 0.20*	0.049	1.27 ± 1.59	0.16

Means with asterisk in the same column were significantly different with baseline value

Means with letter in the same column were significantly different with F3 value

Table 3.2: Plasma cholesterol concentration (mmol/l) in donkeys at baseline, during four days fasting, one - week treatment with 0, 25, and 50 g/day gum Arabic, and after gum Arabic drenching

Days	Control		TG1 (25g/day)		TG2 (50g/day)	
	Mean ±SD	P value	Mean ±SD	p value	Mean ±SD	p value
Baseline	1.94±0.23		1.73±0.55		2.13±0.46	
F_1	2.02±0.44	0.96	2.09±0.48*	0.02	2.71±1.05	0.30
F_2	2.12±0.63	0.77	2.08±0.67	0.34	2.13±0.81	0.82
F_3	2.96±0.98	0.09	2.52±0.65a	0.01	2.68±1.11	0.34
T_1	1.34±0.35a	0.017	1.60±0.57a	0.005	2.45±1.28	0.46
T_2	1.67±0.28a	0.02	1.67±0.63a	0.0008	2.13±1.13	0.19
T_3	1.57±0.28a	0.01	1.28±0.30a	0.001	1.92±0.78	0.09
Post T_1	1.66±0.29*	0.02	1.38± 0.31*	0.002	1.91±0.69*	0.02
Post T_2	1.33±0.18*	0.01	1.26±0.29*	0.0007	1.73±0.83	0.056

Means with asterisk in the same column were significantly different with baseline value

Means with letter in the same column were significantly different with F3 value

Table 3.3: Plasma creatinine concentration (mmol/l) in donkeys at baseline, during four days fasting, one - week treatment with 0, 25, and 50 g/day gum Arabic, and after gum Arabic drenching

Days	Control		TG1 (25g/day)		TG2 (50g/day)	
	Mean ±SD	P value	Mean ±SD	p value	Mean ±SD	p value
Baseline	123.4 ±51.0		111.2 ±43.8		105.9 ±33.9	
F_1	108.0 ±47.3	0.2	97.1 ±36.7	0.7	90.34 ±21.2	0.6
F_2	124.8 ±51.8	0.08	105.0 ±58.8	0.5	99.01 ±24.5	0.8
F_3	144.6 ±119.7	0.8	262.1 ±76.7*	0.02	161.5 ±88.4	0.2
T_1	128.5 ±53.4	0.7	177.2 ±104.7	0.1	96.06 ±42.4	0.2
T_2	112.3 ±60.3	0.4	109.0 ±78.4a	0.02	125.5 ±66.9	0.8
T_3	86.1 ±39.7	0.3	73.7 ±47.0a	0.005	63.12 ±39.3	0.08
Post T_1	48.6 ±19.2	0.1	65.7 ±45.0*	0.008	76.73 ±50.0*	0.03
Post T_2	53.4 ±22.2	0.2	42.1 ±19.1*	0.0008	65.24 ±60.4*	0.04

Means with asterisk in the same column were significantly different with baseline value

Means with letter in the same column were significantly different with F3 value

Table 3.4: Plasma urea concentration (mmol/l) in donkeys at baseline, during four days fasting, one - week treatment with 0, 25, and 50 g/day gum Arabic, and after gum Arabic drenching

Days	Control		TG1 (25g/day)		TG2 (50g/day)	
	Mean ±SD	P value	Mean ±SD	p value	Mean ±SD	p value
Baseline	2.93 1.3		4.01 1.1		3.80 1.1	
F_1	4.02 1.4	0.10	5.55 1.5	0.10	4.86 2.1	0.40
F_2	5.38 2.2	0.06	6.12 1.5*	0.02	5.43 2.6	0.30
F_3	8.71 3.6*	0.03	6.27 1.0**	0.009	6.77 2.8*	0.02
T_1	2.64 1.2a	0.01	3.37 1.2a	0.004	4.21 3.2	0.30
T_2	2.33 1.5a	0.01	2.63 0.9a	0.0006	3.37 2.2	0.20
T_3	2.05 1.4a	0.01	2.35 0.2a	0.0001	5.53 0.9	0.07
Post T_1	1.44 0.4*	0.008	2.53 1.1*	0.0001	2.57 0.9	0.06
Post T_2	1.79 0.6*	0.009	2.67 0.9*	0.0001	2.42 0.4	0.06

Means with asterisk in the same column were significantly different with baseline value

