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RESEARCH ARTICLE

Influence of *Moringa oleifera* flaxseed oil and atorvastatin on hyperlipidemic male albino rats

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ABSTRACT

The main aim of this study was to investigate the beneficial effects of lipitor (LIP), a commercial brand name of atorvastatin drug, flaxseed oil (FSO) and *Moringa oleifera* oil (MO), extract (ME) or meal (Mm) alone or in combination on hyperlipidemia induced in adult male albino rats. Rats were fed for 2 weeks on basal diet as a negative control group or fed on high fat diet (HFD) and a weekly dose of triton X-100 to induce hyperlipidemia as positive controls. The latter group was treated with: LIP, LIP + FSO, LIP + ME, FSO, FSO + ME, ME, MO or Mm for 4 weeks. At the end of the experiment, the livers were quickly excised and processed for analyzing the antioxidative enzymes activities including total superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) and malondialdehyde (MDA). Feeding on MO and ME) showed no effects on the body weight or on anti- oxidative enzyme activities. Feeding on FSO, however, induced only increase in the body weight. ME of dry leaves and roots showed a decrease in the body weight and improved antioxidative enzymes activities. These effects of FSO, ME and MO on the body weight of rats may be attributed to captivity and reduction of the energy expenditure, while their marked effects on the antioxidative enzyme activity may need longer time of treatments. These results indicate that *Moringa oleifera* can be used in packed food supplement products.

Keywords: Antioxidative enzymes, Atorvastatin, Flaxseed oil, hyperlipidemia, *Moringa oleifera*, Lipitor

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INTRODUCTION

Hyperlipidemia, characterized by hypercholesterolemia, is the most prevalent indicator for susceptibility to cardiovascular diseases (Oehami and Mello, 2009). World Health Organization reports that high blood cholesterol contributes to approximately 56% cases of cardiovascular diseases worldwide and causes about 4 million deaths each year. Hyperlipidemia is a metabolic disorder, specifically characterized by alternations occurring in serum lipid and lipid protein profile due to increased concentration of total cholesterol (TC), low density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL) and triglycerides (TG) with a concomitant decrease in the concentration of high-density lipoprotein cholesterol (HDL) in the blood circulation (Aydilek and Aksakal, 2005). Hyperlipidemia is treated with Lipitor (Atorvastatin) which is the commercial brand name of atorvastatin and the active ingredient in this drug is statins. This statin, as 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase inhibitors, belong to a well- established class of drugs that can reduce cholesterol, ameliorate vascular atherosclerosis and improve cardiovascular morbidity and mortality (Amarenco *et al*., 2004). Previous clinical trials demonstrated that statins reduce the first or recurrent stroke risk among patients with known heart disease and subsequently have become cornerstones of therapy for secondary prevention of vascular disease along with antiplatelets and antihypertensives (Amarenco *et al*., 2004). Among statins, atorvastatin is a synthetic type of HMG-CoA analogue that exhibits a substantial efficacy for decreasing total and low-density lipoprotein cholesterol (LDL) levels, triglycerides and modification of lipoprotein composition.

The medicinal plants had played an important role in nearly every culture on earth, including Asia,

Africa, Europe and the Americas. Medicinal plants were based on the premise that plants contain natural substances that can promote health and alleviate illness. Several herbs can help to reduce high blood cholesterol concentrations (Alattar, 2006).

Flax (*Linum usitatissimum*) is a multi-purpose crop. Its seeds contain about 36 to 40% of oil, have long been used in human and animal diets and in industry as a source of oil. Recently there has been a growing interest in the probiotic properties of flax and its beneficial effects on coronary heart disease, some kinds of cancer and neurological and hormonal disorders (Simopoulos 2002). Consumption of flax seeds is beneficial for human health. Among crop plants, flax is rich in polyunsaturated fatty acids (PUFA) which are essential in the human diet. PUFA, however, are highly susceptible to oxidation and formation of reactive oxygen radicales (ROS) (El-Beltagi *et al*., 2007).

Moringa oleifera, which is locally known as shajna, belongs to the monogeneric family Moringaceae Different parts of this plant are used in the endogenous systems of human medicine for the treatment of a variety of human aliments. Ethanolic leaves extract of *Moringa oleifera* are used as hypotensive, hypocholesterolemia and hypoglycemic agent (Nikkon *et al*., 2003 and Abd El-Aziz *et al*., 2015). Based on the above beneficial effects of flax seeds and moringa, this study aimed to investigate the role of flaxseed oil and moringa oleifera extract as a medicinal plants' supplementation on the body weight and oxidation associated with hyperlipidemic induced in male albino rats.

