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Effect of Vegetarian Diet and Functional Meat Based Diet on Serum Lipid Profiles, Liver Functions and Oxidative Stress Related Indices: A Mice Model Study

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ABSTRACT

Epidemiological evidence suggests that intake of high amount of non-vegetarian diet is associated with increased risk of chronic diseases including atherosclerosis, hyperlipidemia and cancers. But, the research study on the effect of functional meat product in physiological functions of health is still pending. Therefore, the aim of this study was to elucidate effect of whole grain cereal flours and some plant derived bioactive compounds intake on antioxidant activity, MDA content, serum lipid profile, hepatic and renal functions and tissue histopathology of vital organs were examined in mice model study. For this, a total thirty six adult male Swiss albino mice were randomly divided into three groups (3×12): one group fed control diet (vegetarian diet, CD), another group fed control wafers (CW) and the third group fed functional wafers (FW). The FW contained whole grain oat and sorghum flours, apple peel paste, oregano, aloe vera gel and vitamin E. Result indicated that FW lowered overall serum total cholesterol (TC) and LDL-C levels but HDL-C remain unchanged. Compared with control group, the TC and LDL-C in FW group were lowered by 15.61 and 16.51 mg/dL, respectively. The TGL was lowest in mice received CD. The serum glucose level was decreased in CW and FW fed mice. The activity of hepatic and renal enzymes were lowered ($P<0.01$) with the feeding of FW. The antioxidant enzymes viz., CAT and GSH-Px, however increased in the FW group by 14.0 and 2.27 U/mL respectively ($P<0.05$). The antioxidant enzymes in FW group mice decreased the MDA formation. This it was concluded that FW intake could greatly help in amelioration of oxidative stress related chronic diseases besides improving most of the lipid profile parameters.

Keywords: *Functional meat products, Glycemic index, Oxidative stress, Antioxidants, Hepatic and kidney disorders, Lipid profiles*

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INTRODUCTION

Recent evidence of epidemiological studies indicates consumption of a diet with high amounts of processed red meats is associated with an increased risk of chronic diseases *viz.,* coronary heart disease, colorectal cancer and type II diabetes (Battaglia *et al.,* 2015). It has been reported that around 32 percent of deaths due to these diseases could be avoided by dietary manipulations (Wolfe *et al.*, 2003). So, functional food products are processed for improving antioxidant activity, chemomodulatory activity, gut acting activity, altering serum lipid profiles by decreasing LDL cholesterol and triglycerides, increasing HDL cholesterol etc (Lui, 2007; Shen *et al.,* 2017). It has been reported that consumption of whole grain flours like Oat flour (Avena sativa L.) rich in β-glucan could reduce total and low density lipoprotein (LDL) and glycemic responses (Jenkins *et al.,* 2002) besides up-regulating immune system through release of cytokine interleukin 1b (IL-1b) (Dongowski *et al.,* 2002) while Sorghum flour (Sorghum bicolour) acts as potential source of antioxidants for human (Awika *et al.,* 2003).

Other research reports also indicated uses of plant parts as functional ingredients in meat foods. Drumstick leaf (Moringa oleifera) powder is rich in polyphenols, β-carotene, iron (Fe), potassium (K), calcium (Ca) and vitamin C while apple (Malus domestica B.) and banana (Musa spp.) peels are major contributors of soluble fibers, phenolic compounds and tannins (Biswas *et al.,* 2015). It has been reported that an increase consumption of fruits and vegetables may reduce the risk of lung diseases (Boeing *et al.,* 2012). Long-term intake of flavonoids in the form of fruits and vegetables prevents type II diabetes, cardiovascular diseases (CVD) and impaired cognitive function (Boeing *et al.,* 2012). The tropical aloe vera vera (Aloe barbadensis Miller) exhibited strong antioxidant activities for scavenging free radicals (Biswas *et al.,* 2015) and reducing lipid per-oxidation. Oregano and Vitamin E is well known for their antioxidant activities in food system (Exarchou *et al.,* 2002).

Thus, variable in-vitro study was conducted for assessment of physiological functional role of many plant based bioactive compounds individually or in combination. However, it remains unclear whether incorporation of these compounds in meat products can improve antioxidant activity in vivo (human). Furthermore, the distinction amongst different meat products needs to be made clear to understand the potential benefit of functional meat product consumption over non-functional meat products or only whole grains. Thus, the objective of this study was to examine and compare the effects of consumption of whole grain based diet, meat wafer and functional meat wafer on human health on mice model study.

