CARNOSINE: A POTENT MODULATOR FOR DIFFERENT DISEASES

Maha Z. Rizk, Hanan F. Aly* and Afaf K. El-Ansary

Therapeutic Chemistry Department, National Research Center, Cairo, Egypt.

CONTENTS

Abstract -------------------------------------------------------------3094
1. Carnosine overview-----------------------------------------------3094
2. Physiological aspects of carnosine-------------------------------3094
2. 1. Biosafety of carnosine administration------------------------3094
2. 2. Carnosine during physiological stress------------------------3095
3. Carnosine and pathological stresses ----------------------------3096
3. 1. Carnosine and toxicity---------------------------------------3096
3. 1.1. Carnosine and compound 48/80 lethal toxicity-------------3096
3. 1. 2. Carnosine and alcohol intoxication------------------------3097
3. 1.3. Carnosine and Monosodium glutamate (MSG) toxicity ------3098
3. 1. 4. Carnosine and gentamicin nephrotoxicity-----------------3099
4. Carnosine and infectious diseases------------------------------3100
4. 1. Carnosine and bacterial infection-----------------------------3100
4. 1. 1. Carnosine and nonspecific bacterial infection----------3100
4. 1. 2. Carnosine and specific bacterial infection---------------3100
4. 2. Carnosine and parasitic infection-----------------------------3101
4. 2. 1. Carnosine and schistosomiasis-----------------------------3101
4. 2. 2. Carnosine and fascioliasis-------------------------------3104
4. 2. 3. Carnosine and trichinellosis-----------------------------3104
5. Possible role of L-carnosine in regulation of blood glucose-3107
5. 1. control of hyperglycemia------------------------------------3107
5.2. Carnosine and its effect on appetite--------------------------3108
6. Role of carnosine in cholesterol abnormalities----------------3108
6. 1. Role of carnosine in hypercholesterolemia-------------------3108
6. 2. Carnosine and hypercholesterolemic cataract----------------3111
7. Carnosine and Cardiovascular diseases--------------------------3112
7. 1. Carnosine and blood pressure (BP)---------------------------3112
7. 2. Cardiac effect of carnosine-------------------------------3113
7. 3. Carnosine and renal vascular stress--------------------------3114
7. 4. Effects of carnosine on brain ischemia----------------------3114
8. Carnosine and teratogenesis-----------------------------------3115
9. Effect of carnosine on partial hepatectomy (PH)----------------3116
10. Carnosine and diabetes----------------------------------------3117
11. Carnosine and Alzheimer’s disease-----------------------------3119
12. Carnosine ameliorates stress-induced glucose metabolism
ABSTRACT
L-Carnosine ((3-alanyl-L-histidine) is an active physiological dipeptide that is distributed naturally in several human tissues, especially, the skeletal muscle, cardiac muscle, nervous tissue and brain. The dipeptide is a potent hydrophilic antioxidant, preventing oxidative damage of membrane lipids and proteins under oxidative stress. Carnosine is also an antiglycating antinflammatory agent, pH buffer and heavy metal chelator. Further, it can prevent aldehyde adducts and nucleic acid oxidation. In addition, carnosine is reported to be involved in natural system of body immune response. One of the most important developments regarding carnosine is its ability to prevent and cure cataract, glaucoma and other age related eye. Carnosine also, has a role in regulation of blood glucose and neuro-protective function. Moreover, carnosine was exploited for its antiparasitic activity against schistosomiasis and fascioliasis.

Key words: Carnosine, antioxidant, oxidative stress, biological activities.

1. Carnosine overview
L-Carnosine ((3-alanyl-L-histidine) is an active physiological dipeptide that is distributed naturally in several human tissues, especially, the skeletal muscle, cardiac muscle, nervous tissue and brain (Gariballa and Sinclair, 2000). The dipeptide is a potent hydrophilic antioxidant, preventing oxidative damage of membrane lipids and proteins under oxidative stress; for example, after using monosodium glutamate as a flavour ingredient in food after alcohol intoxication (Zieba and Wagrowaska, 2003), or by using gentamicin (GM) which is an antibiotic clinically limited by its nephrotoxicity (Soliman and Abdel Monem, 2001; Sorokina et al., 2003). Carnosine is also an antiglycating antinflammatory agent, pH buffer and heavy metal chelator (Boldyrev et al., 1999). Further, it can prevent aldehyde adducts (Hipkiss et al., 2002) and nucleic acid oxidation (Boldyrev et al., 1999) and. In addition, carnosine is reported to be involved in natural system of body immune response (Suzuki et al., 2001).

One of the most important developments regarding carnosine is its ability to prevent and cure cataract, glaucoma and other age related eye (Babizhayev et al., 2000). Furthermore, Nagai et al. (2003) and Yamano et al. (2008) reported the possible role of L-carnosine in regulation of blood glucose while Cheng et al. (2002) indicated the neuro-protective function of carnosine. In addition, carnosine was exploited for its antiparasitic activity against schistosomiasis (Soliman et al., 2000) and Trichinella spiralis and fascioliasis (Soliman et al., 2002). In a related study, Soliman et al. (2007) revealed the promoting effect of canosine on partially hepatectomized mice.

2. Physiological aspects of carnosine
2.1. Biosafety of carnosine administration
The biosafety of L-carnosine (LD50) was recorded to be 18.5g/kg body weight (Soliman et al., 2002).
2. Carnosine during physiological stress

Early studies have demonstrated a link between carnosine, free histidine and histamine synthesis following several types of physiological stresses (Flancbaum et al., 1999). It is proposed that carnosine serves as a non-mast cell reservoir for histidine which becomes available for histamine synth (Fitzpatrick et al., 1980).

Conditions that cause endoplasmic reticulum mal function (ER stress) play a key role in the development of various human diseases including neurodegenerative diseases. Carnosine is an endogenous peptide, present in excitable tissues such as brain and skeletal muscle. Although there are reports suggesting that carnosine has a biological role independent of its antioxidant activity, there have been no reports of the effects of carnosine on the ER stress responses is during periods of stress. It was found that, carnosine almost completely inhibits 6-OHDA-induced ER stress responses and cytotoxicity, and that slight antioxidant activity of carnosine against 6-hydroxydopamine( 6-OHDA) is observed (Yavarj and Subramoniam, 2009).

Although the physiological role of carnosine has not been completely understood yet, many beneficial actions have been attributed to carnosine, such as being an antioxidant, antiglycating and ion-chelating agent, a wound healing promoter and a free-radical scavenger. The role of carnosine in the neuroprotection of oxidative stress-driven disorders has been reviewed. The effects of carnosine have been extensively studied both in vivo and in vitro models of cerebral damages, such as neurodegenerative disorders, hypoxia–ischemia injuries and hypoxia-reoxygenation damage. Beside the classical sacrificial agent, carnosine has been reevaluated as a molecular chaperon and an inducer of antioxidant systems in oxidative stress conditions. Thus, beneficial effects on most of the common biochemical events that characterize neurological disorders make carnosine a very promising molecule among all the endogenous compounds in the treatment and/or prevention of oxidative driven diseases (Fouad et al., 2007 and Bellia et al., 2011) . Urgent need exists for new therapeutic options in ischemic stroke. It was demonstrated that carnosine, an endogenous dipeptide consisting of alanine and histidine, is robustly neuroprotective in ischemic brain injury and has a wide clinically relevant therapeutic time window. The precise mechanistic pathways that mediate this neuroprotective effect are not known. Following in vivo administration, carnosine is hydrolyzed into histidine, a precursor of histamine. It has been hypothesized that carnosine may exert its neuroprotective activities through the histidine/histamine pathway (Bellia et al., 2011).

Carnosine's possible biological activities include scavenger of reactive oxygen species (ROS) and reactive nitrogen species (RNS), chelator of zinc and copper ions, and antiglycating and anticros-linking activities. Carnosine's ability to react with deleterious aldehydes such as malondialdehyde, methylglyoxal, hydroxynonenal, and acetaldehyde may also contribute to its protective functions. Physiologically carnosine may help to suppress some secondary complications of diabetes, and the deleterious consequences of ischemic–reperfusion injury, most likely due to antioxidation and carbonyl-scavenging functions. Other, and much more speculative, possible functions of carnosine considered include transglutaminase inhibition, stimulation of proteolysis mediated via effects on proteasome activity or induction of protease and stress-protein gene expression, upregulation of corticosteroid synthesis, simulation of protein repair, and effects on ADP-ribose metabolism associated with sirtuin and poly-ADP-ribose polymerase (PARP) activities. Evidence for carnosine's possible protective action against secondary diabetic complications, neurodegeneration, cancer, and other age-related pathologies is ascertained (Hipkiss, 2009). Pretreatment with carnosine significantly reduced the infarct volume and the number of terminal-deoxynucleotidyl
transferase-mediated dUTP nick end labeling (TUNEL)-positive cells in the hypoxia–ischemia brain. Carnosine also inhibited mRNA expression of apoptosis-inducing factor (AIF) and caspase-3, which was accompanied by an increase in superoxide dismutase (SOD) activity and a decrease in the malondialdehyde (MDA) level in carnosine-treated rats. Furthermore, carnosine also improved the spatial learning and memory abilities of rats declined due to hypoxia–ischemia. These results demonstrate that carnosine can protect rats against hypoxia–ischemia-induced brain damage by antioxidation (Zhang et al., 2011).

In addition, carnosine can be considered as a potential candidate to protect the liver against the deleterious effect of acute cadmium intoxication (Fouad et al., 2009). Ageing is characterized by a wide variety of physiological changes and, as a consequence, an anti-ageing compound must fulfill a wide variety of roles to be effective. Carnosine is an antioxidant, antiglycating and neuroprotective compound with well-studied clinical benefits. It is becoming a clinically accepted nutritional supplement with uses across a considerable spectrum of chronic diseases, from senile cataract to dementia (Kyriazis, 2010).

3. Carnosine and pathological stresses
3.1 . Carnosine and toxicity
3.1.1 . Carnosine and compound 48/80 lethal toxicity
Several studies showed that administration of compound 48/80 to rats induces lethal stress which was associated with mobilization of myocardial carnosine to histamine (Flanbaum et al., 1990). The histamine release causing death was attributed to production of an anaphylactoid reaction characterized by hypotension, bronchospasm, decreased intravascular volume, diminished venous return and reduced cardiac output which resembles surgical or traumatic shock (Douglas, 1995). Soliman et al. (2002) showed that carnosine produced dose-related protective effect against compound 48/80-induced lethal stress. The authors reported that carnosine might have produced its effect by an action at the level of histamine action on cardiovascular response induced by the compound. Thus, carnosine might have inhibited 48/80-induced histamine release from mast cells either directly or by forming histamine to act on H2-receptors. They concluded that carnosine attenuating the deleterious effect of compound 48/80 protecting rats against its lethal shock that contributed to the same role of the dipeptide in the physiological response to stresses.