Means with letter in the same column were significantly different with F3 value

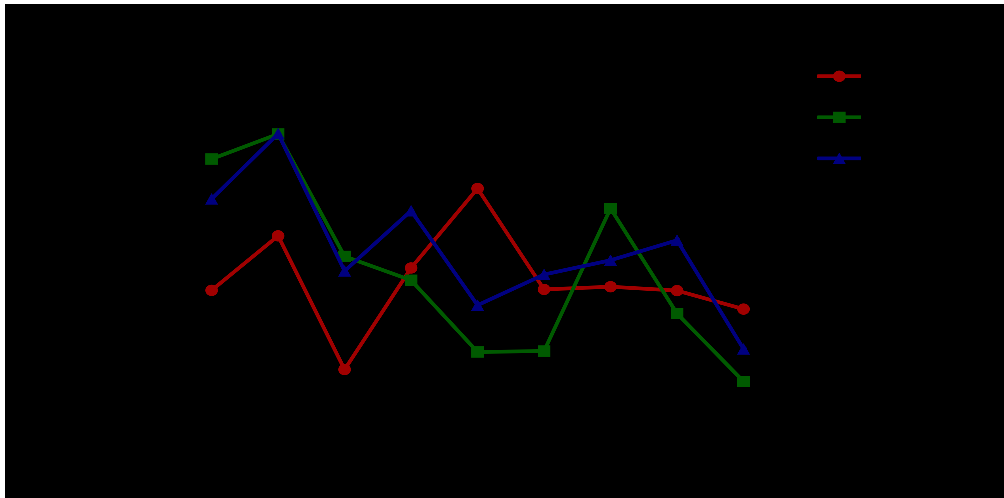

Figure 3.1: Plasma glucose concentration (mmol/l) in donkeys at baseline, during four days fasting, one - week treatment with 0, 25, and 50 g/day gum Arabic, and after gum Arabic drenching

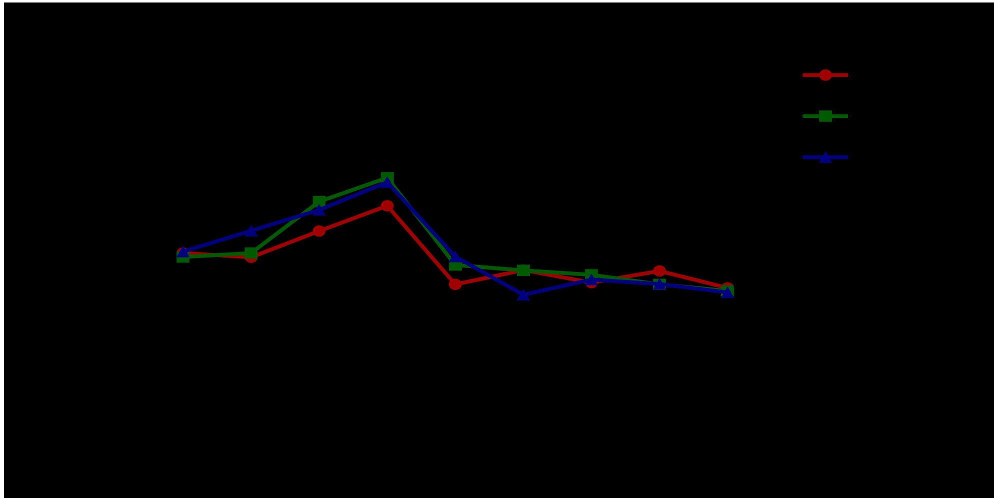

Figure 3.2: Plasma total proteins concentration (g/l) in donkeys at baseline, during four days fasting, one - week treatment with 0, 25, and 50 g/day gum Arabic, and after gum Arabic drenching

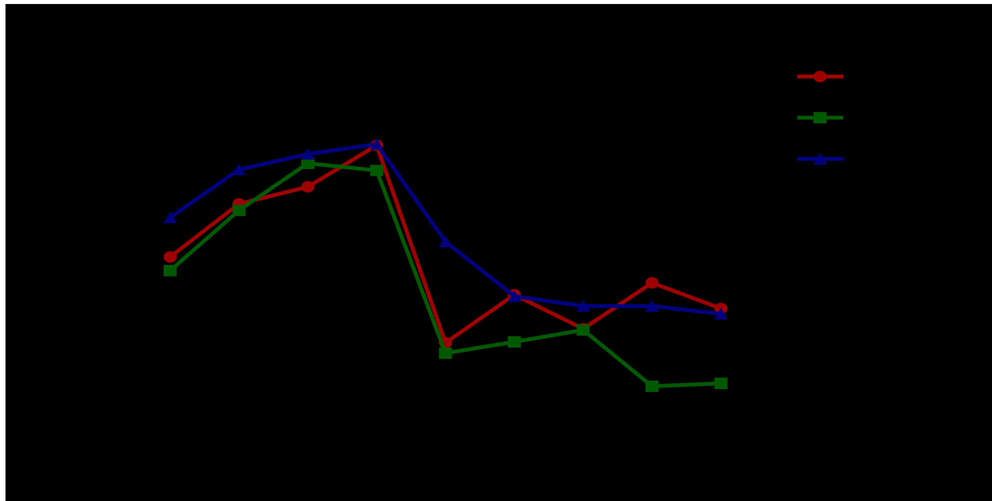

Figure 3.3: Plasma albumin concentration (g/l) in donkeys at baseline, during four days fasting, one - week treatment with 0, 25, and 50 g/day gum Arabic, and after gum Arabic drenching