MATERIAL AND METHODS

Raw materials: Lean meat sample required for the experiments were obtained from adult turkey (Beltsville small white) and spent hens (WLH, above 72 weeks age), whole grains (oat, sorghum, rice, maize and wheat) obtained from local market and apple peel paste, banana peel paste, aloe vera gel and drumstick leaf powder were procured from local botanical garden and processed as the method

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mentioned by Biswas *et al.* (2015). Food grade oregano and basil blend was supplied by Redox Pharmachem Pvt. Ltd., India. Vit. E (Evion, α-tocoferol acetate) procured from Merck Ltd., India.

Chemicals and reagents: All the chemicals, reagents and standards were procured from reputed firms in India and abroad.

Sample preparation: A total three samples were prepared-1. basal diet (BD)- contained corn based formulation as per the stipulated guidelines of the Laboratory Animal Resources (LAR) Section, Indian Veterinary Research Institute (IVRI), Izatnagar, India. 2. control wafers (CW), and 3. functional wafers (FW) samples are meat based diets contained lean meat (chicken and turkey, 30:70), curing ingredients and other seasonings. The CW contained standard formulation (without functional ingredients) while FW contained additional ingredients as mentioned in Table 1. The additional ingredients were quantitatively replaced with the lean meat for CW. The processing techniques for preparation of CW and FW were remained same as reported in our earlier study (Biswas *et al.,* 2015).

Table 1: Ingredients used for preparation of control and functional meat wafers

Sl. No.	Ingredients	Poultry meat wafers	
		Control (%)	Treatment (%)
1	Oat flour	0	4.5
2	Sorghum flour	0	3.0
3	Apple peel paste, Banana peel paste, Aloe Vera gel, Drum stick leaf powder	0	2, 2.5, 2.0 & 0.5 respectively
4	Oregano + basil	0	0.05
5	Vitamin E (α-tocopherol acetate)	0	0.04
6	Table salt	1.3	0.8
7	Potassium chloride)	0	0.5
8	Refined vegetable oil	5.0	0
9	Meat and other non-meat ingredients	93.7	84.11
	Total=	100	100

Experimental animals and diets: The experiment was carried out as per the approved guidelines of IAEC guidelines (No. CAR/CPCSEA/2016/10) has Registration No. /GO/ReBi/S/01/ CPCSEA, dated 25/07/2001). A total of 18 male Swiss albino mice of 6 weeks old were obtained from the LAR Section, IVRI, Izatnagar, India and that were randomly segregated into three different groups. Healthcare and other managerial practices were done as per ARRIVE guidelines. For experimental diets, three different diet were prepared [basal diet (BD), control wafer (CW) with 40% basal diet (CWBD) and functional wafer with 40% basal diet (FWBD)] that were fed to three different groups of mice separately. The chemical composition of the experimental diets is shown in Table 2. Periodical changes in body weight gain and blood serum profiles (glucose index, enzymes, lipids and antioxidants) were assessed at 30, 60 and 90th day of feeding.

Table 2: Nutritional profile of poultry meat wafers

Parameters	CW	FW	SEM	P-value
Moisture (%)	6.78	6.89	0.04	0.248
Protein (%)	42.69^{b}	39.28^{a}	0.66	0.01
Ether extract (%)	12.05^{b}	7.00^{a}	0.39	0.01
Carbohydrate (%)	30.38^{a}	35.76^{b}	0.50	0.01
Crude fiber (%)	1.8^{a}	4.04^{b}	0.42	0.01
Total ash (%)	6.3^{a}	7.03^{b}	0.13	0.01
Total phenolic (GAE mg/100 g)	0.74^{a}	18.55^{b}	3.37	0.01
β-carotene (µg/kg)	307^{a}	407^{b}	19.01	0.01
α-Tocopherol acetate (mg/100 g)	0.15^{a}	3.36^{b}	0.61	0.01
Calorie(Kcal/ 100gm)	401.23	369.37	-	0.01

CW= Control wafer, FW= Functional wafer, SEM=Standard error mean. Mean with different superscript row-wise differ significantly ($p < 0.05$)

Blood collection and biochemical analysis: About 0.2 – 0.3 mL of blood sample from each mice was collected from the orbital sinus of mice using clot activating tubes and serum samples were analyzed for TC, HDL-C, LDL-C, TGL, AST, ALT, ALP, catalase, GSH-Px and glucose concentrations using protocol supplied alongwith the kits (Coral Clinical System, Goa, India). AST and ALT were estimated following the method of Reitman and Frankel (1957). GSH-Px activity was determined by using GSH-Px cellular activity assay kit following the method of Paglia and Valentine (1967) while serum glucose was estimated by the glucose oxidase and peroxidase method as described by Henry *et al.* (1986).

Antioxidant activity: The DPPH and ABTS+ radical scavenging activity and MDA contents of serum samples were determined as per methodology mentioned by Biswas *et al.* (2015).