Furthermore, carnosine treatment significantly reduced blood urea nitrogen and serum creatinine levels elevated by cisplatin administration. Also, carnosine significantly attenuated cisplatin-induced increase in malondialdehyde and decrease in reduced glutathione, and catalase and superoxide dismutase activities in renal cortical homogenates. Additionally, histopathological examination and scoring showed that carnosine markedly ameliorated cisplatin-induced renal tubular necrosis. In conclusion, carnosine can be considered a feasible candidate to protect against nephrotoxicity commonly encountered with cisplatin treatment (Fouad et al., 2009)

The accumulation of malondialdehyde (MDA), a lipid peroxidation by-product that has been used as an indicator of cellular oxidation status, is significantly increased in many neurological diseases such as brain ischemia/reperfusion, Alzheimer's disease and Parkinson's disease in vivo. It was found that MDA treatment in vitro reduced cortical neuronal viability in a time- and dose-dependent manner and induced cellular apoptosis as well as necrosis simultaneously. Furthermore, exposure to MDA led to accumulation of intracellular reactive oxygen species, dysfunction of mitochondria (denoted by the loss of mitochondrial transmembrane potential (ΔΨm)) and activation of JNK and ERK. Carnosine exhibited better
protection against MDA-induced cell injury than antioxidant N-acetyl-cysteine (NAC) with its multi-potency, which alleviated MDA-induced protein cross-linking, Δψm decrease, reactive oxygen species burst, and JNK and ERK activation. Thus, it was suggested that MDA induced cell injury in vitro via protein cross-linking and successive mitochondrial dysfunction, and the activation of reactive oxygen species-dependent MAPK signaling pathway. Carnosine alleviated all these alterations induced by MDA, but NAC merely inhibited Bcl-2 family-related activation of JNK and ERK. These results prompt the possibility that carnosine, but not other conventional antioxidants, can protect neurons against MDA-induced injury through decomposition of protein cross-linking toxicity and may serve as a novel agent in the treatment of neurodegenerative diseases (Cheng et al., 2011).

3.1.2. Carnosine and alcohol intoxication
Chronic alcoholism produces a wide spectrum of stomach, liver and other organ diseases depending on the amount and duration of alcohol intake. Protein deficiency and enzyme activity depression were proved to be associated with chronic alcoholic liver disease (Ozaras et al., 2003). The toxicity of ethanol was mainly attributed to disturbed biochemical interactions of the two ethanol oxidizing enzymes, ADH and cytochrome P450 isoenzymes specially CYP2E1, both involved in ethanol oxidation producing acetaldehyde and ROS (Lieber, 2000 and Roy Chengappa et al., 2012). These products were further proved, in addition to lipid peroxidation, to lead to deleterious modifications of both structural and functional protein molecules by non-specific effect (Niemela, 2001 and Addolorato et al., 2006). Functional protein modifications were reflected as disturbances of many enzyme systems. Depression of both glycolytic and CAC. Pathways were reported by Addolorato et al. (2006). Lowered MDH activity aggravating shift of the oxidoreductive equilibrium of malate/oxaloacetate causing inhibition of CAC was also presented (Volpi et al., 2002). Further, alteration of the hepatocytes redox state, depression of the respiratory chain and limitation of ATP availability were stated in alcoholic intoxication (Soliman et al., 2003). Protein deficiency in alcoholism has been considered for decades to be associated with chronic liver disease characterized by a fall in serum albumin & loss of muscle mass. Studies in this concern have shown that alcoholism caused significant increase in serum ALP, AST and ALT and bilirubin (Soliman et al., 2003).

The effect of carnosine on individual enzymes of different organs in alcohol intoxication was studied by many researchers. Carnosine antioxidant protective effect on the parenchymatous gastric glands in chronic alcoholism was proved (Choi et al., 2009). Fontana et al. (2002) added that carnosine protected pancreatic α-antitrypsin against injurious fate. Similar effects of carnosine on different liver enzymes were reported (Soliman et al., 2004). Carnosine activating transcription could be a further potentiation of carnosine promoting effects on serum proteins (Chen et al., 2002).

Soliman and Mohamed (2004) studied the effect of combination of carnosine before, with or after alcohol administration on certain hepatic biochemical factors. ADH, hexokinase (HK) phosphofructokinase (PFK), MDH, and creatine phosphokinase (CPK) activities were investigated in animal liver tissue (Soliman et al., 2003). The authors deduced that, apart from ADH, for which ethanol is the substrate, all enzymatic activity insults were ameliorated by carnosine treatment with the best results in animal groups receiving the dipeptide at the same time with ethanol. Soliman and Mohamed (2004) further studied the action of carnosine on the glycolytic enzyme LDH & its isoenzymes, CAC enzymes, glycogen content, the glycogenolytic metabolic machineries; glycogen phosphorylase, G-6-Pase, the hydrolytic enzymes; acid & alkaline phosphatase, the amino acid metabolic enzymes; AST & ALT and the nucleic acids
catabolic enzyme 5'-nucleotidase. The authors confirmed the toxic effect of ethanol inducing an oxidative stress on liver tissue and revealed the corrective action of carnosine on these studied parameters. In a related study, Soliman and Mohamed (2004) deduced the effect of L-carnosine on hepatic tissue lipid peroxides (LPO) and both intrinsic and extrinsic antioxidant factors namely, catalase activity, glutathione as well as vitamins C and E after ethanol intoxication. They found that LPO and vitamin C were increased while GSH, vitamin E & catalase were reduced by administration of alcohol. Carnosine relieved enzyme activities from the toxic action of ethanol, improved the levels of antioxidants and decreased LPO. This found support in its membrane protecting activity (Nagasue et al., 1987 and Huang et al., 2005) due to carnosine potent biological antioxidant effect (Chen et al., 2002).

This corrective action of carnosine could be attributed to its reported multibeneficial effects under different stressful conditions similar to its ability to protect glycolytic enzymes in anoxia and hyperthermia (Deev et al., 1997). This was explained by carnosine stimulating glycolysis, protecting protein modification mediated by peroxyl radicals generated by ethanol toxicity (King and Mahmoud, 1999). Le Blanc and Soucy (1994) previously recorded significant increase in serum albumin after carnosine administration. Carnosine activating transcription could be a further potentiation of carnosine promoting effects on serum proteins (Chen et al., 2002). Silaeva et al. (1992) showed that carnosine also increased mitotic activity in hepatocytes and accelerated liver recovery from toxic methanol hepatitis in rats. In addition carnosine could play a role in delaying apoptosis by decreasing protein denaturation as well as in participating in the disposal of glycated protein in the affected tissue (Yeargans and Seidler, 2003).

The dipeptide could also combine with harmful active acetaldehyde thus preventing its combination with protein carbonyl groups (Hipkiss, 2009). Silaeva et al. (1992) showed that carnosine also increased mitotic activity in hepatocytes and had accelerated liver recovery from toxic methanol hepatitis in rats.

It was found that, polaprezinc (PZ), which consists of l-carnosine and zinc, is widely used to treat gastric ulcers. In a rat model of ethanol-induced gastric mucosal damage, polaprezinc administration ameliorated ethanol-induced mucosal injury and showed protective effects on the mucosa by reducing the levels of inflammatory cytokines and increasing the expression of antioxidant enzymes and growth factors. Furthermore, PZ showed cytoprotective effects by increasing the heat shock proteins (HSP) levels (Choi et al., 2001).

3. 1. 3. Carnosine and Monosodium glutamate (MSG) toxicity
Monosodium glutamate (MSG) is one of the main flavour enhancers used as an ingredient of various food products. Recently MSG toxicity has gained a lot of interest because of its association with Chinese restaurant syndrome in human. It was further reported to induce oxidative stress (Ahluwalia et al., 1996).

Soliman et al. (2003) studied the protective effect of carnosine against MSG toxicity in rats by evaluating the effect of the drug on some biochemical and ultrastructural parameters. Their study revealed that MSG caused increased LPO and superoxide dismutase (SOD) enzyme activity, lowered activities of glutathione peroxidase (GPX) and glutathione reductase (GRX) and decreased glutathione (GSH) concentration. The activities of both GRX & GPX were significantly increased with increase in GSH content. These deteriorations resulted from MSG inducing excess liberation of reactive oxygen species (ROS) emphasizing its toxic action and inhibition of the antioxidant defense system (Shaheen et al., 2000). The increased SOD
activity after MSG could be a compensatory response to oxidative stress causing accumulation of its reaction product H$_2$O$_2$. The latter would thus be involved in exhaustion of GPX and GRX (Shaheen et al., 2000). Furthermore, histochemically, MSG causes increased collagen deposition, numerous big vacuoles, and dilatation of endoplasmic reticulum. In support, frequent association of the pathogenesis of tissue fibrosis with enhanced lipid peroxidation and damaged antioxidant defense system was reported (Poli and Parola, 1997).

Carnosine given with MSG was effective in protecting all biochemical machineries. There was a sharp decrease in LPO and SOD activity. The increased GSH content and its redox-cycling enzymes (GPX and GRX) might provide the liver with greater protection from excess ROS liberation and actions. The micrographic picture of the liver showing that carnosine administrated with MSG efficiently minimizes the number & size of vacuoles, disappearance of collagen fibrils, and prevention of increased fibrinogenesis. This could reflect both normal structure and metabolic reactions of hepatic cells.

Its beneficial antioxidant potency against MSG toxicity indicated that the rate of free radical production was decreased in carnosine protected rats than in those receiving MSG alone.

3.1.4. Carnosine and gentamicin nephrotoxicity

Gentamycin (GM) is an antibiotic whose clinical use is limited by its nephrotoxicity. GM produced functional failure in rats shown as increased blood creatinine and urea. Histopathological, ultrastructure and enzyme histochemical studies proved this failure. Specifically, the excessive insult of the proximal tubules could result from being the primary site of drug accumulation (Maldonado et al., 2003). It was found that O$_2^\cdot$, H$_2$O$_2$ and hydroxyl radicals were increased with GM treatment (Martinez-Salgado et al., 2002,2007).

Experimentally induced oxidative stress was shown to produce tubular damage (Cuzzocrea et al., 1998). GM, structurally disturbing cellular mitochondrial membranes could lead to alteration in most enzymes of oxidative phosphorylation that caused suppression of metabolic activity and reduction of ATP production (Cohn et al., 2004). Madden et al. (2000) also reported that GM induced apoptosis in the renal proximal tubular cells. Soliman et al. (2007) showed by histopathological and ultrastructural examinations that GM- nephrotoxicity presented apoptotic reaction. Their histochemical results showed that sections of GM injected animals, reflected activation of LDH (Morel and Friday, 1994 and Soliman et al., 2005) and inhibition of SDH, ATPase, ACP and ALP (Soliman et al., 2005), that GM decreased ATP and SDH was also previously recorded in chronic liver insult (Soliman et al., 2002).

Varalakshmi et al. (2008) attributed ACP decrease to cellular degeneration & necrosis of renal tubules. The authors added that, GM inhibition of ALP was explained by binding to anionic phospholipids present on the brush-border membrane.