MATERIALS AND METHODS

Animals

The experiments were performed on 100 male Sprague-Dawley albino rats weighting 150±10 g of start week age. The rats were obtained from the Holding Company for Biological Products & Vaccines (Vaccera), Helwan, Egypt. The rats were housed in plastic mesh cages for one week before the experimental work and lasts for 6 weeks. Animals were fed on high fat diet (HFD) with constituents of protein (20-22%), fats (6.4%), fibers (3.78%), carbohydrate (60%) as a source of energy, starch (5%), salt mixture (5%), vitamins (5%) and tap water was supplied ad libitum. The temperature in the animal room was maintained at 25±3^{o}C with a relative humidity of 55±5% at the normal light dark cycle. Experiments were performed according to the Institutional Animal Ethical Committee approved by Faculty of Science, Tanta University, Egypt. Animals were carefully observed every day for accommodation. Their body weights, food consumption, and water intakes were registered precisely every week to follow up any signs of toxicity or abnormality during the experiment.

Atorvastatin, Medicinal plants and other Chemicals

Atorvastatin (Lipitor drug; LP) was obtained from Pfizer company (Cairo, Egypt). Flaxseed oil (FSO) was obtained from the local market (Tanta Company for Flax and oil). Dry roots and leaves of *Moringa oleifera* were obtained from National Research Center (Giza, Egypt) and oil was obtained from the local market and used according to Mona *et al*., (2015). Other chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, Mo, USA) and Fisher bio reagent company (USA).

Experimental protocol

After 7 days of acclimation to laboratory conditions, the rats were divided randomly into ten groups of 10 rats each as follow: Group (A): The negative control group (basal diet) treated with PBS. Group (B): The positive control group which was fed on high fat diet (HFD) for 2 weeks and a weekly dose of triton x-100 (to induce hyperlipidemia as positive control). The rest of rats were fed on the same diet as in group B and then divided randomly into (8) groups (1-8), which were treated for 4 weeks as follows: Gp (1): HFD + 50 mg/kg Lipitor (LP), Gp (2): HFD + 50 mg/kg LP +500 mg/kg (1 ml/rat) of FSO), Gp (3): HFD + 50 mg/kg LP + 150 mg/kg *Moringa oleifera* extract (ME), Gp (4): HFD + 500 mg/kg (1 ml/rat) of FSO, Gp (5): HFD + 500 mg/kg (1 ml/rat) of FSO + 150 mg/kg *Moringa oleifera* extract (ME), Gp (6): HFD + 150 mg/kg ME, Gp (7): HFD + 100 mg/kg of *Moringa oleifera* meal (Mm). Gp (8): HFD + 0.4 mL/rat of *MO*.

Doses of LP, FSO, MO, ME and Mm

LP was suspended in 1% carboxy methyl cellulose solution in distilled water and administered in a dose of 50 mg/kg/d body weight and gavages by P.O for 8 weeks based upon the method of

Mansurah, 2011. FSO was orally given in a dose of 500 mg/kg (1 ml/rat/day for 8 weeks) according to Abdel Moneim (2012). Dry roots and leaves (1kg) of ME were prepared and add to 1.5 L of water and boiled in a pressurized container. The resulting extract from this process was given orally in a dose level of (150mg/kg body weight for 8 weeks) on the basis of Gupta *et al*., (2012). Dry roots and leaves (100 g) of *Moringa* oleifera were mixed with 100 g of basal diet and given for one meal in 72 hours for 4 weeks according to Chinedu *et al*., (2013) and Villarruel-López *et al*., (2018). MO dose was given to rats (0.4 mL/rat) for 8 weeks on the basis of Mansour *et al*., (2012).

Induction of hyperlipidemic

Hyperlipidemia was induced by administration of Triton X-100 at a dose of 400 mg/kg, p.o which we found to induce maximum hyperlipidemia after 48 hours according to Vidyadhara *et al*., (2014).

Measurement of body weight changes

The weight of the individual rats of each group was recorded after the 2nd and the 6th week of the experiment.