Histopathological study: The liver, kidney, spleen, intestine and heart were dissected from the mice, excised, washed with physiological normal saline, fixed in 10% neutral formalin, dehydrated in ascending grades of alcohol and imbedded in paraffin wax. Paraffin sections were taken at 5 µm thick and were stained with Haematoxylin and Eosin (H and E). The sections were examined for histopathological changes (×10) under light microscope. The live fields were scored according to Field *et al.* (2008).

Statistical analysis: Data generated from the experimental study were subjected to statistical analysis following the standard procedures of Snedecor and Cochran (1994) with the help of SPSS 20 software package. Data generated were analyzed using two-way ANOVA, homogeneity test and Duncan's Multiple Range Test (DMRT) for comparing the means to find the effects between treatment, between feeding periods and their interactions and a P-value less than 0.05 was taken to indicate statistical significance.

RESULTS AND DISCUSSION

Effect on serum lipid profile: To study functional activity of basal diet (BD), CWBD and FWBD, the lipid profile in serum were determined at every 30 days interval until end of the experiment. As shown in Table 3, the inclusion of the functional wafer in basal diet decreased ($p< 0.01$) serum total cholesterol (TC) and LDL-C. HDL-C though had higher for BD but differed non-significantly from FWBD. The triglycerides (TGL) content was significantly low for BD fed group. These results indicate that functional wafer and BD could reduce TC and LDL-C levels, but had very little influence on HDL-C levels. At the end of the 90 days feeding trials it was observed that compare to CWBD group, TC, LDL-C, and TGL of mice in the FWBD group were decreased by 76.46, 76.87 & 22.82%, respectively, whereas HDL-C were increased by 12.76 %.Similarly, while compared with BD group, the TC and LDL-C in FWBD were lowered by 8.08 and 16.63 %, respectively, whereas HDL-C was increased by 20.28%. The TGL was lowest in mice fed with BD and it was decreased by 16.33% as compared to FWBD. These results demonstrated that incorporation of FW in BD have greatest effect in reduction of hypolipidemic effects.

The alterations in serum lipid profile by feeding of these functional ingredients were reported. Hyson (2011) reported that consumption of an apple peel powder daily for more than one month significantly reduced the serum triglycerides, total cholesterol content in woman having high lipid content. Similarly, Bobek *et al.* (1998) also showed that supplementation with apple alongwith a high cholesterol diet in rats, reduced the amount of triglyceride, low density of lipoprotein LDL and total triglyceride and increased HDL concentration. These effects can be due to antioxidant activity of bioactive compounds of food constituents that were linked, probably by inhibiting lipid peroxidation and decrease production ofcholesterol, LDL and triglycerides. Flavonoids content in banana showed hypolipidemic activity evidenced by decrease in cholesterol, triglycerides (TG), free fatty acids and phospholipids levels in serum, liver, kidney and brain of rats. The cholesterol lowering effect was attributed to a higher degradation rate of cholesterol than synthesis. Alinejad-Mofrad *et al.* (2015) reported that total cholesterol, LDL- cholesterol and triglycerides levels were reduced in human following consumption

Table 3: Serum lipid profile of mice fed with different experimental diets

Group/ Period	30d	60d	90d	Group Mean	P-value			SEM
					G	P	G*P	
Total cholesterol (mg/dl)								
BD	176.68^{q}	82.33^{p}	111.57^{p}	123.53^{b}	0.005	0.001	0.001	7.536
CWBD	117.08^{p}	88.87^{p}	192.71^{q}	132.88^{b}				
FWBD	119.68^{p}	100.85^{p}	103.22^{p}	107.92^{a}				
Period Mean	137.81^{y}	90.68^{x}	135.84^{y}					
HDL-Cholesterol (mg/dl)								
BD	90.32^{t}	71.70^{qrs}	59.33^{p}	73.78^{b}	0.001	0.001	0.001	2.460
CWBD	64.67^{pqr}	63.50^{pqr}	59.78^{p}	62.65^{a}				
FWBD	78.41^{s}	62.61^{pq}	74.43^{rs}	71.82^{b}				
Period Mean	77.80^{y}	65.94^{x}	64.51^{x}					
LDL-Cholesterol (mg/dl)								
BD	116.35^{r}	56.88^{p}	134.12^{s}	102.45^{b}	0.001	0.001	0.001	8.083
CWBD	88.03^{q}	59.36^{p}	203.39^{t}	116.92^{c}				
FWBD	87.76^{q}	55.05^{p}	114.99^{r}	85.94^{a}				
Period Mean	97.38^{y}	57.10^{x}	150.83^{z}					
Triglycerides (mg/dl)								
BD	192.09^{pq}	174.45^{p}	174.43^{p}	180.33^{a}	0.014	0.080	0.009	8.198
CWBD	142.06^{p}	257.39^{r}	249.26^{qr}	216.24^{c}				
FWBD	197.34^{pqr}	202.94^{pqr}	202.94^{pqr}	201.07^{b}				
Period Mean	177.16^{X}	211.59^{Y}	208.88^{Y}					