However, Soliman et al. (2007) showed that carnosine presented great modulating actions, massively correcting the damaged renal histological elements, the three enzymes LDH, SDH and ATPase. The of carnosine on the three energy machinery related enzymes reflected equal hypercorrections that could help more energy production for utilization in defending GM damage. In support, carnosine proved to cause increased liberation of ATP in cardiac and skeletal muscle (Millar and Rice-Evan, 1997) and in the liver (Churchill et al., 1995). Soliman et al. (2003 and 2004) also reported the correctively stimulatory action of carnosine on CAC enzymes. The variabilities of protective actions of carnosine that lead to the favourable normalization of both renal function and structure could be related to selectivity of carnosine actions (Soliman et al., 2005).
4. Carnosine and infections diseases

4.1. Carnosine and bacterial infection

4.1.1. Carnosine and nonspecific bacterial infection

For both types of bacterial non-specific infection, Fitzpatrick et al. (1980) recorded that in acute infection reduction in leg muscle carnosine much lagged behind that in chronic infection amounting to 25 and 95% respectively. This exhaustion of carnosine in the chronic type clearly pointed to a more role of the dipeptide in chronic infections than in acute ones. Stadnikov et al. (2000), demonstrated the suppressive effect of carnosine with respect to the pathogen and its positive influence on the processes of the regeneration of eukaryotic tissues. In this concerns, Kashimura et al. (1999) and Suzuki et al. (2001) found that, polaprezinc inhibited Helicobter pylori-associated gastric mucosal oxidative inflammation, including initial micro-vascular leucocyte activation, in Mongolian gerbils.

Babizhayev and Deyev (2012) and Babizhayev et al. (2013), reported that histidine-containing compounds can modulate the Influenza virus release from neutrophils and reduce virus dissemination through the body of the organism. This review points the ability of therapeutic control of Influenza viral infections associated with modulation by oral non-hydrolized forms of carnosine and related histidine-containing compounds of Polymorphonuclear neutrophils (PMN) apoptosis which may be involved at least in part in the pathophysiology of the disease in animals and humans. Thus, these findings may have implications for global influenza surveillance and planning for pandemic influenza therapeutic prevention with oral forms of non-hydrolized natural L-carnosine as a suitable alternative to the conventional vaccination for various flu ailments.

4.1.2. Carnosine and specific bacterial infection

The above mentioned effect of carnosine on chronic non specific infection research matched the series of work investigating one type of specific bacterial infection, namely tuberculosis. Carnosine in-vivo action proved effective for both liver and lung affection in guinea pigs, even more than the traditional streptomycine-isoniazid (INH) combination therapy. Although streptomycine-INH treated animals showed normal spleen and liver, yet the exacerbation of tuberculous lesions in the lung tissue was a major drawback (Atabai and Matthay, 2002). The authors recorded that carnosine treatment caused microscopic abatement of tuberculosis in 6 out of 8 animals. These two animals still presented mild occasional hepatic regressive tuberculous granulomata. The regression was accompanied by signs of increased immunity; presence of epithelioid cells, few binucleated cells and also infiltration with macrophages.

However, a favourable non tuberculous manifestation in all the eight-carnosine treated animals was the smooth liver enlargement. The increase of liver weight mounted to 80 to 100%. Microscopically, this enlargement proved to be due to ubiquitous intracellular increased glycogen storage. The excess glycogen however, was shown to be hydrolysable as the liver regained the normal size and weight after a night fast and microscopic examination showed abatement of cellular glycogen engorgement (Atabai and Matthay, 2002).

Comparing this carnosine in-vivo antituberculous effect with similar researches on other imidazole compounds; histidine (Abdel Kader et al., 1977), histamine (Abdel Kader et al., 1979) and the mother imidazole ring itself (Abdel Kader et al., 1993). Although all showed marked antituberculous action yet, carnosine proved to be the most effective. The drawback of imidazole treated group was the presence of some remnants of tuberculous lymphocytic follicles (Abdel Kader et al., 1993). The drawback of histidine treatment were areas of hepatic central atrophy (++) and few scattered pulmonary tubercles limited by thick fibrous
tissue and immature new alveoli (Abdel Kader et al., 1977). The drawback of histamine treatment was the presence of moderate tuberculous insults in spleen, liver and lung (Abdel Kader et al., 1977). Further no increased hepatic glycogen was observed with any of the other tested compound other than carnosine.

4. 2. Carnosine and parasitic infection
Although, the above mentioned carnosine effects on chronic bacterial infections (both non-specific and specific) were earlier studied yet, researches on other chronic infections (parasitic) were delayed to 2001.

4. 2. 1. Carnosine and schistosomiasis
The effect of carnosine administration on Schistosoma mansoni (S. mansoni) infected hamsters, was studied by Soliman and Abdel Monem (2001). Liver examination showed that the dipeptide effectively reduced worm burden and egg count. The authors observed that the earlier the treatment by carnosine, the more diminished the ability of both male and female worms to couple (as extracted by liver perfusion). This explained the relative diminished number of fertilized eggs. The authors concluded that this uncoupling effect could be one of the carnosine mechanisms combating infection. They also recorded that carnosine normalized the liver tissue antioxidant status indicated by correction of MDA concentration, adenylate energy charges (AEC) and glycogen content.

Further, the ability of carnosine to improve the disturbed serum liver parameters also induced by S. mansoni parasite in hamsters was tested by Soliman et al. (2002). The authors indicated that infestation induced depletion of liver glycogen and lowered blood glucose concentration. This low glucose in Schistosoma infested hamsters and the diminished glycogen content of hepatocytes were also previously found by Skelly et al. (1998). The decrease of both carbohydrate parameters was attributed to two different mechanisms by the host as wellas by the parasite; the host inflammatory response exhausting blood glucose & hepatocyte glycogen (Soliman and Abdel Monem, 2001) and the adult schistosomes depending on host blood glucose absorbed across their outer body tegument (Skelly and Shoemaker, 2000). Soliman et al. (2002) also reported disturbed levels of liver function tests. There were increased activities of serum AST, ALP and gamma-glutamyl transferases. Infestation also increased serum procollagen III peptide (PIMP). PIMP was stated as a marker of liver fibrosis, induced in schistosoma parasitized subjects (Modha et al., 1998). PIMP was further recorded as as the most relevant factor in assessing severity of liver injury in chronic hepatic inflammations (Mahmoud et al., 2001).

Administration of carnosine either concurrent with, 2 and 4 weeks post infection was effective in reducing differential worm burden (Soliman et al., 2001), in improving blood glucose level (only when administered 2 and 4 weeks after infestation), reducing serum PIMP level and correcting all tested enzyme activities except gamma-glutamyl transferase (Soliman et al., 2002). Histopathological sections showed abatement of schistosomal infection; diminution in number and size of granulomata, destruction of schistosoma ova in the granuloma, with, diminution of its fibrotic a collagen content, accompanied by evidence of enhancement of the host immune response evidenced by increased histocytic and lymphocytic content and a lymphocytic cuff surrounding the granulomta. However, slight degree of increased portal fibrosis was noticed in the late-treated group. These actions support supplementation of schistosomiasis therapy by carnosine, being a chronic inflammatory disease (Soliman et al., 2002).
The enhancement of immune response was further proved by studying the effect of carnosine on individual Schistosoma mansoni antigens; SEA (soluble egg antigen), CAP (cercarial antigen preparation) and SWAP (soluble worm antigen) was studied in rabbit (Soliman et al., 2003). Carnosine treatment caused variable individual modifications of serum immunoelectrophoretic pattern in response to each of the three tested Ags. The patterns of SWAP, CAP and SEA presented disappearance of two, one and no band respectively in comparison to untreated group. This variable action of carnosine treatment could be explained by the dipeptide reported function as a universal buffer. Carnosine involved in body defense machineries logically reacts according to two biological evolutionary factors governing body response. Here, they are exposure to the stress agent and time. Concerning direct exposure, it is obvious that mammals, on natural infection, are in direct contact with the body of both cercaria, then the worm. The egg contents, on the other hand, being covered by its shell does not directly come in contact with the host tissues. Thus, the body and consequently its general defense molecule, carnosine, do not elicit any immunological response to SEA. The time of exposure to the cercaria is shorter than that of the adult worm; being the final stage chronic inhabitant in the liver. Hence, the immunological response to SWAP is double that of CAP i.e. disappearance of two bands in comparison to one respectively.

The reduction in number of detectable antigens as recognized with anti-sera of rabbits treated with carnosine could confirm the role of carnosine in modulating immune response to the antigenicity of the two stages of S. mansoni antigens, CAP and SWAP. The modulating action of carnosine on the natural immune resistance was previously studied (Nagi and Suda, 1988 and Silaeva et al., 1992). Carnosine was proved to have the ability to bind to macrophage and lymphocyte receptors stimulating their synthetic and secretory abilities and liberating immune modulator intermediates; cytokines and interleukins (Suzuki et al., 2001). Antigen were tested as vaccines against the invading larvae to prevent or reduce infection, but only little protection has been achieved (Attalah et al., 1999, Qui-Lishue et al., 1999 and Bethony et al., 2006).

Histopathological examination showed that increased response in the SEA animals could be indicated by the marked increased lymphoid follicle number and size in both the spleen and lymph nodes that could be supported by the persistence of all Ag-Ab bands. On the other hand, the marked decrease of lymphoid follicle number and size as well as the disappearance of one Ag-Ab band in CAP groups presented a decreased immunological reaction by carnosine. In SWAP animals, the increased sinus histocytosis and the disappearance of the two protein bands might be due to the phagocytic action of histiocytes (Golasby et al., 2001) engulfing the Ag-Ab complex molecules. These findings could be interpreted as being proportionate to the importance of the host-pathological reaction and clinical benefit.

The short period of the cercarial stage in the final host presenting the primary insult might explain the action of carnosine (Muller, 2001). These changes in the three I-C groups could be a reasonable involvement of carnosine in the actual natural sequence of biological events. The possible mechanisms by which carnosine regulates these action was previously supported by several authors demonstrating the ability of carnosine to improve both liver histopathological and some biochemical disorders induced by S. mansoni (Soliman et al., 2002 and Diazgroundosca et al., 2003). In addition carnosine has the ability to bind to macrophage and lymphocyte receptors stimulating their synthetic and secretory abilities, which may eventually lead to the activation of the natural systems of body immune resistance (Silaeva et al., 1992). It induces liberation of immune modulator intermediates, cytokine and interleukin (Shimida et al., 1999, Suzuki et al., 2001 and Hsien et al., 2002). Carnosine
demonstrates a protective effect on T-cell that is attributed to diminished oxidative DNA damage (Hyland et al., 2000). It is also effective in diminishing apoptotic cell death (Boldyrev et al., 1999). Carnosine modulation of immune response and decreasing apoptosis could, in part, be due to its antioxidant property.

The effect of carnosine in relation to the three Schistosoma antigens (SEA, CAP and SWAP) was further extended by Soliman et al. (2005) studying some biochemical parameter changes induced in rabbits by immunization and the influence of carnosine co-administration on these parameters. Namely total protein, glycogen and glycogen phosphorylase b in liver & also some serum protein fractions with varying molecular weights the authors deduced that CAP and SWAP have energy suppressive abilities while the passive effect of SEA might reflect the difference of native biological activities between the three parasite stages, cercariae & worms on one side and egg on the other towards energy metabolism of the animal host. This might be explained by the eggs being metabolically quit, while the cercariae & adult worms do actively complete their hepatic reside (Mullar, 2001).