Collection of tissue samples

At the end of the experiment, rats were killed using euthanasia and the liver was quickly excised and washed by ice-cold isotonic NaCl saline and processed for investigations. The liver was placed on ice, washed in ice-cold isotonic NaCl saline, blotted dry with filter paper and weighed to determine weigh change. Apart of the liver was separated to use it for immediate enzyme assays. The UV/VIS spectrophotometer (JENWAY 6505, UK) was used for the measurements of enzyme activities.

Antioxidative enzyme activities and biochemical markers assays:

The activity of Total superoxide dismutase (SOD) was assessed according to the method of Paoletti and Mocali (1990). The catalase (CAT) activity was assessed according to the method of Xu *et al*. (1997). Glutathione peroxidase (GPX) activity was measured by the method of Hafeman *et al*. (1974). Glutathione reductase (GR) activity was measured by the method of Smith *et al*. (1988). Malondialdehyde (MDA) level was measured according to the method of Buege and Aust (1978).

Statistical analysis

The data obtained were statistically analyzed and presented as means ± Standard deviations (SD). The statistical evaluation of all data was done using one- way analysis of variance (ANOVA) followed by the student's *t* test using a computer program (Graphpad InState Software, Inc) to compare the body weights between the controls and treated groups. For all comparisons, p-values less than 5% ($P<0.01$) and ($P<0.05$) were considered to be statistically significant.

RESULTS

Effect of treatments on hyperlipidemia

The results of the body weight in all groups after the 2nd week of treatments showed insignificant increase ($p>0.05$) as shown in Figure 1. Also, the results of the body weight in groups (1, 2, 3, 5, 6, 7, 8) after the 6th week of treatments showed insignificant increase ($p > 0.05$) as compared to GP (A). But the results of the body weight in GP (4) after the 6th week of treatments) showed a significant increase ($p < 0.05$) as compared to GP (A as shown in Figure 2.

Effect of treatment on antioxidative enzyme

After the 6th week of treatments compared to GP (A) showed a significant increase ($p<0.01$). On the contrary, the comparison between groups (1, 2, 3 and 7) after the 6th week of treatments compared to GP (A) showed insignificant decrease ($p>0.05$) as shown in Figure 7.

activity and the biomarker

The superoxide dismutase (SOD) activity (U/gm./50% inhibition /wet weight tissue) of liver in groups (4, 5, 6, and 8) and GP (B) after the 6th week of treatments showed a significant increase ($p<0.01$) as compared to GP(A). On the other hand, the comparison between groups (1, 2, 3 and 7) after the 6th week of treatments compared to GP (A), the SOD showed insignificant decrease ($p>0.05$) (Figure 3).

Catalase (CAT) activity (µm/min/gm/wet weight tissue) of liver in groups (4, 5, 6 and 8) and GP (B) after the 6th week of treatments compared to GP (A) showed a significant decrease ($p<0.01$). On the other hand, the comparison between groups (1, 2, 3 and 7) after 6th week of treatments to GP (A) showed insignificant increase ($p>0.05$) (Figure 4).

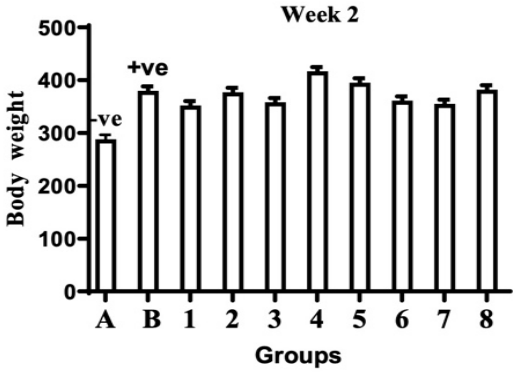

Figure 1. Effect of Atorvastatin, Flaxseed oil and *Moringa oleifera* on the body weight in Hyperlipidemia male albino rat groups after 2nd week of treatments. Each reading represents Mean ± SD of 10 rats. Asterisk indicates a significant increase compared to Gp (A) at P<0.01 and P<0.05 determined using ANOVA followed by Dunnett s' test.