^{pqrs}Means bearing different superscripts differ significantly ($p <0.05$). ^{abc}Means bearing different superscripts differ significantly ($p <0.05$). ^{xyz}Means bearing different superscripts differ significantly ($p <0.05$). BD=basal diet, CWBD=control wafer with 40% basal diet, FWBD=functional wafer with 40% basal diet.

of 500 mg of aloe vera extract for eight weeks and suggested that these effects were due to its suppressive effect on adipogenesis gene. Jain *et al.* (2010) found that the serum cholesterol, triacylglyceride, VLDL, LDL and atherogenic index were reduced by *M. oleifera* but HDL level was increased. This hypocholestrolemic effect was attributed that *M. oleifera* increase the excretion of fecal cholesterol.

Effect on liver and antioxidant enzyme activity: The effects of feeding of BD, CWBD and FWBD in mice on circulating levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were diverse (Table 4). The activity of serum enzyme AST, ALT and ALP were found to be significantly (p <0.01) lowered in FWBD fed group than the other two groups. However, activities of these enzymes were significantly increased in all groups during the course of feeding. The activity of ALP in CWBD fed group though statistically non-significant from BD group, it was highly significant from FWBD fed group at the day 90. Similarly, AST and ALT was increased greatly in CWBD fed group than the other two groups and significantly (p < 0.01) increased at all intervals. As shown in Table 4, compared with the CWBD group, AST, ALT and ALP in the serum of the FWBD group were decreased (p<0.01). This indicated that bioactive compounds of functional wafer protect the liver damage *in vivo*, there by decreased formation of these enzymes.

Feeding of different experimental diets significantly (p <0.05) influenced the serum catalase and glutathione peroxidase (GSH-Px) levels in mice (Table 4). The activity of both these antioxidants enzymes were steadily increased at each feeding intervals during entire period of study in all the fed groups of mice. However, the activity of catalase and GSH-Px were found significantly higher in FWBD fed group than the other two groups. As shown in Table 4, catalase (CAT) and glutathione peroxidase (GSH-Px) levels in the serum of the BD group were increased by 16.67% and 5.30% respectively (p< 0.05), whereas the same enzymes in the serum

Table 4: Test for enzymes responsible for liver damage and antioxidant enzymes

Group/ Period	30d	60d	90d	Group Mean	P-value G	P	G*P	SEM
AST (IU/L)								
BD	33.52^{p}	174.20^{rs}	257.60t	155.11^{b}	0.001	0.001	0.001	16.161
CWBD	29.46^{p}	188.85^{s}	309.06u	175.79^{c}				
FWBD	42.37^{p}	140.48^{qr}	115.61^{q}	99.49^{a}				
Period Mean	35.12^{x}	167.84^{y}	227.43^{z}					
ALT (IU/L)								
BD	59.15p^{q}	93.57^{r}	162.73^{s}	105.15b	0.001	0.001	0.001	8.976
CWBD	67.04^{q}	67.67^{q}	210.27^{t}	114.99b				
FWBD	43.92^{p}	91.85^{r}	70.04^{q}	68.60a				
Period Mean	56.71^{x}	84.36^{y}	147.68^{z}					
ALP activity (K.A Units)								
BD	42.67^{q}	84.21^{r}	130.22st	85.70^{b}	0.001	0.001	0.001	12.652
CWBD	39.54^{q}	97.37^{s}	141.21^{t}	92.70^{c}				
FWBD	29.90^{p}	83.81^{r}	77.31^{qr}	63.67^{a}				
Period Mean	37.37^{x}	88.59^{y}	116.24^{z}					
Catalase activity (U/ml)								
BD	0.54p	0.70q	0.65pq	0.63a	0.001	0.001	0.001	8.254
CWBD	0.54p	0.75qr	0.80r	0.69b				
FWBD	0.65pq	0.78qr	0.90s	0.77c				
Period Mean	0.57x	0.74y	0.78z					
Glutathione peroxidase activity (U/ml)								
BD	11.50^{p}	12.05^{pq}	12.11^{pq}	11.88^{a}	0.001	0.001	0.001	12.258
CWBD	12.11^{pq}	13.55^{q}	14.15^{qr}	13.27^{b}				
FWBD	12.27^{pq}	13.87^{q}	16.33^{r}	14.15^{c}				
Period Mean	11.96^{x}	13.15^{y}	14.19^{z}					

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of the CWBD and FWBD group were increased by 48.14 and 16.84%, and 38.46 and 33.08%, respectively ($p< 0.05$) at the end of the study. Indeed, functional wafer incorporated with 40% BD seems to have potent antioxidant enzyme activity in animal system.