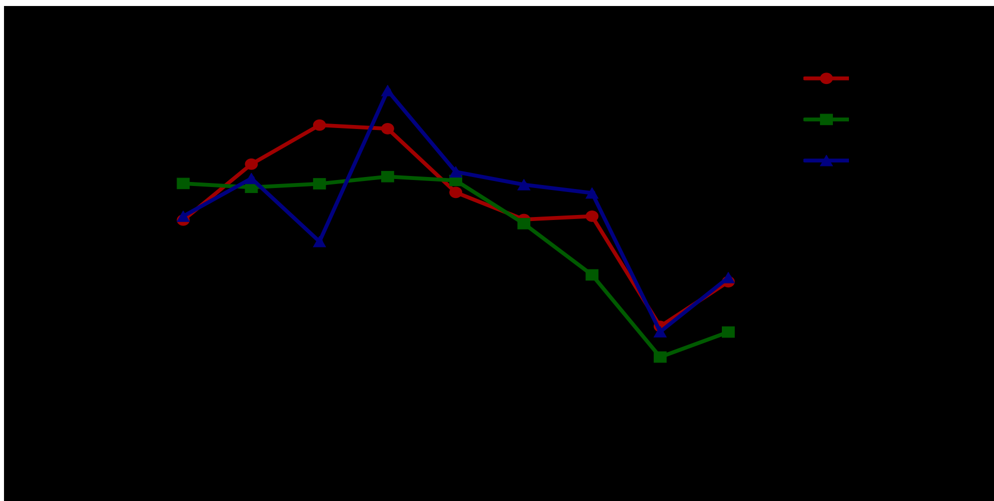

Figure 3.4: Plasma AST activity (U/l) in donkeys at baseline, during four days fasting, one – week treatment with 0, 25, and 50 g/day gum Arabic, and after gum Arabic drenching

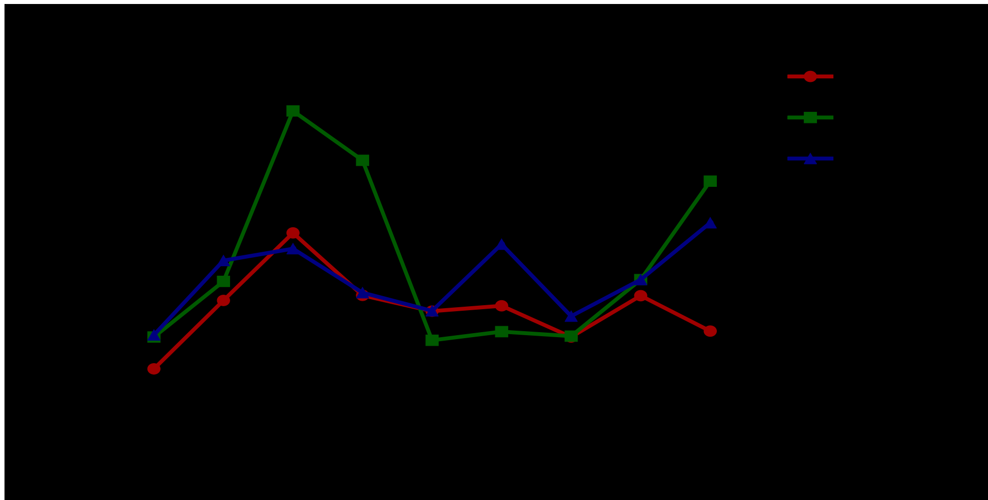

Figure 3.5: Plasma ALT activity (U/l) in donkeys at baseline, during four days fasting, one – week treatment with 0, 25, and 50 g/d gum Arabic, and after gum Arabic drenching

3.2 Second experiment

In this experiment, animals were either drenched with gum Arabic for seven successive days or left without treatment and then subjected to five days fasting.

During gum Arabic supplementation triglycerides concentration showed fluctuation with no significant (P>0.05) change. Significant increase (P<0.05) during the 2nd, 3rd, and 4th days of fasting was observed in the control and the PG1, while the level increased significantly (P<0.05) only at the 4th day of fasting and to a level that was noticed to be lower when compared with the level of the control and PG1 (Figure 3.6).

As shown in Figure (3.7) a similar pattern for cholesterol was observed in the control and PG1, where significant (P<0.05) increase was observed at the 2nd, 3rd, and 4th day of fasting; while the significant increase was only observed at the fourth day of fasting in the PG2. The level returned to normal by the end of the experiment.

Urea concentration increased significantly (P<0.05) in the three groups during fasting period and returned to normal level by the end of the experiment as shown in Figure (3.8). Here it is noticeable that urea level in PG2 was much lower than that of PG1 and control.

ALT activity was significantly (P<0.05) increased during fasting in the control and the first group (PG1), while the increase in the second group (PG2) did not reach the level of significance (Figure 3.9).

The fluctuation in glucose concentration did not reach significant level (Table 3.5), while creatinine level showed considerable non significant increase in the control and PG1, and decrease in PG2 (Table 3.6). Albumin concentration did not show any significant difference following either administration of gum Arabic or five days fasting (Table 3.8).

Significant increase in total protein concentration was observed in the first prophylactic group (PG1) during fasting. The level returned to non-significant change by the end of the experiment (Table 3.7).

Significant increase in AST activity was observed only in the second prophylactic group (PG2) during fasting period and at post fasting the level returned to a non significant ($P>0.05$) change by the end of the experiment (Table 3.9).