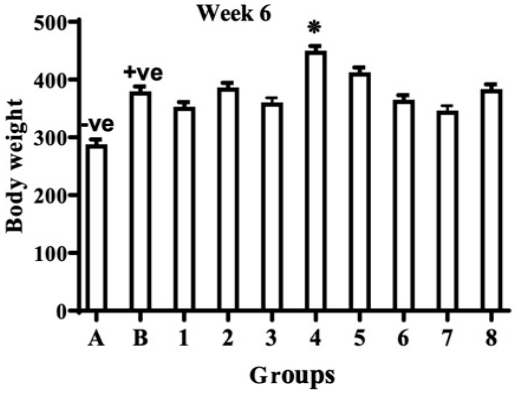

Figure 2. Effect of Atorvastatin, Flaxseed oil and Moringa oeifera on the body weight in Hyperlipidemia male albino rat groups after 6th week of treatments. Each reading represents Mean ± SD of 10 rats. Asterisk indicates a significant increase compared to Gp (A) at P<0.01 and P<0.05 determined using ANOVA followed by Dunnett s' test.

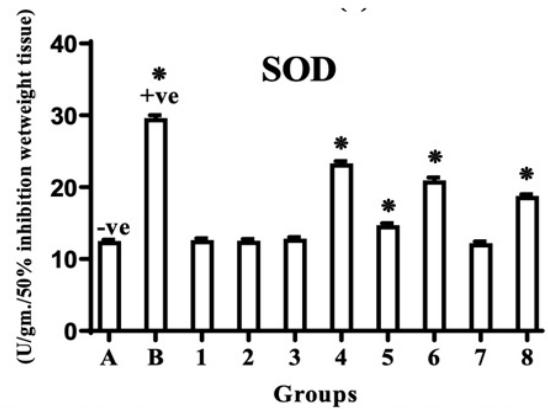

Figure 3. Changes in SOD activity (U/gm./50% inhibition wet weight tissue) in liver of Hyperlipidemic male albino rat groups after 6th week of treatments. Each reading represents Mean ± SD of 10 rats. Asterisk indicates a significant increase compare to Gp (A) at P<0.01 and P<0.05 determined using ANOVA followed by Dunnett s test.

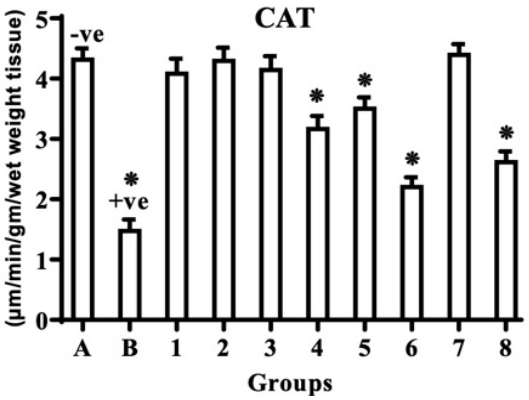

Figure 4. Changes in CAT activity (µm/min/gm/wet weight tissue) in liver of Hyperlipidemic male albino rat groups after 6th week of treatments. Each reading represents Mean ± SD of 10 rats. Asterisk indicates a significant decrease compared to Gp (A) at P<0.01 and P<0.05 determined using ANOVA followed by Dunnett s' test

Glutathione reductase (GR) activity (µm/min/gm/ wet weight tissue) of liver in groups (4, 5, 6 and 8) and GP (B) after 6th week of treatments compared to GP (A) showed a significant decrease (p<0.01). On other hand, the comparison between groups (1, 2, 3 and 7) after the 6th week of treatments compared to GP (A) showed insignificant increase (p>0.05) as shown in Figure 5.

Glutathione peroxidase (GPX) activity (µm/min/ gm/wet weight tissue) of liver in groups (3, 4, 5, 6 and 8) and GP (B) after the 6th week of treatments compared to GP (A) showed a significant decrease (p<0.01). While the comparison between groups (1,2 and 7) after the 6th week of treatments compared to GP (A) showed insignificant increase (p>0.05) as shown in Figure 6.

The malondialdehyde (MDA) level (nm//gm/ wet weight tissue) of liver (lipid peroxidation biomarker) in groups (4, 5, 6 and 8) and GP (B) in the present study, insignificant increase was observed in the body weight of atorvastatin treated hypercholesterolemia male albino rats. Some studies showed that statins increase the body and liver fat accumulation in obese Zucker rats (Aguirre *et al*., 2013). Also, other studies reported a significant increase in the body weight after one year of atorvastatin randomization (Dzien *et al*., 2013 and Ong *et al*., 2014). It is widely accepted that statins significantly decrease the incidence of cardiovascular complications, and the great benefit of statins outweigh the smaller risk of glucose impairment or weight gain in high-risk populations (Belalcazar *et al*., 2009).