ALP, AST and ALT are the major serum hepatic enzymes used for assessment of liver function. The elevated activities of these enzymes in serum are an indication of liver damage. Based on study conducted by Cefarelli *et al.* (2006), flavonoids such as quercetin and its derivatives are the anti-free radical of apple. So, decreasing the liver enzymes may be due to the antioxidant feature and phenolic compounds and also the high percentage of potassium and phytoestrogens of apple. Similar findings were reported by Martin *et al.* (2007). It was found that the flavonoids from banana stimulates the activities of superoxide dismutase (SOD) and catalase which might be responsible for the reduced level of peroxidation products such as MDA, hydroperoxides and conjugated dienes. Previous studies of aloe vera gel have documented its anti-inflammatory and anti-oxidant and these were agreed with previous studies conducted by (Rajasekaran *et al.,* 2005; Saritha and Anilakumar, 2010) and in those studies it has been concluded that aloe vera maintains normal levels of AST and ALP in rat. But activity of *Moringa oleifera* is multifarious. It protected the hepatocytes from alcohol-induced liver injury and subsequent leakage of enzymes in to the circulation. These compounds also quench ROS and regenerate membrane-bound antioxidants levels in serum. Singh *et al.* (2000) reported that the increase in the levels of antioxidant profiles i.e. GPx, GR, SOD and catalase by *Moringa oleifera* extract may be attributed to have biological significance in eliminating reactive free radicals that may affect the normal functioning of cells. Thus, it is proposed that GPx is responsible for the detoxification of hydrogen peroxide in low concentration whereas catalase comes into play when GPx is saturated with the substrate.

Effect on serum glucose: The serum glucose level in mice fed with different experimental diets was observed (in Table 5) to understand the influence of feeding of BD (grains alone), control wafer (meat based) with 40 % BD (CWBD) and functional wafer (meat based) with 40 % BD (FWBD). It has been observed that the circulating level of serum glucose was increased in mice fed with BD, whereas that was decreased ($p<0.05$) in CWBD and FWBD groups at the end of the 90 days feeding trials. A significant variation was also observed in glucose level amongst the different groups at each feeding intervals. As evident from the Table 4 serum glucose levels in mice fed with BD were increased by 11.93 and 6.52% at day 60 and 90, respectively, whereas glucose levels in the serum of CWBD group mice were decreased by 30.74 and 10.42 % respectively. The glucose levels in the serum of FWBD group mice were 19.41 and 21.23% for the same periods.

Glyceamic index (GI) is the relative ranking of carbohydrate in a food how actually they affect the blood glucose levels. GI of 55 or less relatively slowly digested, absorbed and metabolized, so chances of increase of blood glucose level is slow. It has been observed that apple peel supplemented diets reduced blood glucose levels while they also reduced insulin levels in CF-1 mice. These results suggest apple peels had greater a role in glucose metabolism, however the mechanism for this improvement remains elusive. While Chauhan *et al.* (2010) reported that a pectin type polysaccharide obtained from the banana peel extract exhibited hypoglycaemic activity in normal and streptozotocins induced diabetic mice by stimulating the secretion of insulin and reducing the glycogen content in the mice Ali *et al.* (1993) reported that aqueous extract of *M. oleifera* leaves reduces the blood glucose level in normal rats and normalizes the high blood glucose levels in sub, mild and severely diabetic rats. It also improves glucose tolerance in normal, sub and mild diabetic animals.

Effect on antioxidant activity and MDA content: The 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^+$) 2, 2-diphenyl-1-picrylhydrazyl (DPPH) are the two assays widely used to evaluate free radical scavenging activity of the antioxidant compounds. The circulating levels of serum ABTS+ (% inhibition) and DPPH (% inhibition) activities for BD group were increased by 4.43% and 3.0% respectively, whereas in the serum of CWBD and FWBD groups were increased by 5.84% and 6.96%, and 30.22% and 18.55%, respectively (Table 5).The highest and the lowest $ABTS^+$ scavenging activities were found in the mice serum fed FW with 40 % basal diet and BD group, respectively. The highest DPPH activity was found in the serum of FWBD group, followed by CWBD and BD groups. Thus, these results showed that feeding of functional wafer markedly improved antioxidant activity *in vivo*.

Feeding of functional wafer significantly influenced MDA contents in the serum of experimental mice and they are highly correlated with the activities of antioxidant enzymes (Table 5). However, feeding of experimental mice for 90 days the MDA contents in the serum of BD group were decreased by 7.69%, whereas in the serum of CWBD and FWBD groups MDA contents were decreased by 30.0% and 41.66%, respectively ($p < 0.05$). These indicate that bioactive compounds of functional wafer had protective effect on lipid oxidation, thereby formed very little peroxides in the blood.