However, those groups which were co-administered carnosine showed more pronounced changes in the serum protein fractions compared to those immunized with the different antigen only. Although, the dipeptide regained the normal levels of depleted glycogen in S. mansoni infected hamsters (Soliman et al., 2003a). This repletion in glycogen was more remarkable in CAP than in SWAP which might indicate that carnosine was more capable to nullify the strong action of CAP that could possibly be attributed to short life duration of the cercariae in the animal host.

In case of the studied serum protein, SEA and CAP showed induced reduction in most electrophoretic protein fractions while SWAP induced only lowered level of one protein. The decrease of almost all parameters in SEA and CAP opposite to the small number in SAWP might be attributed to the biological interrelations between both parasite & host tissues. The duration of the two developmental stages, eggs & cercariae, being transient inhabitant of host tissues is very short (Mullar, 2001). On the host side, the animal body constituents had evolutionary, long been exposed to worm Ag. Thus carnosine, a natural body component, became unable to compete with such high dosages of egg or cercarial antigens that the dipeptide might have not ever experienced during evolution. Carnosine, then, faced with these unnatural experimental high amounts of either antigen, which it had never acquired the proper ability to manage, was perplexed and unable to normalize the decreased protein parameters. It even caused further numerous decreases (Leinonen et al., 2004).

A special pereculiarity in results of Soliman et al. (2005) was the record of the presence of both favourable increase in glycogen content (antioxidant action) as well as decrease in serum protein concentrations (moderate proxidant effect). However, this difference could be explained by the previously reported selectivity of carnosine in regulating animal body biochemical machinerics in pathological insults (Quinn et al., 1992, Soliman et al., 2003a and soliman and Aly, 2003c).

The mechanisms by which carnosine could cause selective changes are multiple the dipeptide facilitates the removal of deleterious proteins inactivated by forming protein carbonyl carnosine adducts (Hipkiss et al., 2002). Carnosine prevents oxidation and glycation, both of which could contribute to protein cross-linking promoting beta-amyloid plaque formation (Hobart et al., 2004).
4.2.2. Carnosine and fascioliasis
Comparing the antibody (Ab) response to *Fasciola gigantica* in experimentally infected rabbits treated 14 weeks with mirazid or carnosine by using ELISA. Results showed highly significant difference between the numbers of *Fasciola* worms in liver of treated and control rabbits. A reduction of 54.5% in worm burden was detected in the carnosine treated group. However, mirazid treated group showed complete worm eradication in the liver. It is concluded that the natural compound carnosine potentiates the immune response against infection and presented more increased antibody level than mirazid. However, mirazid has anthelmintic effect against *F. gigantica* infestation and carnosine was effective in declining the number of worms. Thus, a combination of both carnosine and mirazid might only be recommended in patients presenting other causes of diminished immunity in addition to *Fasciola* infection (Soliman et al., 2004).

4.2.3. Carnosine and trichinellosis
The ability of carnosine to affect the infectivity as well as certain metabolic disturbances induced by *Trichinella spiralis* (*T. sp.*) infection in rats as compared to the antitrichinellosis drug, abbendazole was studied by Soliman et al. (2007). The search involved investigation of worm and encysted larvae burdens of both intestinal and muscular parasitic phases (after 1 and 6 weeks respectively) as well as serum metabolic parameters. These parameters included; peroxidation/antioxidant parameters; MDA as an index of lipid peroxidation, tissue damage, and the antioxidant factors; vitamin C, GSH and G6PDH activity has an important role in generation of NADPH needed for the maintenance of GSH in its reduced form (Ristof et al., 2001). Activities of other four enzymes CPK (a marker of muscle function), LDH (a marker of both liver and muscle functions) and ALT and AST (markers of liver function) were also estimated in addition to, glucose and total proteins.

Both intestinal worms and muscular encysted larvae phases of *T. sp* invasion showed oxidative damage represented by different degrees of increased MDA levels positively correlated with the intensity of the helminthic infection. This finding matched the report stating increased MDA in parasitic invasion (Grudunski et al., 2003) that was accompanied by proportionate decreases of G6PDH activity, concentrations of GSH and vitamin C. These decreased antioxidant levels were stated to be due to intensification of infestation inducing host phagocytes to generate large amounts of ROS that might exceed the capability of host antioxidants to catabolize them (Sharma and Agarwal, 1996). The deficient antioxidant levels during trichinillosis were stated by other authors as an initial non-specific defense reaction of the host toward parasitic invasion (Derda et al., 2004). An increase in ALT, AST as well as LDH activities were observed during the two phases of trichinellosis. This found support in similar increases in serum of infected patients (Sohn et al., 2000). The increased serum enzymes were attributed to two successive insults affecting the liver. The presence of the parasite within the intestine was stated to primarily elicit an immune response that subsequently affects the liver. Afterwards, newborn larvae merging to circulation, release substances that are hepatotoxic (Wang et al., 2000). The development of large areas of multifocal coagulative necrosis in the liver further documented both statements (Bliss et al., 2003).

That CPK activity (marker of muscle function) increased only later, in the muscular phase of invasion, indicated the arrival of larvae to muscles and coinciding with the muscular disorder (Garacia et al., 2003 and Guillermo et al., 2008). CPK activity insult chronologically lagged 6 weeks beyond that of LDH to increase only in muscular phase. This found a previous support stating that the increment of serum CPK was attributed to oxidative stress causing inflammatory reactions in muscles (Daoud et al., 2000). This could indicate the more
tolerance and defense ability of muscular system to strongly withstand stresses in other organs (liver and intestine). The high increases of serum LDH activity in both infection phases matched deteriorated liver (in both phases) and muscle (in muscular phase) functions.

Both phases of T sp. infection reflected low serum glucose concentration, indicating that infection with this parasite is hypoglyceamia inducer (Nishina and Suzukim, 2002). The lowered blood glucose by parasitic infection could be attributed to three factors: 1-the reduced glucose intestinal absorptive capacity of the insulted intestine (Maden et al. 2004) 2- high glucose consumption by the parasite 3- liver glycogen depletion (Reina et al., 1989 and Bliss et al., 2003). Both disturbances in two tested energy substrate & the energy building enzyme in muscle (CPK) might be a factor explaining the presentation of general easy fatigability of patients infected with helminthic parasites (King and Mahmoud, 1999 and Guillermo et al., 2008).

In general, the deterioration of all tested biochemical parameters, except total protein that presented normal levels in all rat groups, showed more advanced defect in the muscular phase. This could be attributed to longer period of infection, the insult of the huge mammalian muscle bulk as well as to the possible 95% depletion of body carnosine by the chronic infection (Fatzpatric et al., 1980). The great diminution of body carnosine, could deplete the body from an important protective ability.

The pathochemical atiology of Trichinella spiralis invasion of both intestine and muscles was studied. Daoud et al. (2000) and María et al. (2011) stated that infection primarily caused inflammatory responses in both tissues. Both intestinal and muscular stages of T. spiralis and different host cell types of all host tissues, established complex interaction that lead to general immunological, pathological and metabolic disturbances (Mitreva et al., 2004 and Zhao et al., 2013). An additional weakening factor might be due to muscle tissue losing its store of carnosine. The majority of muscle carnosine content (95%) was previously found to be lost in chronic infection (Fatzpatric et al., 1980). This absence of carnosine could be a participating factor in the generalized stress phenomenon of T. spiralis infection.

Results showed that administration of albendazole effectively resulted in 100% cure rate against intestinal worms and muscle larvae. This agrees with the report of several authors that benzimidazoles, including albendazole, effectively eradicated both worms and larvae in early and late stages of T spiralis infection (Chung et al., 2001, Maherani et al., 2012and Siriyastien et al., 2003). However, concerning derangement of metabolic parameters, the peroxidation (MDA) in both invasion stages after treatment with albendazole, remained equal to that of infection-untreated group. The antioxidant status evaluated by vitamin C, GSH and G6PDH proved to remain deteriorated in the intestinal phase similar to infected group. Even GSH activity became more deteriorated. Vitamin C, sill lagging, below normal showed mild increase. In the muscular phase the improvement of the antioxidant status was better than in the intestinal one as indicated by acquiring significant increased differences, in vitamin C and GSH, to that of infected group. CPK, only affected in the muscular phase was mildly improved by the drug. ALT, AST as well as LDH and G6PDH remained deteriorated although milder than the infected animals. However, the activities of these four enzymes were much increased by albendazole treatment than those of infected control, indicating specific hepatic toxic action of the drug, a fact which was further emphasized by other reports stating more deterioration of liver function after treatment with the benzimidazoles drugs (Davis et al., 1989, Reuter et al., 2003 and Mido et al., 2012).
CPK being normal when albendazole was given early in the intestinal phase denoted that muscles are not yet insulted by the parasite larvae. However, when the treatment was given after beginning of muscular phase the improvement was only moderate in spite of complete eradication by elbendazole (Bruschi, and Murrell, 2011).

Serum glucose level showing midway improvement in alendazol animals could be attributed to nullification of glucose utilization by parasite. This action was stated to result through 2 mechanisms; impairment of the intestinal uptake of glucose thereby increasing the parasite glycogen depletion & hampering its ability to form ATP which is used as the energy source by the worms (Bolas-Fernandez et al., 2004). This impairment of worm glucose absorption and utilization by albendazole was further completed by the drug eradicating the parasite. Eradication of the parasite was explained by selective binding of the drug to nematode tubulin, inhibiting the tubulin polymerase, thus impeding cell division. Serum host glucose level was still not normalized and remained in a hypoglycemic level, could feasibly be due to hepatotoxic action of albendazole (Lacy, 1990, Bolas-Fernandez et al., 2004 and Mido et al., 2012).

Oral administration of carnosine either before, concurrent with or after T. spiralis infection effectively reduced the intestinal adult worm burden & completely eradicated muscle larvae, indicating its feasible biocidal activity against trichinellosis. Metabolically the carnosine group showed great amelioration of the damage indicated by a subnormal level of MDA pointing to high antioxidant protective effect of the dipeptide. Carnosine, as well, correctively increased the concentrations of both vitamin C and GSH and G6PDH activity. Carnosine presented more protection of GSH than that of vitamin C and even increased above normal in both the Co-and post infection treatment groups. In addition carnosine normalized G6PDH activity being in line with the corrected GSH concentrations. That carnosine preferably protected GSH than the other vitamin C. Although both acting as antioxidant agents, GSH is the most abundant non protein thiol in cells and the first line against oxidative stress. This ensured the selective action of the dipeptide previously stated by Soliman and mohamed (2004).

Carnosine administration restored glucose level to normal was in full agreement with the previous results of Soliman et al( 2002) reporting the involvement of carnosine in the repletion of liver glycogen level in hamsters under the effect of S. mansoni infection. The mechanism might be explained by the finding of Ikada et al. (1980) that carnosine could inhibit phosphorylase b at pH 7 through changing its conformation. Carnosine through its buffering action against changes in muscular pH safety prevented tissue acidification, thus slowing the rate of action of phosphorylase b and hence helping glycogen repletion (Parkbouse et al., 1985 and Jia et al., 2009).