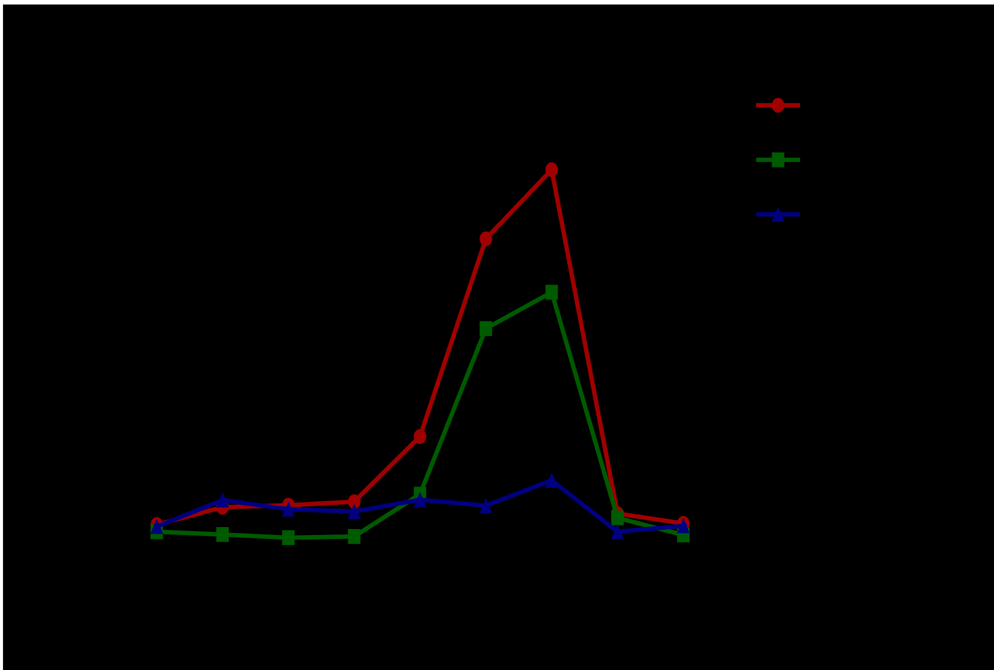

Figure 3.6: Plasma triglycerides concentration (mmol/l) in donkeys at baseline, one - week treatment with 0, 25, and 50 g/d gum Arabic, during five days fasting, and after re-feeding

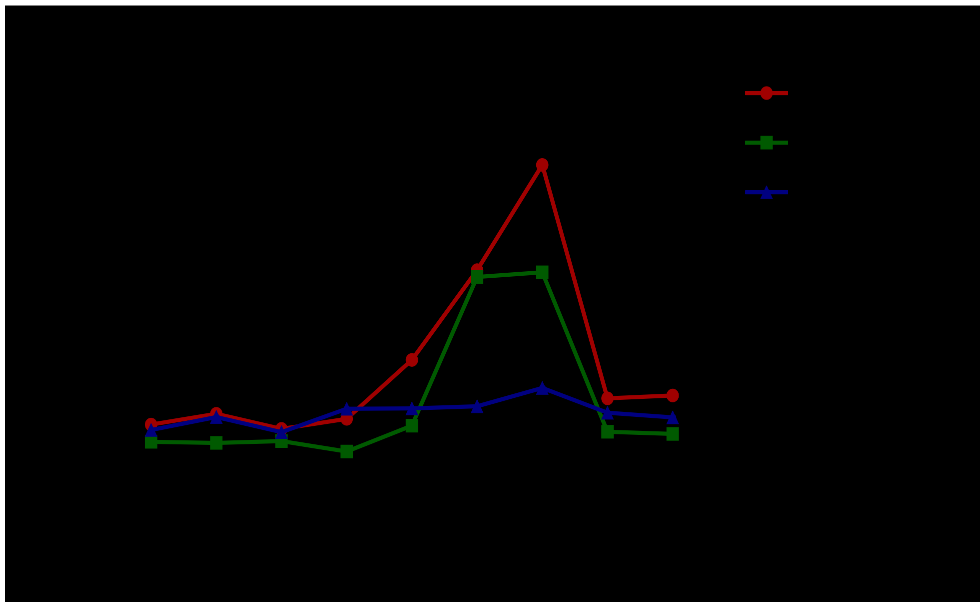

Figure 3.7: Plasma cholesterol concentration (mmol/l) in donkeys at baseline, one - week treatment with 0, 25, and 50 g/d gum Arabic, during five days fasting, and after re-feeding

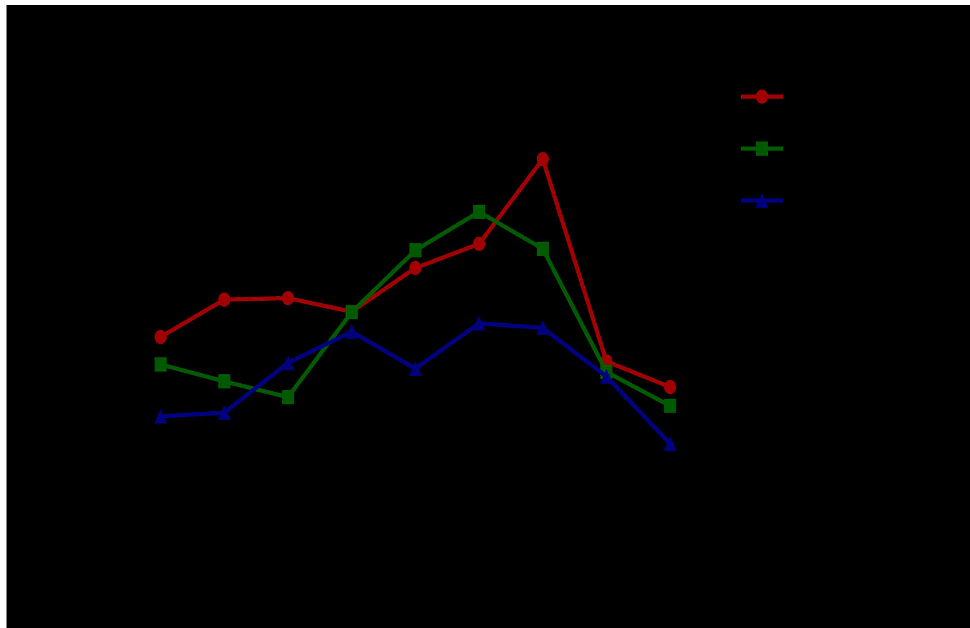

Figure 3.8: Plasma urea concentration (mmo;/l) in donkeys at baseline, one - week treatment with 0, 25, and 50 g/d gum Arabic, during five days fasting, and after re-feeding