Antioxidant potential of apple peel, banana peel, aloe vera and drumstick leaf powder is well documented. Antioxidant activity of banana peel was reported due to presence of vitamin C, vitamin A, glutathione, flavonoids and phenolics which have potent antioxidant property. It could also be due to its glycosides and monosaccharide components (Mokbel and Hashinaga, 2005). *Moringa oleifera* leaf extract significantly reduced DPPH radicals. Recently, Charoensin and Wongpoomchai (2012) reported that the aqueous extract of *Moringa oleifera* leaves contained polyphenols and had DPPH and ABTS free radical scavenging activity. Antioxidants, on interaction with DPPH, either transfer an electron or hydrogen atom to DPPH, thus neutralizing its free radical character. TBARS value is used for the estimation of lipid per oxidation (LPO) and expressed in terms of malondialdehyde (MDA) content. In a study, it was suggested that banana peel contained large amounts of dopamine and L-dopa,

Table 5: Serum glucose level, antioxidant activity and TBARS (MDA content) value

Group/ Period	30d	60d	90d	Group Mean	P-value G	P	G*P	SEM
Serum Glucose (mg/dl)								
BBD	92.52^{rs}	103.56^{s}	60.04^{p}	85.37^{ab}	0.0.32	0.002	0.001	3.081
CWBD	92.56^{rs}	64.10^{pq}	82.91^{qrs}	79.86^{a}				
FWBD	102.17^{s}	82.34^{qrs}	80.48^{qr}	88.33^{b}				
Period Mean	95.75^{y}	83.33^{x}	74.48^{x}					
ABTS (% inhibition)								
BD	12.15^{p}	14.25^{p}	16.58^{pq}	14.32^{a}	0.001	0.001	0.001	8.541
CWBD	18.41^{q}	20.54^{qr}	24.25^{r}					
FWBD	22.25^{qr}	46.57st	52.47st					
Period Mean	17.61^{x}	27.12^{y}	31.10^{z}					
DPPH (% inhibition)								
BD	8.24^{p}	10.25^{pq}	11.24^{pq}	9.91^{a}	0.001	0.001	0.001	12.125
CWBD	10.29^{pq}	15.64^{qr}	17.25^{r}	14.39^{b}				
FWBD	24.21^{rs}	41.25^{t}	42.76^{t}	36.07^{c}				
Period Mean	14.24^{x}	22.38^{y}	23.75^{y}					
TBARS (mg MDA/dl sample)								
BD	0.012^{pq}	0.013^{pq}	0.013^{pq}	0.012^{b}	0.001	0.001	0.001	8.256
CWBD	0.014^{r}	0.018^{s}	0.020^{s}	0.017^{c}				
FWBD	0.007^{p}	0.009^{p}	0.012^{pq}	0.009^{a}				
Period Mean	0.011^{x}	0.013^{y}	0.015^{z}					

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catecholamines with a significant antioxidant activity (Chauhan 2010). Similarly, aloe vera increased levels of antioxidant enzymes and thereby significantly reduced lipid per-oxidation products in streptozotocin-induced diabetic rats, showing the relationship between antioxidant activity and the onset of diabetes (Rajasekaran *et al.,* 2005).It has been reported that pre-treatment with M. oleifera extract before alcohol administration significantly decreased the levels of lipid per-oxidation (TBARS) in the rat blood. The anti-peroxidative effect may be due to the presence of antioxidants in the leaves such as β-carotene, α-tocopherol and vitamin C (Nadro *et al.,* 2006). Oregano extracts contain high concentrations of polyphenols; primarily rosmarinic acid, carvacrol and thymol constitute its major antioxidant capacity (Vekiari *et al.,* 1993).

Tissue Histopathology: To investigate the effect of incorporation of functional meat product in normal diet (basal diet), various vital organs were collected to elucidate the changes occurred after 90 days of experimental study. Fig. 1 shows that no histopathological changes were observed in liver, spleen, kidney, heart and intestine of Albino mice in different groups. The architecture of liver parenchyma was intact and the hepatocytes, sinusoids and portal area were also found normal. Kidney section showed normal glomeruli, proximal and distal convoluted tubules. Similarly, there was no microscopic lesion observed in the sections of spleen, intestine and heart. These indicated that functional meat product can be safely included in the diet to the albino mice.