Carnosine improved ALT, AST and LDH activities, presenting a regular change pattern for the three animal groups of each phase. The correction of both Co and post infection groups showed complete recovery of enzyme activities opposite to their more deterioration result by albendazole.

This could give a clue that carnosine has the ability to ameliorate pathological disorders as emphasized by previous studies (Soliman et al., 2002 and Iovine et al., 2012). All these present biochemical serum parameters improvement could indicate equal abilities of carnosine to alleviate both toxic effects of adult worm inhabiting the gut as well as those of larvae inhabiting muscles.

That carnosine correction of parameters was more presented in the muscular phase, starting 6
weeks after infection, matched the old reports that the dipeptide presented more abatement of chronic infection than acute one (Fatzpatric et al., 1980 and Seo et al., 2012).

Having a back-comparing notice to the pathological reactivity of either organs; muscle and liver, in the untreated group, could very feasibly indicate that muscles, not as much affected by liver insults, could be attributed to it being the store house of carnosine, the marvelous general defensive buffer against variable body stresses. Many researches proved the variable abilities of exogenous carnosine administration to selectively combating insults of all body organs (Soliman et al., 2007). Here one might state that carnosine imparting reasonable defense power to different body organs, logically present more protection towards its host tissue!, the muscle! Often, carnosine had proved to be a wise agent (Presenting selective actions) and now additionally, proved to be just and generous for its main host, the muscle.

The possible additional mechanisms by which carnosine regulates these actions are numerous. It binds with macrophage and lymphocyte receptors, stimulating their synthetic and secretory abilities, leading to the activation of body immune resistance against the parasite (Silaeva et al., 1992, Soliman et al., 2003 and Williams et al., 2012). It induces liberation of immune modulator intermediate, cytokine and interleukin (Suzuki et al., 2001). It is capable to combat non specific (Fitzpatrick et al., 1980 and Seo et al., 2012) as well as specific chronic infection (Soliman et al., 2002).

5. Possible role of L-carnosine in regulation of blood glucose
5.1. control of hyperglycemia
L-Carnosine (0.01-0.001%) proved to be implicated in the control of hyperglycemia produced by lateral cerebral ventricular (LCV) injection of 2-deoxy-D-glucose (2DG). This hyperglycemia was attributed to disturbance of autonomic nerves control, to excitation of sympathetic nerves and inhibition of parasympathetic nerves leading to suppression of insulin secretion and enhancement of adrenalin and glycogen secretion (Chum, 1998).

The mechanism of carnosine action was stated to be through lowering activities of sympathetic nerves and facilitating those of parasympathetic nerves (Yamano, 2001 and Niijima, 2002). The suppressive action of the dipeptide might also be due to its transformation to L-histidine, then histamine, both of which cause inhibition of 2DG-hyperglycemia when injected into LCV through the stimulation of the histaminergic H₃ receptor (Nagai et al., 2003).

In patients with type I diabetes mellitus, Gayova et al. (1999) showed that carnosine levels in red blood cells decreased, which suggests that the dipeptide is less available for metabolic processes, therefore it should be supplemented. Moreover, in streptozotocin-induced diabetic rats, carnosine (50 mg/ kg/ day) orally for 7 days was shown to prevent the decrease in the hemolytic stability of red blood cells. The authors also showed that the dipeptide normalized the acid erythrogram parameters in these diabetic models (Korobov, 2000).

In addition, carnosine as well as GABA and taurine levels in serum of diabetic mothers and their off springs were significantly below normal (Aerts and Van-Assche, 2001). This deficiency in carnosine, previously entitled as neuroprotector (Quinn et al., 1992) and both neurotransmitters involved in the hypothalamo-hypophyseal regulation of insulin secretion, might contribute to development of impaired glucose tolerance and gestational diabetes, thereby, transmitting the effect to the next generation.
The advanced glycation end-product (AGE) hypothesis proposes that accelerated chemical modification of protein molecules by glucose during hyperglycemia contributes to the pathogenesis of diabetic complications. The two most commonly measured AGE are N-(espition)-carbonxy-methyl lysine and pentosidine. L-Carnosine showed inhibition of AGE formation that result from chelating or antioxidant activity than from nucleophilic trapping for reactive carbonyl intermediates in the formation of AGE₃ (Price et al., 2001). Hipkiss et al. (2001) and Yamano et al. (2001), showed that carnosine combining with small carbonyl compounds (or groups) on glycated/oxidized proteins and other molecules inhibited cross-linking of glycoxidized polypeptides. The authors termed this process carnosinylation. They also found that carnosine suppressed diabetes-associated increase in blood pressure in fructose-fed rats. On the other hand, Sztanke and Pasternak (2003) stated that carnosine is a natural substance that inhibits the AGE cross-links as a result of Maillard reaction but do not seem to break AGE-derived protein cross-links already formed. Therefore, they reported that the dipeptide might not be effective in patients with a long history of the disease.

5.2. Carnosine and its effect on appetite
Nagai et al (2003) reported that IP-injection of 100mg (0.44mmol) of the dipeptide inhibited the food intake for one hr after injection. On the contrary, other researches proved that L-histamine suppressed appetite in rats and mice (Yoshimatsu, 1999 and 2000). In support Tagboto and Townson (2001) reported the inhibition of food intake in a dose dependent manner. Therefore, these contradictory results could possibly be explained that smaller doses of L-carnosine might enhance appetite via 113 receptors in rats.

6. Role of carnosine in cholesterol abnormalities
6. 1. Role of carnosine in hypercholesterolemia
Diniz et al. (2004) supported the concept that the susceptibility of body tissues to oxidative stress might depend on dietary factors. The authors' emphathized that the surplus oxidative metabolism of fuels results in excess ROS production. Hypercholesterolemia, a common metabolic error of common occurrence, causes oxidative stress, resulting in functional and pathological disturbances in most organs. This elicits widespread cytotoxic damage to cell constituents such as membrane lipids, structural proteins as well as enzymes. The disturbance of many biochemical parameters in chronic HC was reported by several authors in different body organs as well as in blood of chronic hypercholesterolemic (HC) subjects (Lee et al., 2003, Lim et al., 2003 and Yanni et al., 2003). The oxidative stress is not only associated with increased unsaturated fatty acid peroxidation (MDA production) but also, dependeds on metabolic pathways shifting energy production (Yeda et al., 2004).

Soliman and mohamed (2004) proved increased MDA and NO production as well as decreased activities of some energy machineries enzymes in the liver and kidney tissues namely, hexokinase (HK), aldolase (ALD) phosphoglucoisomerase (PGI), and creatine phosphokinase (CPK). Disturbances of other serum functional and metabolic parameters of both organs were proved in HC rabbits. Serum analysis documented impaired functions of both organs. AST and ALT amounted to 80 and 59.5 IU/L respectively and bilirubin was much increased (12mg/dl). Uric acid and creatinine were highly increased (25 and 4.5 mg/dl respectively). These disturbances were supported by histological examination revealing deleterious pictures in both liver and kidney of HC rabbits. The hepatic architecture was disturbed. The hepatocytes were swollen, some cells showed darkened cytoplasm and others presented cytoplasmic vacuolations. The central veins were congested. Microscopic sections of the kidney showed distended hypercellular glomerioli, swollen elongated edematous tubules with obliterated lumina. Their epithelial lining was degenerated with karyolysis of
Previous researches on protein metabolic parameters in HC showed the decrease of serum total proteins and amino acids (Farmer and Gotto, 1995). Dadimarz et al. (1998) stated that under stressful HC conditions, these amino acid concentrations changes in either blood or liver indicated stress-induced organ pathology. Decreased serum total proteins concentration and disturbed levels of most serum amino acids were also recently stated by Soliman et al. (2005) The authors found 11 disturbed amino acid levels out of the 17 tested. Nine amino acids, including arginine, were decreased while, only 2 were increased.

In correlation with the decreased total proteins and most amino acid levels, there was increased serum transaminases and urea cycle enzymes (Fan et al., 2003 and Soliman et al., 2005). Increased serum ALT and AST, in HC-animals would possibly indicate liver dysfunction or degeneration of muscle, either of which could result in disturbed amino acid metabolism (Pearce, 2003). In addition, the reported increased urea cycle enzymes participated in increased catabolism of most amino acids and hence, their decreased serum levels (Fouad et al., 1983). This was supported by Soliman et al. (2005) proving increased activities of three urea cycle enzymes (OAT, ASS and arginase). These increased activities of serum metabolic protein enzymes in both these two HC-groups could be explained by three mechanisms. 1- Metabolic disturbances of the liver (Crespo et al.,1999, Soliman and Mohamed, 2004 and Soliman etal., 2005). 2- ROS liberation leading to accumulation of calcium in mitochondria causing the discharge of enzymes in circulation (Alabovskii et al., 1999). 3- Hyperactivation of arginase causing more production of urea and liver dysfunction leading to discharge of enzymes. Arginine, presenting low serum level, could be documented by the associated increased arginase activity (Soliman et al., 2005). Decreased arginine could disturb endothelial NO production, a potent inhibitor of platelet aggregation (Ueda et al., 2001 and Reid Sutton et al., 2003), leading to endothelial dysfunction and atherosclerosis (Spieker et al., 2002). Some researches indicated that increased urea cycle activity could anticipate in HC being a risk factor for atherosclerosis (Novelli et al., 2002 and Ogita and Liao, 2004).

The effect of treatment of by an authorized hypocholesterolimic drug, fluvastatin, in reducing the above mentioned disturbances in both liver and kidney tissue; was studied comparing its efficacy to that of the natural dipeptide carnosine (Soliman and Mohamed, 2004 and Herculano et al., 2011). The study involved MDA level, NO production and the three tissue energy enzymes GPI, ALD, CPK as well as serum functional tests of both organs. Serum transaminases activity, albumin and bilirubin evaluated liver function. Serum creatinine and uric acid evaluated kidney function. Soliman et al. (2005), further compared serum total proteins, 17 individual amino acids levels, transaminases and urea cycle enzymes.
The corrective action of carnosine on the same parameters in kidney tissue was extremely evident. Normalization of all three enzyme activities indicating complete correction of the kidney faculties. Normalization of renal function by carnosine was explained by several functional mechanisms (Soliman et al., 2004). 1- Increase in GFR caused by carnosine arteriolar dilation (Ririe et al., 2000) and hence increased capillary pressure (Kaplan et al., 1990 and Rahman et al., 2001). 2- Improvement in endothelial vasodilator functions by carnosine inhibitory effect on lipid peroxidation and preventing further formation of free radicals. On the other hand, renal microscopic examination showing normal histological structure of renal glomeruli, tubules and interstitial spaces documented complete renal recovery.