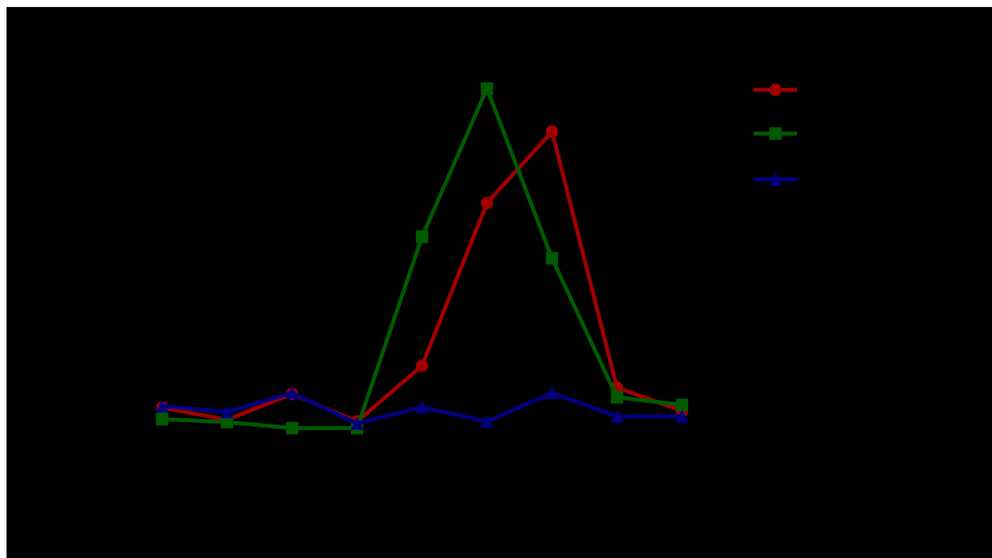

Figure 3.9: Plasma ALT activity (U/I) in donkeys at baseline, one - week treatment with 0, 25, and 50 g/d gum Arabic, during four days fasting, and after re-feeding

Table 3.5: Plasma glucose concentration (mmol/l) in donkeys at baseline, one - week treatment with 0, 25, and 50 g/d gum Arabic, during five days fasting, and after re-feeding

Days	Control		25g treated		50g treated	
	Mean ±SD	P value	Mean ±SD	p value	Mean ±SD	p value
0	3.78±0.59		3.28±1.08		3.65±0.84	
T1	3.69±0.88	0.85	2.65±0.49	0.08	3.35±0.96	0.82
T2	3.51±0.50	0.50	3.15±0.82	0.55	3.56±0.33	0.76
F1	3.89±0.47	0.84	3.17±0.94	0.65	3.00±0.42	0.53
F2	3.64±1.73	0.83	3.06±0.65	0.43	3.55±0.44	0.37
F3	4.56±2.75	0.57	3.76±1.14	0.58	3.18±0.47	0.41
F4	4.48±1.14	0.26	3.65±2.06	0.85	4.04±0.37	0.24
Post F1	4.18±0.94	0.64	2.83±1.17	0.31	4.07±0.43	0.93
Post F2	3.95±0.40	0.34	2.91±0.63	0.06	3.90±0.35	0.70

Table 3.6: Plasma creatinine concentration (mmol/l) in donkeys at baseline, one - week treatment with 0, 25, and 50 g/d gum Arabic, during four days fasting, and after re-feeding

Days	Control		25g treated		50g treated	
	Mean ±SD	P value	Mean ±SD	p value	Mean ±SD	p value
0	81.52±26.88		91.58±19.95		77.26±16.10	
T1	88.17±15.80	0.79	103.8±11.76	0.07	70.15±9.19	0.94
T2	80.20±20.68	0.35	86.46±14.19	0.83	85.57±22.49	0.07
F1	79.45±11.23	0.39	94.07±23.96	0.44	77.79±2.50	0.27
F2	90.79±7.84	0.96	100.8±24.22	0.28	83.09±13.07	0.053
F3	83.76±14.80	0.61	100.5±56.83	0.70	75.13±11.04	0.45
F4	85.88±19.22	0.71	96.77±17.67	0.46	71.60±10.04	0.73
Post F1	74.47±22.53	0.36	92.08±13.29	0.42	53.92±17.59	0.78
Post F2	90.31±4.80	0.64	107.2±14.96	0.19	87.96±9.06	0.07

Table 3.7: Total protein concentration (g/dl) in donkeys at baseline, one -week treatment with 0, 25, and 50 g/d gum Arabic, during four days fasting, and after re-feeding