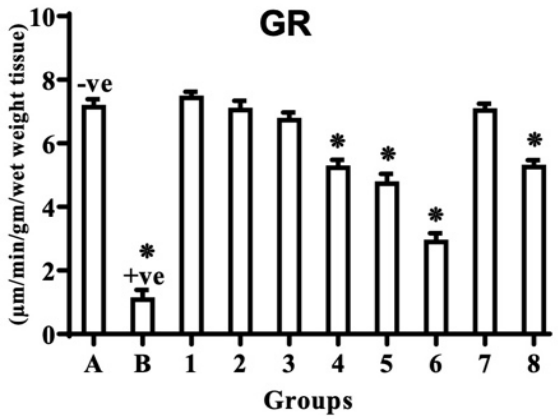

Figure 5. Changes in GR activity (µm/min/gm/wet weight tissue) in liver of Hyperlipidemic male albino rat groups after 6th week of treatments. Each reading represents Mean ± SD of 10 rats. Asterisk indicates a significant decrease compared to Gp (A) at P<0.01 and P<0.05 determined using ANOVA followed by Dunnett s' test.

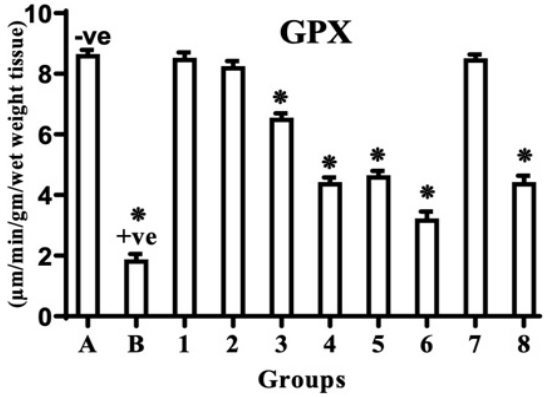

Figure 6. Changes in GPX (µm/min/gm/wet weight tissue) in liver of Hyperlipidemia male albino rat groups after 6th week of treatments. Each reading represents Mean ± SD of 10 rats. Asterisk indicates a significant decrease compared to Gp (A) at P<0.01 and P<0.05 determined using ANOVA followed by Dunnett s' test.

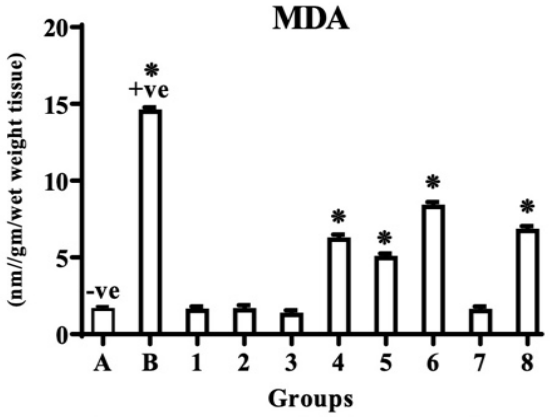

Figure 7. Changes in MDA level (nm/gm/wet weight tissue) in liver of Hyperlipidemic male albino rat groups after 6th week of treatments. Each reading represents Mean ± SD of 10 rats. Asterisk indicates a significant increase to Gp (A) at P<0.01 and P<0.05 determined using ANOVA followed by Dunnett s' test.

From our study, atorvastatin significantly improved the level of antioxidative enzyme activities (SOD, CAT, GR, and GPX) and the biomarker (MDA). Passi *et al*., (2003) and Danesh and Kanwar (2004) reported that the protective effects of atorvastatin on reactive oxygen species (ROS) included cholesterol dependent and non-cholesterol dependent antioxidative properties. For example, Wassmann *et al*., (2002) concluded that atorvastatin caused a significant increase in the CAT activity. Meanwhile, Sakabe *et al*., (2003) found that insignificant correlations between the observed changes in the pleiotropic effect of atorvastatin regarding antioxidative properties and the improvement in the lipid profile**.** It was suggested that the pleiotropic effect of atorvastatin was due predominantly to inhibition of isopreniods (treatments) but not cholesterol synthesis (Jozef *et al*., 2005).