There is very little information was found for the effect of dietary supplementation of functional meat products on histopathological changes in vital organs of mice. In a study, it has been reported that feeding of 2% cholesterol in the diets to hypercholesterolemic rat significantly damaged the hepatic and heart tissue since in that study fatty deposits and congestion of hepatic sinusoids, disrupted cells, disrupted hepatic strands, vacuolated cytoplasm, and necrosis were observed (Abulnaja and El Rabey, 2015). However, this study the mice fed with different diets (BD, CWBD and FWBD) did not show any histological and pathological change of different vital organs. Thus, it was felt that basal diets supplementation with control or functional wafers did not show any significantly changes in vital organs rather basal diets alongwith functional wafers could improve functional status of vital organs beyond normal condition.

CONCLUSIONS

This study demonstrated that basal diet supplemented with functional meat wafers lowered serum TC and LDL-C levels but increased HDL-C in Swiss albino mice. TGL level, however remained relatively constant, through significantly increased in the serum of CWBD group mice. Further, positive responses were also

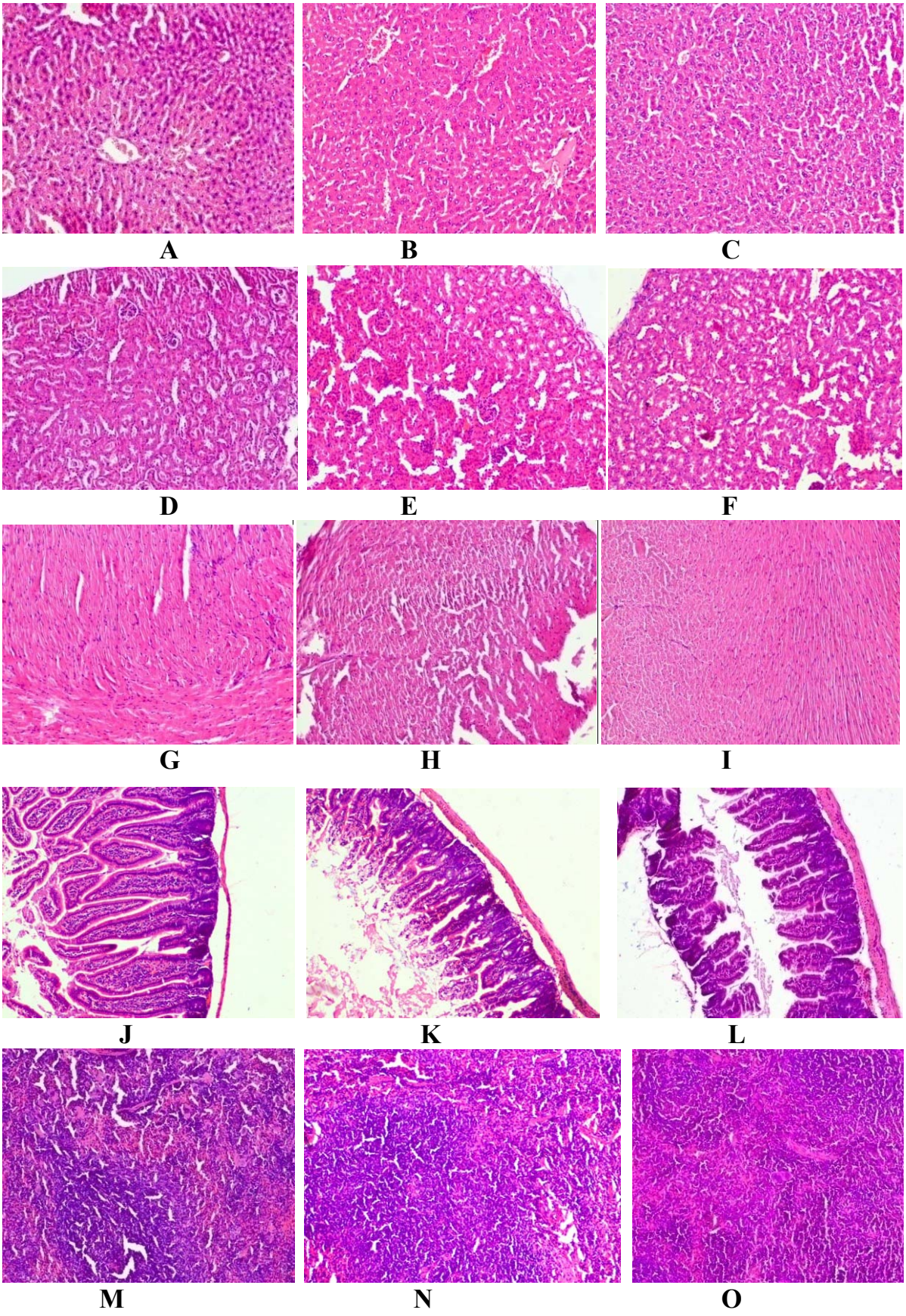

Fig 1. Histopathology of vital organs of mice fed with experimental diets (10 X H & E)