Days	Control		25g treated		50g treated	
	Mean ±SD	P value	Mean ±SD	p value	Mean ±SD	p value
0	61.8±12.3		61.1±7.7		54.2±10.5	
T1	66.8±6.8	0.30	51.2±8.5	0.15	55.7±11.8	0.63
T2	63.7±15.7	0.57	46.2±7.2	0.03	60.1±7.1	0.75
F1	57.8±11.4	0.97	52.1±7.8	0.07	59.6±10.5	0.95
F2	68.0±19.4	0.40	66.7±5.4*	0.0002	57.7±7.5	0.70
F3	66.3±9.6	0.29	62.1±10.7	0.19	51.3±14.9	0.43
F4	75.7±15.1	0.16	68.7±7.8*	0.003	56.8±3.3	0.69
Post F1	58.6±9.9	0.08	60.4±9.1	0.14	56.4±10.9	0.96
Post F2	62.9±4.9	0.06	65.2±7.4	0.32	48.8±5.5	0.11

Table 3.8: Albumin concentration (g/l) in donkeys at baseline, one - week treatment with 0, 25, and 50 g/d gum Arabic, during four days fasting, and after re-feeding

Days	Control		25g treated		50g treated	
	Mean ±SD	P value	Mean ±SD	p value	Mean ±SD	p value
0	27.6±7.8		22.1±8.4		24.4±5.7	
T1	30.1±8.6	0.6	19.7±3.2	0.5	27.5±6.9	0.5
T2	26.4±6.7	0.5	20.7±3.3	0.6	22.2±6.9	0.2
F1	23.3±5.6	0.5	19.7±2.3	0.5	21.8±3.7	0.1
F2	25.5±11.2	0.9	24.3±2.7	0.8	22.3±6.4	0.09
F3	30.7±7.2	0.3	30.1±6.3	0.2	20.8±5.4	0.1
F4	28.4±3.3	0.4	27.9±5.9	0.1	21.8±4.6	0.1
Post F1	23.3±6.6	0.1	21.5±5.5*	0.04	25.8±7.3	0.2
Post F2	27.9±6.1	0.7	23.9±6.8	0.3	22.5±3.9	0.7

Table 3.9: Plasma AST activity (U/I) in donkeys at baseline, one - week treatment with 0, 25, and 50 g/d gum Arabic, during four days fasting, and after re-feeding

Days	Control		25g treated		50g treated	
	Mean ±SD	P value	Mean ±SD	p value	Mean ±SD	p value
0	132.7±60.1		131.9±36.7		80.3±12.8	
T1	117.9±32.30	0.9	131.4±21.9	0.9	88.6±6.8	0.6
T2	114.0±38.9	0.7	122.8±18.3	0.7	97.1±46.9	0.6
F1	135.9±31.0	0.9	114.8±17.9	0.4	166.4±19.0*	0.0007
F2	172.7±58.6	0.1	110.6±17.1	0.3	122.8±14.7*	0.001
F3	196.5±108.1	0.2	165.4±51.7	0.3	186.8±25.8*	0.001
F4	231.6±60.3	0.1	125.7±17.9	0.7	323.5±140.0*	0.005
Post F1	206.4±112.7	0.5	114.4±17.3	0.4	282.8±105.4	0.1
Post F2	226.5±84.1	0.7	109.9±31.1*	0.04	175.1±42.9	0.1

Chapter four

Discussion

The most characteristic change in the blood of equine with hyperlipaemia is increased plasma triglycerides (TG). Animals in the current study expressed significant increase in TG concentration following four days fasting. A result which was in agreement with that obtained by Bulldan *et al.*, (2013) in donkeys subjected to four or five days fasting. They reported that there was significant increase in TG during the post fasting period.

The plasma total triglyceride concentration measured in the present study were lower than values reported previously, with a range 0.33±0.13 - 4.51±2.85. This could be attributed to the fact that most of the animals in this experiment had body score ranging from fair to good. The degree of increase in plasma triglyceride (TG) appears to differ substantially among equids with similar primary causes and clinical presentation leading to hypertriglyceridaemia/hyperlipaemia. Experimental induction of hyperlipaemia by starving (fasting) of healthy animals resulted in only a moderate increase of plasma total triglycerides. The values reported for fasted health donkeys (4.24±0.56 mmol/l; Forhead *et al.*, 1994) are in the same line with our results. In naturally occurring hyperlipaemia, the elevation of total triglycerides exceeded the values obtained from fasting normal animals (Dunkel and Mckenzie, 2003).

Treatment with gum Arabic with the dose rate of 25g/day for seven successive days significantly decreased the level of triglycerides, while animals in the second treatment group (TG2) exhibited no significant decrease. Triglycerides level in the control group also decreased significantly with re-feeding of animals, with slow rate when compared with TG1. A result that is in agreement with that of Topping *et al.,* (1985)

who reported that plasma triglyceride concentration in plasma was significantly lower in rats fed with GA than in controls. Abd-Razig *et al.*, (2010) reported significant decrease in triglyceride concentration in the serum and egg yolk of hens supplemented with 5% or 7% gum Arabic for three months. It is worth to mention that the plasma triglycerides level in the second treatment group (TG2) exhibited no significant (P>0.05) decrease. Annison *et al.*, (1995) reported that plasma triacylglycerol concentrations were higher in rats fed Gum Arabic, whereas liver triacylglycerol were lower in rats fed the gums.

Following fasting there was statistically insignificant slight increase in cholesterol level in the control and TG2 treated group. In TG1 the increase in cholesterol level is statistically (*p value* 0.01) significant by the end of the fasting period. A result that is in harmony with that obtained by Bulldan *et al.*, (2013) who indicated significant increase in total cholesterol concentration in the group that was subjected to five days fasting.