HC-carnosine animals presented much amelioration of the other disturbed parameters. Examining serum protein metabolic parameters, plasma cholesterol concentration was decreased although, it was still higher than control (Steiner et al., 2001). Soliman et al. (2005) recorded normalization of total serum proteins and most amino acids (13 amino acids out of 17). AST and ALT showed significant improvements, although they were not normalized. The corrective ability of carnosine on urea cycle enzymes reflected lowering of OAT and ASS enzyme to their normal activities. This normalization of both activities could denote much amelioration of the cycle action. Arginase activity, not completely decreased to control levels might be attributed to plasma cholesterol not completely normalized. Thus marked correction of both urea cycle machinery and serum transaminases activity could point to normalization of protein catabolism by carnosine reflected as normalization of serum total proteins and most a. a. levels.

For HC-fluvastatin animals, Soliman et al. (2004) stated that although, all fluvastatin data reflected improvement yet, they still presented lagging deviations from control and HC-carnosine results. The authors reported marked amelioration of MDA and NO concentrations and the 3 energy metabolic enzyme activities; PGI, ALD and CPK of both liver and kidney tissues. In support, several reports proved that statins, like many drugs, carry the risk for adverse effects on the liver being the target organ for drug detoxication. Previous reports of untoward effects of statin therapy included; increased liver cholesterol and triglycerides (Farmer and Gotto, 1995), alteration of hepatic key enzymes of carbohydrate metabolism (Crespo et al., 1999), increased serum liver function tests (Steiner et al., 2001). Soliman et al. (2005) proved increased both bilirubin (11mg/dl) and the two transaminases (ALT andAST; 62: 76 IU/L respectively) by fluvastatin. Histopathological reports of liver necrosis and gall blader inflammation with mucosal hyperplasia (Zagoya et al., 1999) and vaculated heptocytes with pyknotic or karyolitic nucleoli as well as engorged central veins (Soliman et al., 2005) indicated hepatotoxicity.

Other biochemical blood examinations proved an incomplete down-regulating role of cholesterol concentration. by statin (Steiner et al., 2001). This down-regulatation of blood cholesterol concentration lagged behind that of carnosine although; both were higher than control animals. A harmful increase in cholesterol content of the liver by fluvastatin was previously recorded (Zagoya et al., 1999). This increased cholesterol liver tissue content was then explained by the statin hypocholesterolemic mechanism. Statin action was through inhibiting HMGCOA reductase leading to down regulation of cholesterol synthesis and re-regulation of hepatic high affinity receptors for low density lipoproteins followed by increased catabolism of LDL cholesterol (Ness and Chambers, 2000). This increased liver tissue cholesterol could indicate diminished fluidity of plasma membranes revealing impaired hepatocyte metabolic abilities (R++) thus, explaining the still marked lowering of serum total
proteins and nine amino acids after fluvastatin treatment. This statin, on the other hand showed correction of 6 amino acids only; a score similar to the insulted HC group. The numerical ratio between the numbers of normal amino acids was 6: 13: 6. for HC: HC-carnosine: HC-statin. In document, Yilimaz et al. (2004) stated that fluvastatin treatment was accompanied by few a. a. improved levels.

Of the five catabolic protein enzymes tested (AST, ALT, OAT, ASS and arginase), only one namely, ASS was down-regulated (Soliman and Mohamed, 2004). This might impart a minor beneficial effect to fluvastatin as it did not save serum protein nor a. a. concentrations. Both these results much lagged behind that of carnosine. In support, records about drawback effects of statin were increased serum liver enzymes, increased cholesterol content in liver and disturbances of key enzymes of liver carbohydrate metabolism (Gespo et al., 1999 and Zagoya et al., 1999).

In another research (Soliman et al., 2005) on seven serum parameters; amino acids, total protein concentration, the two transaminases (AST and ALT) and three enzymes of urea cycle (OAT, ASS and arginase), also selective improvements were noticed. Only three parameters; total protein, OAT and ASS were normalized. However, the majority of amino acids were also normalized. The dipeptide improved both transaminases and arginase.

Thus, HC-carnosine groups presented the best ameliorated results of both tested agents.

6. 2. Carnosine and hypercholesterolemic cataract

Morel (1994) and Vendemil et al. (1996) reported that HC rabbits showed increased LPO and reduced glutathione levels respectively in the fluids and tissues of the eye due to exhaustion of the natural antioxidant mechanisms. In hyperlipidemia, the lense was more susceptible to oxidative stress that caused development of cataract (Atef et al., 1998). The authors attributed it to enrichment of the lens with lipid constituents particularly cholesterol Serum HDL-cholesterol level was also associated with posterior subcapsular cataract in men (Hiller et al., 2003). Another explanation was previously stated by Putilina et al. (1999) that epithelial cells degeneration could cause more severe disruption of Na/ K ATPase pump causing efflux of Na and water. This leads to vacuolation and separation of lens fibers which could be indicative of edema. High lens cholesterol content presented self-associated forming immusible domains in the plasma membrane, a phenomenon that contributed to the pathologic cellular processes participating in cataractogenesis (Pertson et al., 2003).

The effect of prophylactic carnosine administration on cataract induced by hypercholesterolema was tested in comparison to fluvastatin (Soliman and Mohamed, 2004). In HC-carnosine rabbits, the lenses remained transparent. Light and SEM examination revealed intact capsule and epithelial cells as well as regular arrangement of lens fibers. These findings are a good evidence that carnosine could help in maintaining the transparency of the lens. These data could be confirmed by those of Quinn et al. (1992) that rabbits fed a high cholesterol diet were well protected against atheroscherosis and cataract if given carnosine supplements.

The ability of carnosine was also reported to eliminate existing cataracts by actually restoring the the lens proteins (Babizhayev et al., 2002). Wang et al,(2000) reported 100% effect of carnosine eye drops on primary senile cataract and 80% on mature senile in different cataract types with no side effects. This potential carnosine role to its ability to penetrate to the lens and increases Na-K-ATPase activity and thus had a potential role in treatment & prevention
of cataract. However, an essential factor in this carnosine cataract preventing effect could be referred to its potency in lowering blood cholesterol level in HC rabbits (Soliman and Mahmoud, 2004). The authors advised the conjoined systemic carnosine administration in addition to topical NAC (N-acetyl carnosine) eye drops for cataract treatment.

Fluvastatin showed mild changes in the form of thickened lens capsule, vacuolation and deformation of epithelial cell nuclei as well as swollen cortical lens fiber (Soliman and Mohamed, 2004) that was previously referred to inhibition of cholesterol biosynthetic pathway (Cendella, 1996 and Chang et al., 2003). Novelli et al. (2002) recorded that alteration in fuel food constituents leading to inhibition of cholesterol biosynthetic pathway. The mechanism of action of carnosine being corrective of lens cataract might be mainly attributed to its antioxidant effect, having a di-potent antioxidant action (Salganik et al., 2001 and Hipkiss, 2011). Oxidation of proteins initiates the process of cross-linking forming inactive carboxylated and glycosylated protein end products which can further adduct to other native protein molecules causing more tissue damage (Yeargans and Seidler, 2003 and Maherani et al., 2012 ). Carnosine protects the tissues from these “second-wave” chemicals. It activities disposal of the damaged proteins by forming protein-carbonylcarnosine adducts (Hipkiss et al., 2001, Hipkiss et al., 2002 and Hipkiss, 2011).

7. Carnosine and Cardiovascular diseases

7.1. Carnosine and blood pressure (BP)
Carnosine proved a dose dependent effect on the BP. Smaller doses presented hypotensive effect (Nishina and Suzuki, 2002, Soliman et al., 2003). Bae and Majid (2013) correlated this hypotensive effect of carnosine to its vasodilatory action. The authors observed that carnosine (0.625-20mM) produced a dose-dependent vascular relaxation of rat aorta that was independent of endothelium, and was in part mediated via cyclic GMP. Ririe et al. (2000) showed that low IV dose (16.7mg/kg) of carnosine produced insignificant change in blood pressure in rats whereas reduction in blood pressure was observed with higher IV dose (33.3mg/kg). This hypotensive action was proved to be through vascular muscles and not through endothelium. However, they demonstrated that carnosine produced concentration-dependent relaxation of the isolated rat aortic rings pre-constricted with phenylalanine, an effect which was endothelial independent, and decreased by soluble guanylate cyclase. The authors' proved that the effect of carnosine was at least in part mediated via cyclic GMP production in vascular smooth muscle. Niijima et al. (2002) found that a diet containing 0.0001% or 0.001% L-carnosine decreases blood pressure elevation in DOCA-salt hypertensive rats. They postulated that L-carnosine may be an endogenous factor controlling blood pressure in a manner possibly antagonistic to the obesity-associated hypertensive effect of leptin.

In intact anaesthetized animals, the results of Soliman et al. (2003) were in accordance with these data where dose-dependent short-term hypotensive effect of carnosine (250 µg-128mg/kg) was observed in intact anaesthetized cats. Matching with this hypotensive effect of small doses of carnosine in producing hypotension, the authors reported an insignificant increase was observed in heart rate with IV carnosine doses up to 16 mg/kg while a mild significant increase was produced using 32, 64 and 128 mg/kg doses (indicating hypotensive action). Tanida et al. (2004), stated that carnosine in doses ranging from 250-128 µg/kg produced a significant dose dependent short-term reduction in the carotid blood pressure of chloralized anesthesized cats.
In the spinal cat carnosine IV injection still produces hypotensive effect although, less than that observed in intact anaesthetized cats (Soliman et al., 2003). Previously Quinn et al. (1992) reported that neither transection of both trunks of vagus nerve nor high transection of spinal cord affected the hypotensive carnosine effect.

The hypotensive effect however, was completely antagonized by I V injection of mepyramin suggested the involvement of H1-agonistic activity of carnosine. This pointed that the hypotensive effect of carnosine could be mediated centrally and peripherally. Although H1 receptor mediated hypotensive effect was suggested, the possibility then remained that carnosine could be acting at a novel receptor with coincidental sensitivity to mepyramine. The possible mechanism involved in this observed hypotensive effect of carnosine was also investigated by Tanida et al. (2004). The authors found that intralateral cerebral ventricular injection of low doses of L-carnosine (1µg I. V; 0.01µg) significantly suppressed renal sympathetic nerve activity (RSNA) and reduced the blood pressure. The authors further studying the effects of antagonists of histaminergic receptors (H1 and H3) on L-carnosine-induced effects. They observed that an H3 receptor antagonist (thioperamide) given peripherally and centrally, blocked RSNA and any lowering of blood pressure induced by smaller doses of peripheral L-carnosine. On the contrary, higher doses (1001.tg I. V; 101.tg LCV) elevated the RSNA and blood pressure (Tanida et al., 2004). Diphenylhydramine (an H1 receptor antagonist) inhibited increases induced by higher doses of L-carnosine. However, it could be concluded that the equilibrium between carnosine and its constituent amino acids in vascular tissues may represent a new and novel mechanism for modulation of vascular tone and treatment of hypertension.

Soliman and Abdel Monem (2001) revealed that in-vitro, large doses of carnosine induced concentration-dependant contractions of isolated rabbit aortic spiral strips which was not affected by the addition of both a-adrenergic and angiotensin receptor blockers. On the other hand, methyl sergid (5 HT1-a2 antagonist) and mepyremain (H1-anagonist) produce a rightward shift of the contractile response curves of carnosine reflecting the involvement of serotoninergic-like a (or and) H1-receptors.