The antioxidative potency of the flaxseed has been shown to reduce total cholesterol (Katare *et al*., 2012) as well as platelet aggregation. The flaxseed lignin and mammalian lignans enter diol and enterolactone were previously shown to be effective antioxidants against DNA damage and lipid peroxidation. The data of flaxseed oil (FSO) showed a significant increase of the body weight gain and insignificant improve of antioxidative enzyme activities (SOD, CAT, GR, GP_X) and the biomarker (MDA). Hala and Thanaa (2013) reported that the FSO increase of body weight gain, induces a good antioxidant activity and alleviates oxidative stress in rats. Oral administration of (FSO) to rats with experimental oxidative stress improved total cholesterol and triglycerides, produced a good antioxidant activity (Abd El-Ghany *et al*., 2011**,** Naqshbandi *et al.,* 2012). The lipid lowering effect and MDA reduction of FSO were reported (Prasad, 2005 and Khan *et al*., 2012). The antioxidant activity of FSO could be attributed to its high content of omega-3 polyunsaturated fatty acid (PUFA). However, Lee and Prasad, (2003) concluded that FSO (Omega Nutrition) suppresses oxygen radical production by white blood cells and improves cardiovascular health due to its powerful antioxidant activity. It was found that dietary flaxseed may have significant health-related benefits due to its high content of the omega-3 fatty acid, alpha-linolenic acid (Austria *et al*., 2008; Abdel Moneim *et al*., 2011). Also, it was reported that the flaxseed oil

significantly increased the activity of antioxidative enzyme glutathione peroxidase, superoxide dismutase and catalase in the liver tissue of rats with oxidative stress.

Moringa oleifera leaves act as a good natural source of antioxidant vitamins/compounds such as ascorbic acid, flavonoids, phenolics and carotenoids. The higher concentration of ascorbic acid, estrogenic compounds and others such as methionine, cysteine, tryptophan and lysine present in *Moringa oleifera leaves* make it virtually an ideal nutritional supplement (Abd El-Aziz *et al*., 2015). This would explain the therapeutic use of *Moringa oleifera* edible parts in many regions of Africa as well as Asia by patients affected by diabetes, hypertension, or HIV/AIDS (Kasolo *et al*., 2010). It possesses various biomedical properties such as anti- inflammatory, antioxidant, antimicrobial, antifertility, anticancer, antihepatotoxic, and antiulcer activities (Goyal *et al*., 2007).

Rats fed on Moringa oleifera (meal) showed a significant decrease in the body weight and improved lipid profile and antioxidative enzyme activities (SOD, CAT, GPX and GR) and oxidative biomarker (MDA). The leaf powder consumption brings new knowledge about the safety of this plant while providing options for plant preservation without the loss of nutrients, since the leaf of this plant can be used as a vegetable in soup preparations, cooked and mixed in with ground peanut cake or packaged in powder pills (Villarruel-López *et al*., 2018). It was observed that oral administration of extract of *Moringa oleifera* significantly reduces blood glucose concentration and inhibits weight loss (Olayaki *et al*., 2015). Another study showed a significant increase in the body weight, specifically essential amino acids and vitamins. In contrast, the body weights of rats were further increased significantly after increasing *Moringa oleifer* concentration in the diet. The highest body weight gain of rats in AL3 could support earlier reports that *Moringa oleifera* is of a high nutritional value (Anwar *et al.,* 2007). The increase in the body weight of rats might be due to the fact that *Moringa Oleifera* is rich in amino acids, vitamins and minerals particularly iron (Faye *et al*., 2011). The significant increase in body weights of rats might also be attributed to captivity, where energy expenditure is minimal (Fadi *et al.,* 2010). While the significant reduction in the body weight by the extract in female rats fed with high fat diet was partly attributed to inhibition of cholesterol deposition in body tissues or inhibition of 3-hydroxy-3- methylglutaryl coenzyme A (HMG CoA) reductase activity which is the key regulatory enzyme in cholesterol biosynthetic pathway (Patil *et al*., 2010).

It can be concluded that flaxseed oil increased the body weight with less effects on antioxidative enzyme activities. Its action is ineffective in short term treatments but it may take a longer time to show a marked effect. Also, *moringa olefira* extract and oil may give the same effect of flaxseed oil on antioxidative enzyme activities but can decrease the body weight. However, *Moringa olefiera* (meal) can decrease the body weight and improve the antioxidative enzyme activities. For that reason, it might be included in a wide scale in food and can be commercially used in different food products.

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