Following treatment for seven continued days cholesterol level in TG1 group dropped to statistically significant level (*p value* 0.001). A daily intake of 25 and 30 g of GA for 21 to 30 days was reported to reduce total cholesterol by 6 and 10.4% in humans, respectively (Ross *et al.*, 1983, Sharma 1985). Furgał-Dierżuk (2004) reported that LDL-Cholesterol and Triglycerides level in serum were significantly (P≤0.01) lower in pigs supplemented with guar gum. However, Topping *et al.,* (1985) and Annison *et al.*, (1995) reported that plasma cholesterol concentration was not affected by the supply of GA.

The mechanism involved is clearly linked to the increased bile acid excretion and faecal neutral sterol or a modification of digestion and absorption of lipids (Moundras *et al.*, 1994). Various mechanisms have been proposed to explain the hypocholesterolemic effect of GA (Annison

et al., 1995, and Tiss *et al.*, 2001). Some studies have suggested that the viscosity of fermentable dietary fibre contributes substantially to the reduction of lipids in animals and humans (Gallaher *et al.*, 1993, and Moundras *et al.*, 1994).

In donkeys, with naturally occurring hyperlipidaemia/hyperlipaemia, a positive correlation between plasma insulin and STG concentration has been reported (Forhead *et al*., 1994). Activities of lipoprotein lipase and hepatic lipase are higher in hypertriglyceridemic, feed-deprived horses than in fed horses (Frank *et al.*, 2003). This suggests that overproduction of triglycerides, possibly complicated by defective catabolism, is the predominant cause of hypertriglyceridemia (Watson *et al*., 1992b).

Following induction of hyperlipidaemia, there was prominent increase in plasma creatinine and urea concentration in the three groups. Treatment with gum Arabic resulted in reduction in urea and creatinine level below the baseline values. Similar results were obtained by Bulldan *et al.*, (2013) who reported significant increase in urea, total bilirubin and creatinine level as well as AST activity, during the fasting period. Bilirubin and creatinine continued to increase significantly at post fasting. In the second group where animals were subjected to five days fasting, urea, AST and bilirubin exhibited non significant ($P>0.05$) change, while, creatinine showed significant increase during the fasting period (Bulldan *et al.*, 2013). Tarrant *et al.*, (1998), reported increase in urea (31.2 mg/dl) and decrease in AST (270 UI). Azotaemia has been associated with hypertriglyceridaemia in several reports. A statistically significant association has been found between serum creatinine and serum triglycerides (STG) in horses (Naylor *et al.*, 1980) and ponies with hyperlipaemia (Watson *et al.*, 1992a). Eight horses had an elevated serum creatinine (mean 0.46 mmol/l) concurrent with peak STG

measured; 12 horses had an elevation of the serum creatinine concentration at least once during their hospitalization (Dunkel and McKenzie, 2003). Bliss *et al*., (1996), reported significant decrease in serum urea nitrogen level during supplementation with gum Arabic (50g/day) in chronic renal failure (CRF) subjects compared with low protein diet (LPD) or supplementation with pectin; a result that may support our finding. The decrease in serum urea nitrogen during the gum Arabic supplementation period is consistent with previous observations of a decrease in serum urea nitrogen in CRF patients consuming a fiber supplemented LPD (Rampton *et al.*, 1984, and Little and Trafford 1991).

Induction of hyperlipidaemia in donkeys resulted in no significant ($P>0.05$) change in glucose and total protein concentration as well as AST and ALT activities in all groups. Samia *et al.*, (2006) indicated that 5% Gum Arabic have some positive effect on decreasing glucose level in the blood stream of the animals studied. Treatment with the two doses of gum Arabic did not affect glucose level significantly. Albumin concentration increased significantly ($P<0.05$) by the end of the fasting period in all treatment groups. During re-feeding albumin decreased gradually in all groups but the decrease wasn't significant ($P>0.05$) in the group treated with 50 mg/day GA (TG2 group). Physiologically, these results could be justified as the animals reduce water intake during fasting which may lead to haem-concentration, and following re-feeding the animals tend to drink water as usual, resulting in haem-dilution.

Four days fasting, resulted in no significant ($P>0.05$) decrease in the total protein concentration, but the level increased significantly following re-feeding. While, in the donkeys subjected to five days fasting there was significant decrease in total protein and albumin concentration (Bulldan *et al*., 2013). The fluctuation in total protein and albumin concentration could be attributed to negative energy balance where

animals tend to use protein as source of energy. Tlak *et al.*, (2008) reported significant lower total protein concentration as a result of a six-day fasting period in Peking duck ducklings. Also, significantly lower AST activity was found after the 5th day of fasting, and lower ALT activity after the 4th day of fasting in the same trial. The low concentrations of total proteins over the entire trial period as well as reduced activities of the afore mentioned enzymes were probably a consequence of a reduced protein regeneration in the body due to the deficiency of amino acids from the gastro-intestinal tract during fasting (Tlak *et al.*, 2008).

In the second experiment, animals were either drenched with gum Arabic for seven successive days or left without treatment and then subjected to five days fasting.

The significant increase ($P<0.05$) in triglycerides during the fasting period in the control and the PG1, and the delay in rise of triglycerides may indicate dose dependent effect of gum Arabic. In a study conducted to explore the lipid lowering effect of water soluble plants fibers, consumption of GA at a dose of 15 g/day for 4 weeks by healthy men had no significant effect on plasma lipids (Haskell *et al.*, 1992). Topping *et al.*, (1985) has shown that plasma triacylglycerols were significantly lower in rats fed gum Arabic than in controls. Abd-Razig and his colleagues (2010) reported significant decrease in triglycerides and cholesterol in serum and egg yolk in laying hens associated with increase in dose of gum Arabic.