Moreover, Tanida et al. (2004) recorded that bilateral lesions of hypothalamic suprachiasmatic nucleus eliminated both effects on RSNA and blood pressure induced by low and high doses of peripheral carnosine they suggested the involvement of hypothalamic suprachiasmatic and histaminergic nerves in the activity of L-carnosine and that it acts in the brain and possibly other organs. Furthermore, the authors found that H3 and H1 histaminergic receptor antagonists (thioperamide and diphenylhydramine respectively), inhibited any decreases or increases in BP induced by carnosine, which proved the involvement of histaminergic nerve in the activities and that carnosine acts in the brain hypothalamic suprachiasmatic nucleus. They also found that carnosine suppressed diabetes-associated increase in blood pressure in fructose-fed rats. On the other hand, Sztanke and Pasternak (2003) Lee et al. (2005), stated that carnosine is a natural substance that inhibits the AGE cross-links as a result of Maillard reaction but do not seem to break AGE-derived protein cross-links already formed. Therefore, they reported that the dipeptide might not be effective in patients with a long history of the disease.

7.2. Cardiac effect of carnosine
The dipeptide also proved a cardioprotective effect during stressful conditions. A decreased incidence of ischemia-induced cardiac arrhythmia was reported with carnosine perfused hearts compared to those perfused with either control buffer of beta antagonist (Zaloga et al.,
In the same beneficial direction, carnosine proved a protective effect during cardioplegia (Alabovakki et al., 1999). It was also reported that in stressed hearts, carnosine significantly improved the functional recovery of perfused isolated rat hearts after 40 min global ischemia (Lee et al., 2009). During reoxygenation, after experimental hypoxia in intact animal, the addition of carnosine on top of the perfusing solution increased the coronary blood flow and decreased contractile activity. This may be due to the replenishing of the blood supply in order to wash accumulated hypoxia-metabolites for the benefit of cardiac muscle recovery.

In addition, Zeiba and Wagrowska (2003) and Kalaz et al. (2012) studied the influence of carnosine on the cardiotoxicity of doxorubicin (DOX), which induces congestive heart failure. They found that Co-administration of carnosine with DOX normalized the values of the mean arterial pressure in rabbits and increased the cardiac index and stroke index while its effect on total peripheral resistance was non-significant. The histopathological examination of cardiac muscle cells revealed smaller damage of cardiac muscle in rabbits which received DOX with carnosine compared to those receiving DOX alone (Zieba and Wagrowska-Danilewicz, 2003).

These cardio-protective actions of carnosine could be related to a previously proved carnosine regulatory action on intracellular calcium. It had a direct action on the major calcium release channel in the sacroplasmic reticulum since it increases the intracellular cytoplasmic calcium level which causes increased cardiac muscle contractility (O'Dowd and Miller, 1998, Robert and Zaloga, 2000 and Kalaz et al., 2012).

7. 3. Carnosine and renal vascular stress

Fujioka et al. (2003) studied the preventive effect of L-carnosine on ischemia/ reperfusion induced acute renal failure in rats (by occlusion of the left renal artery and vein for 45 minutes followed by reperfusion-2 weeks after contralateral nephrectomy. Pre-ischemic treatment with L-carnosine (1-10µg/ kg IV) attenuated the induced renal dysfunction. Histopathological examination of the kidney of untreated acute renal failure rats revealed severe renal damage which was significantly suppressed by pre-treatment with L-carnosine at each dose given. Moreover, the authors recorded that the effect of the dipeptide is accompanied by suppression of the enhanced norepinephrine release in the kidney immediately after perfusion. Thus, the preventing effect of L-carnosine in ischemic acute renal failure is probably through suppression of enhanced renal sympathetic nerve activity induced by ischemia reperfusion.

The effect of carnosine in complete kidney recovery (function & structure) in HC-animals was also reported by Soliman and mohamed (2004) and Harini et al. (2011).

7. 4. Effects of carnosine on brain ischemia

Carnosine had a protective effect on rat brain (Stadnikov et al., 2000, Zhang et al., 2011 and Nam- Bae and Majid, 2013). The authors investigated the effect of carnosine on rat brain subjected to 45min. global ischemia. They found that this type of ischemia characterized by decreased Kp-nitrophenyl-phosphate monamine oxidase B & A, disordering of membrane bilayer by reactive oxygen species attack and lead to 67% mortality in animals. Pretreatment with carnosine offered a protection of the brain against these oxidative injuries and increased the % survival.
8. Carnosine and teratogenesis
The effect of maternal treatment with phenytoin on the neonatal rats varied from small sized completely distorted non viable one (about 10% of animals) to others with manifest gross congenital malformation which appeared in clubbing of foot. Microscopic examination neonatal rat's liver and kidney showed marked degenerative changes. The liver's capsule became disturbed and the hepatic cords became disorganized most of hepatocytes showed marked cytoplasmic vacuolations. Their nuclei appeared small and faint but some cells lost their nuclei. The blood sinuosoids could not be detected but, the haemopoietic cells appeared normal. In addition, the central veins were dilated and congested and their endothelial lining was disturbed. The portal area was surrounded with infiltrated cells. The portal vein was markedly dilated and congested. The collagen fibers deposition around the portal tract and in the capsule was moderately increased while hepatocyte glycogen was markedly reduced. Administration of phentoin (PHT) induced a highly significant decrease in the perimeter nucleus of hepatocytes in the liver and the nucleus of the tubules of the kidney. Carnosine given to the mothers with PHT caused improvement of body weight and length of neonates. No dead offspring or congenital anomalies were detected. Microscopic examination of neonatal rat's liver showed marked improvement. The hepatic architecture, blood sinusoids and hepatocytes were normal. Polychedral in shape, they had eaisinophilic granular cytoplasm and had large vesicular basophilic nuclei with one or more nucleoli. The blood sinusoids were decreased in size by hepatocytes distended with cytoplasmic glycogen. The sinusoids were lined with flat endothelial cells and large Von kupper cells. The central vein was slightly congested and mildly dilated. The portal tract was normal and the cells of bile duct have a large vesicular nucleus. The portal vein presented normal size with slight congestion. The distribution of the collagen fibers in the capsule and around the portal tract remained moderately increased. The effect of carnosine was dose dependant. The lower dose caused more vascular congestion as well as markedly increased collagen fibers and glycogen engorgement (Soliman et al., 2003b).

Microanatomy of the neonatal rat's kidney of treated mothers with PHT showed severe degenerative changes which could be manifested by areas of cells with ill-defined boundaries formed a syncytium of eosinophilic cytoplasm containing faint nuclei were seen within the cortex and medulla. Some distal convoluted and collecting tubules appeared. Both thick and thin loops of Henle within the outer medulla appeared small in size and the identification of them was difficult in the inner medulla. The cells of these loops had deeply eosinophilic stain and their nuclei had eosinophilic stain. The vasa recta could not be recognized while the interstitial cells appeared clearly. The majority of collecting ducts within the inner medulla presented partially interrupted lumina and their cells boundaries were lost. Some collecting ducts had obliterated lumina with irregular arrangement of cells in their wall. The collagen fibers were not seen in the glomerular tuft of capillaries, in between the renal parenchyma nor within the capsule. The PAS positive reaction appeared less than the control in the basement membrane of renal tubules, in the luminal borders of proximal convoluted tubules and in the renal corpuscle (Lee et al., 2009).

Carnosine "10mg or 5 mg" and PHT showed mild improvement in the renal parenchyma. The glomeruli appeared normal in both groups. An apparent improvement of renal tubular structure was noticed in these groups. Many tubules acquired an improved epithelial lining but the epithelium was not completely normalized. The proximal convoluted tubules lost their brush luminal borders. Few cells of the distal convoluted tubules had vacuolated cytoplasm. Few of collecting tubules appeared with obliterated lumena and disrupted cells. Few cells had vacuolated cytoplasm. The thick loops of Henle appeared differentiated but most of cells had
vacuolated cytoplasm. The thin loops of Henle appeared in the outer and inner medulla with large oval nuclei. The majority of collecting ducts within the inner medulla were differentiated but their cells became cubical with less basophilic nucleus, some of them had disturbed cell. The vasa recta appeared congested. The interstitial cells appeared increased with large nucleus. There were an increase in the amount of the collagen fibers in the renal capsule which became thick and in between the renal improvement findings were dose dependent except for collagen fiber formation and cytoplasmic glycogen were more hown with the small carnosine dose. The small carnosine dose group less improvement parenchyma. The PAS positive reaction was seen in the basement membrane of all renal tubules, in the glomerular tuft of capillaries, in the parietal layer of Bowman's capsule and in the lumens of proximal convoluted tubules (Soliman et al., 2003b and 2009).

Morphometric showed that administration of PHT induced a highly significant decrease in the erimeter nucleus of hepatocytes in the liver and the nucleus of the tubules of the kidney. On the other hand, the administration of either carnosine dose 5mg or 10mg with PHT induced a marked improvement in the mean perimeter nucleus of hepatocytes in the liver and the nuclei of the tubular cells of the kidney. The mean gray of PAS positive material in the hepatocytes was depressed, basal laminae of kidney tubules and its brush borders convoluted (Soliman et al., 2003b).

PHT induced significant decrease of the mean gray of PAS positive material in the hepatocytes of liver, basal laminae of kidney proximal convoluted tubules and its brush borders when compared with the control group. On the other hand, the administration of carnosine 5mg or10mg with PHT induced a marked improvement where the mean gray of PAS positive material in the hepatocytes of liver spatially in group treated with 5mg carnosine. The basal laminae of kidney proximal convoluted tubules and its brush borders showed a highly significant increase the mean gray of PAS positive material when compared to the control group(Soliman et al., 2003b).

9- Effect of carnosine on partial hepatectomy (PH)
Soliman and mohamed(2004), due to the above wide range of biological effects of carnosine on various metabolic and biochemical aspects, the ability of carnosine to prevent biliary sludge in liver transplantation (Barton et al., 1995) and its wound healing effect (Roberts et al., 1998) investigated the role of the histidine dipeptide on mice liver regeneration after partial hepatectomy (PH). Liver regeneration in insults, including surgical trauma, was governed by acute phase response (Fujioka et al., 2001) being for regeneration and limitation of inflammation. It is usually orchestrated cytokines and hepatocyte stimulating factors associated with characteristic metabolic changes in liver protein synthesis. Individual acute phase reactant proteins have different biochemical functions. Some are protease inhibitors; al-macroglobulin, al-acid glycoprotein, al- antitrypsin. Others are transporters α and β -lipoproteins ceruloplasmin, hemopexin, haptoglobin, transferrin (Ganong, 1999). They have different regulatory mechanisms that are differentially expressed within hours or days after PH (Campbell et al., 2001). Hence, they are good indicators of liver condition as they are affected by its regeneration rate.