A similar pattern for cholesterol was observed in the control and PG1, where significant ($P<0.05$) increase was observed during fasting; while the significant increase was only observed at the fourth day of fasting in the PG2. The effects of GA on lipid metabolism are variable. Feeding rats with 5 per cent GA has significantly decreased the

absorption of dietary cholesterol by 17 per cent (Kelly and Tsai, 1978). The results obtained by Ross *et al.,* (1983) and Sharma (1986) who reported reduction of total serum cholesterol by 6 and 10.4 per cent, when subjects received 25 g/day and 30 g/day of GA for a period of 30 and 21 days, respectively. Here although the reduction in cholesterol was only in PG2, the delay in cholesterol increase might indicate a dose dependent and/or duration effect.

Mee and Gee (1997) reported a significant 10 per cent reduction in serum cholesterol and a significant 14 per cent reduction in LDL-cholesterol concentrations with the mixture of apple fibre and acacia gum (10 g/day, approximately half apple fibre and half acacia gum) in a crossover study in 27 men

Urea concentration increased significantly ($P<0.05$) in the three groups during fasting period; here it is worth of mention that the level of urea in PG2 during the fasting period was lower than in the other two groups. Plasma urea N concentrations were increased in horses during 4 days and 21 to 69 h of feed deprivation (Patterson *et al.,* 1985; and Sticker *et al.*, 1995). This rise in plasma urea N is presumably related to increased catabolism of body protein stores for use as energy and the production of plasma proteins (Guyton, 1991). Others have reported increases in plasma urea N during 5 and 7 d in cattle (Blum and Kunz, 1981, and Blum *et al.*, 1981). Ali and co-workers (2004) reported that GA alleviates adverse effects of chronic renal failure in human patients consuming GA at doses of 50 g/ day for 3 months. Serum urea, creatinine and uric acid levels have significantly decreased in the groups of gum users. In the current study the duration of treatment was seven successive days that is to be considered shorter than that of Ali and his colleagues (2004).

The fluctuation in creatinine and albumin concentration did not show any significant difference following either administration of gum Arabic or five days fasting. The no significant decrease in glucose level in donkeys treated with gum Arabic was supported by the previous reports of Wadood *et al*., (1989) and Nasir *et al*., (2010).

Significant increase in total protein concentration was observed in the first prophylactic group (PG1) during fasting, an observation that could be attributed to haemo-concentration as animals deny drinking water during starvation. The level returned to non-significant change by the end of the experiment.

The significant increase in ALT and AST activity observed in the current study could be attributed to the increased breakdown of proteins to provide energy, and also reflects the increase in urea level.

Cells contain different types of aminotransferases. Many are specific for α-ketoglutarate as the amino group acceptor but differ in their specificity for the L-amino acid. The enzymes are named for the amino group donor (alanine aminotransferase, aspartate aminotransferase, for example). ALT and AST are important in the diagnosis of heart and liver damage caused by heart attack, drug toxicity, or infection.

Alanine also plays a special role in transporting amino groups to the liver in a nontoxic form, via a pathway called the glucose-alanine cycle. In muscle and certain other tissues that degrade amino acids for fuel, amino groups are collected in the form of glutamate by transamination. Glutamate can be converted to glutamine for transport to the liver, as described above, or it can transfer its α-amino group to pyruvate, a readily available product of muscle glycolysis, by the action of alanine aminotransferase. The alanine so formed passes into the blood and travels to the liver. In the cytosol of hepatocytes, alanine aminotransferase transfers the amino group from alanine to α-

ketoglutarate, forming pyruvate and glutamate. Glutamate can then enter mitochondria, where the glutamate dehydrogenase reaction releases $NH4^+$, or can undergo transamination with oxaloacetate to form aspartate, another nitrogen donor in urea synthesis.

Alanine aminotransferase (ALT) catalyzes the reversible transamination of L-alanine and 2-oxoglutarate to pyruvate and L-glutamate. ALT, along with other transaminases, plays a role in amino acid catabolism and interorgan nitrogen transport (Duncan, 1994).

Chapter five

Conclusion and Recommendations

5.1 Conclusions

It is to be concluded that the use of gum Arabic at 25 g/day in donkeys with experimentally induced hyperlipidaemia resulted in significant decrease in triglycerides level, while the use of GA at 50g/day had no significant effect on triglycerides level. The concurrent decrease in urea and creatinine level may indicate a further effect in the kidney functions.

In the other hand, use of gum Arabic as prophylactic measurement against hyperlipaemia proved to be of value with a dose rate of 50 g/day for seven successive days. In the current study gum Arabic was used for a short duration of time compared to relevant studies

5.2 Recommendations

According to results obtained we may recommend the following concerning hyperlipaemia in equine:

1. Gum Arabic is of value in controlling hyperlipaemia and associated azotaemia.
2. Controlled study using donkeys with natural hyperlipaemia is recommended to evaluate the same and/or other doses to control the condition.
3. Formulation of gum Arabic in drip forms for intravenous infusion could be of value in controlling hyperlipaemia.
4. Other hypothesized lipid lowering agents: chromium, L-carnitine could also be evaluated in controlled experiments.

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