PH operation was recorded to elicit the specific acute phase response of serum proteins associated with characteristic metabolic changes in liver protein synthesis (Fujioka et al., 2001). Soliman and Ali (2004) studied the effect of carnosine administration to both normal mice and those 70% partially hepatectomized. Biochemical investigations of 16 liver tissue and serum parameters were tested after 15 and 30 days after operation: liver weight, DNA ,
RNA as well as serum total protein, albumin and protein profile were examined. Serum protein profile using two dimensional immuno-electrophoresis (Fouad et al., 1983) so, quantitate separation of 11 protein fractions was investigated ; prealbumin, α and β-lipoproteins α-macroglobulin, acid glycoprotein, α-antitrypsin, cholinesterase, ceruloplasmin, hemopexin, haptoglobin and transferrin.

Healthy mice treated with carnosine for 15 days (Hlth-15 C) showed the increase of 4 parameters, while those treated with carnosine for 30 days (Hlth-30 C) showed the increase of 13 parameters. This was attributed to the selective protein promoting action of carnosine on liver tissue proteins. Some proteins were increased while the catabolic enzymes were suppressed (Soliman and mohamed, 2004).

This was assured by the increased concentration of DNA in PH-C (15 days) to that ofPH-C (30 days). However, in the later group it was only promoted to normal level. Thus, the more actively regenerating liver cells in the operated (PH-C-15 days) mice were more labile to stimulation by carnosine treatment. This indicated that embryonic cells are more responsive to carnosine action.

10. Carnosine and diabetes

It was found that, carnosine (50mg/kg/day orally for 7 days) prevent the decrease in the hemolytic stability of red blood cells in streptozotocin-induced diabetic rats (Korobov, 2000). It normalizes the acid erythrogram parameters in this diabetic model in rats. This finding suggested usefulness of carnosine as a possible treatment for diabetic patients (Korobov et al. 2000),In patient with type I diabetes mellitus, Gayova et al.,(1999) and Kamei et al .(2008) showed that the plasma carnosine levels were not significantly increased compared to the levels in healthy population, while the levels in red blood cells decreased. Lowered levels of carnosine in red blood cells could point out similar deficit in other cells. Due to its lower levels in cells, the authors suggested that carnosine is less available for metabolic processes, like antioxidant reactions. Its participation in antioxidant defense reactions is limited, non-enzymatic glycosylation of proteins. Therefore, it should be supplemented.

Carnosine as well as GABA and taurine levels in serum of diabetic mothers and their offspring were found significantly below normal (Aerts and Van-Assche (2001). The investigators concluded the low serum levels of the endogenous neuroprotector and both neurotransmitters taurine , GABA and the endogenous neuroprotector.

Carnosine in diabetic mothers and their fetuses might compromise the normal structural and functional development of the fetal brain. The effect of gestational diabetes on fetal development and induction of diabetogenic effect in the offspring are not limited to glucose and insulin metabolism. It appears to be modulated by alterations at the hypothalamohypophyseal axis. Therefore, the deficiency of the circulating levels of these neurotransmitters involved in the hypothalamohypophyseal regulation of insulin secretion, might contribute to development of impaired glucose to tolerance and gestational diabetes, thereby, transmitting the effect to the next generation (Aerts and Van-Assche, 2001).

The advanced glycation end-product (AGE) hypothesis proposes that accelerated chemical modification of proteins by glucose during hyperglycemia contributes the pathogenesis of diabetic complications. The two most commonly measured AGE; N(epsition)-Carboxymethyl lysine and pentosidine are glycoxidation reaction products (Price et al.,2001 and Rashid et al
L-carnosine showed inhibition of AGE formation. That results from chelating or antioxidant activity than nucleophylic trapping for reactive carbonyl intermediates in the formation of AGE (Price et al., 2001). Another study by Hipkiss et al. (2001 and 2002) showed that carnosine react with small carbonyl compounds (aldehyde and ketones) and protects macromolecules against their cross-linking action. These authors found that carnosine can react non-enzymatically with carbonyl groups on glycated / oxidized proteins and other molecules inhibit cross-linking of glycoxidized polypeptides. This reaction was termed "carnosinylation". Those authors reported that carnosine suppressed diabetes-associated increase in blood pressure in fructose-fed rats, an observation consistent with carnosine's antiglycating actions.

On the other hand, Sztanke and Pasternak, (2003) found that carnosine is one of the substances of natural origin that inhibit the AGE cross-links as a result of Maillard reaction but do not seen to break AGE-derived protein cross-links that already formed. Therefore, it might not be effective in patients with a long history of the disease.

A study by Babizhyev et al. (2000) showed that naturally occurring compound N-alpha-acetyl carnosine is proposed as a pro drug of L-carnosine that is resistant to enzymatic hydrolysis by carnosinase. Topical ocular administration of this compound in rabbit eyes by different treatment techniques, instillation, sub-conjunctiva injection. In aqueous humor, L-carnosine might act as an antioxidant and enter the lens tissue when present at effective concentration (5-15 mm). The advantage of the ophthalmic prodrug N-alpha-acetylcarnosin and its bio-activated principle L-carnosine as universal antioxidant related to their ability to give efficient protection against oxidative stress both in the lipid-phase of biological membranes and in aqueous environment. Therefore, these agents are proposed for treatment of ocular disorders that have a component of oxidative stress in their genesis as complications of diabetes mellitus, cataract, glaucoma, retinal degeneration and ocular inflammation.

Thus, Nagai et al. (2003) examining this possibility, they tested the effects of dephenhydramine (H1-blocker) and FMH (a specific inhibitor of histidine decarboxylase) on the 2DG-hyperglycemia. Consequently, both treatments significantly inhibited the hyperglycemia. This suggests that the histaminergic H1-receptor is involved in the mechanism of the 2DG-hyperglycemia by elevating the blood glucose level. It was previously stated that lipolysis in rat white adipose tissue was stimulated by facilitating the sympathetic nervous system functions through H1-receptors of histaminergic neurons (Tsuda et al., 2002). The authors also proved that LCV-injection of a higher amount of histamine enhanced the 2DG-hyperglycemia.

In contrast, previously Arrang (1988) reported that the histaminergic H3 receptor has a higher affinity for lrisialnine Arun H1 and H2 receptors and that it was implicated in inhibition of histaminergic nerves as an inhibitory presynaptic receptor for the endogenous histamine release in the central and peripheral nerves. Therefore, it is possible that LCV-injections of smaller amount of L-carnosine or L-histidine suppressed the 2DG-hyperglycemia through the stimulation of the histaminergic H3 receptor (Nagai et al., 2003). In accordance with this possibility, it was found that thioperamide, an antagonist for histaminergic H3 receptor, could inhibit both suppressive action of L-carnosine and L-histidine on the 2DG-hyperglycemia (Yamano, 2001).
11. Carnosine and Alzheimer’s disease
The dipeptide carnosine (β-alanyl-L-histidine) has contrasting but beneficial effects on cellular activity. It delays cellular senescence and rejuvenates cultured senescent mammalian cells. However, it also inhibits the growth of cultured tumour cells. Based on studies in several organisms, we speculate that carnosine exerts these apparently opposing actions by affecting energy metabolism and/or protein homeostasis (proteostasis). Specific effects on energy metabolism include the dipeptide’s influence on cellular ATP concentrations. Carnosine’s ability to reduce the formation of altered proteins (typically adducts of methylglyoxal) and enhance proteolysis of aberrant polypeptides is indicative of its influence on proteostasis. Furthermore these dual actions might provide a rationale for the use of carnosine in the treatment or prevention of diverse age-related conditions where energy metabolism or proteostasis are compromised. These include cancer, Alzheimer's disease, Parkinson's disease and the complications of type-2 diabetes (nephropathy, cataracts, stroke and pain), which might all benefit from knowledge of carnosine’s mode of action on human cells (Hipkiss et al., 2013).

12. Carnosine ameliorates stress-induced glucose metabolism disorders
Carnosine could ameliorate stress-induced glucose metabolism disturbance. It is presumable that carnosine exerts its anti-stress effects by indirectly affecting the histaminergic neuron system, modulating the stress-activated hypothalamic–pituitary–adrenal axis and improving glucose metabolism through regulation of the enzymes in the glucose metabolic pathways (Tosi et al., 2011).

13. L-Carnosine and Autism
Oral supplementation with L-Carnosine resulted in demonstrable improvements in autistic behaviors as well as increases in language comprehension that reached statistical significance. Although the mechanism of action of the amino acid is not well understood, it is believed that it acts to modulate neurotransmission and affect metal ion transfer of zinc and copper in the entorhinal cortex. This may enhance neurological function or act in a neuroprotective fashion (Chez et al., 2013).

14. Carnosine the best antiaging
Carnosine is considered as the most important anti-aging supplements available to us today. The therapeutic potential of carnosine supplementation has been tested in numerous diseases in which ischemic or oxidative stress are involved. For several pathologies, such as diabetes and its complications, ocular disease, aging, and neurological disorders, promising preclinical and clinical results have been obtained. The carnosine system has evolved as a pluripotent solution to a number of homeostatic challenges. L-Histidine, and more specifically its imidazole moiety, appears to be the prime bioactive component, whereas β-alanine is mainly regulating the synthesis of the dipeptide (Boldyrev et al., 2014).

CONCLUSION
It could be concluded that carnosine is a potent antiglycating antinflammatory agent, pH buffer and heavy metal chelator. It can prevent aldehyde adducts, nucleic acid oxidation and enhanced immune response. Also, it prevent and cure cataract, glaucoma and other age related eye. Carnosine also, has a role in regulation of blood glucose and has neuro-protective function as well as antiparasitic and antiaging activities. Thus, Supplementation with carnosine and food rich
(Poultry, beef and pork and fish) protects against several diseases via reducing oxidative stress through its effects as free radical scavengers. Also, the antioxidant can activate neurons and preserve cellular viability. Eating fish once a week is recommended for reducing the risk of Alzheimer's disease due to carnosine rich and its polyunsaturated fatty acids contents. These types of fatty acids may suppress brain inflammation and have a role in brain development and protecting nerve cells degeneration.

REFERENCES

Bae O and Majid A (2013). Role of histidine/histamine in carnosine-induced neuroprotection during ischemic brain damage Brain Research, 1527, 21, 246-254


Hipkiss AR (2011). Energy metabolism, proteotoxic stress and age-related dysfunction – Protection by carnosine Molecular Aspects of Medicine, 32, s 4–6,267-278.


Lee S, Kim M, Yon J, Baek I, Park CG, Lee BJ, Yun YW, Nam S (2009). Black ginseng inhibits ethanol-induced teratogenesis in cultured mouse embryos through its effects on antioxidant activity Toxicology in Vitro, 23, 1, 47-52


Mido S, Fath EM, Farid AS, Nonaka N, Oku Y, Horii Y (2012). T richinella spiralis: Infection changes serum paraoxonase-1 levels, lipid profile, and oxidative status in rats Experimental Parasitology, 131, 2, 190-192

Miller NJ, Rice-Evans CA (1997). The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food. Chem. 60, 331–337.


Tsoi B; Rong He R; Yang D; Li Y; Li X; Li W; Abe K; Kurihara H.(2011). Carnosine Ameliorates Stress-Induced Glucose Metabolism Disorder in Restrained Mice. Journal of Pharmacological Sciences; 117( 4):223-229.


This work is dedicated to the soul of Kawther M. Soliman, Prof. Dr. of Biochemistry, Faculty of Medicine, Cairo University.