A, B, C; D, E, F; G, H, I; J, K, L & M, N, O representing cross section of vital organs (Liver, Kidney, Heart, Small Intestine and Spleen), respectively. For each organ 1st, 2nd and 3rd picture representing the mice fed with CD, CWBD and FWBD, respectively.
CD=control diet; CWBD=control wafer with 40% basal diet; FWBD=functional wafer 40% basal diet

found in the activity of all the hepatic and antioxidant enzymes in the serum of FWBD group mice, and thus reduce oxidative stress, thereby decreased formation MDA contents. The histopathological study revealed normal appearance for vital organs from all groups of mice. So, consumption of functional meat wafers alongwith basal diet could greatly help in amelioration of the oxidative stress and improving liver enzymes functions besides improving lipid profiles.

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ETHICS STATEMENT: The protocol and procedures employed were ethically reviewed and approved by the appropriate Institutional Animal Ethical Committee confirming compliance with all requirements.

REFERENCES

Abulnaja KO, El Rabey HA (2015) The efficiency of barley *Hordeum vulgare*) bran in ameliorating blood and treating fatty heart and liver of male rats. Evid-Based Complementary Altern Med 2015: 1-13

Ali L, Khan AKA, Mamun MIR, Mosihuzzaman M, Nahar N, Nur-e-Alam M, Rokeya B (1993) Studies on hypoglycemic effects of fruit pulp, seed, and whole plant of *Momordica charantia* on normal and diabetic model rats. Planta Med 59(5): 408 – 412.

Alinejad-Mofrad S, Foadoddini M, Saadatjoo SA, Shayesteh M (2015) Improvement of glucose and lipid profile status with *Aloe vera* in pre-diabetic subjects: a randomized controlled-trial. J Diabetes Metab Disord 14(1): 22.

Awika JM, Dykes L, Gu LW, Rooney LW, Prior RL (2003) Processing of sorghum (*Sorghum bicolor*) and sorghum products alters procyanidin oligomer and polymer distribution and content. J Agric Food Chem 51(18): 5516–5521.

Battaglia RE, Baumer B, Conrad B, Darioli R, Schmid A, Keller U (2015) Health Risks Associated with Meat Consumption: A Review of Epidemiological Studies. Int J Vitam Nutr Res 85(1-2): 70 – 78.

Biswas AK, Beura CK, Yadav AS, Pandey NK, Mendiratta SK, Kataria JM (2015) Influence of novel bioactive compounds from selected fruitby-products and plant materials on the quality and storability of microwave-assisted cooked poultry meat wafer during ambient temperature storage. LWT-Food Sci Technol 62: 727-733.

Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A, Leschik-Bonnet E, Muller M J, Oberritter H, Schulze M, Stehle P, Watzl B (2012) Critical review: vegetables and fruit in the prevention of chronic diseases. Eur J Nutr 51(6): 637–663.

Bobek P, Ozdin L, Hromadova M (1998) The effect of dried tomato, grape and apple pomance on the cholesterol metabolism and antioxidative enzymatic system in rats with hypercholesterolemia. Mol Nutr Food Res 42(5): 317 – 320.

Chauhan A, Sharma PK, Srivastava P, Kumar N, Dudhe R (2010) Plants Having Potential Antidiabetic Activity: A Review. Der Pharmacia Lettre, 2(3): 369-387

Cefarelli G, D'Abrosca B, Fiorentino A, Izzo A, Mastellone C, Pacifico S, Piscopo V (2006) Free-radical-scavenging and antioxidant activities of secondary metabolites from reddened cv. Annurca apple fruits. J Agric Food Chem 54(3): 803 – 809.

Charoensin S, Wongpoomchai R (2012) Effect of aqueous extract of Moringa oleifera leaves on quinone reductase activity. Naresuan Phayao J 5: 101-109.

Dongowski G, Huth M, Gebhardt E, Flamme W (2002) Dietary fiber rich barley products beneficially affect the intestinal tract of rats. J Nutri 132: 3704–3714.

Exarchou V, Nenadis N, Tsimidou M, Gerothanassis IP, Troganis A, Boskou D (2002) Antioxidant activities and phenolic composition of extracts from greek oregano, greek sage, and summer savory. J Agric Food Chem 50 (19): 5294–5299.

Field KM, Dow C, Michael M (2008) Part I: Liver function in oncology: biochemistry and beyond. Lancet Oncol 9: 1092 –1102.

Hayson DA (2011) A comprehensive review of apples and apple components and their relationship to human health. Adv Nutri 2(5): 408 – 420.

Henry RR, Wallance P, Olefsky JM (1986) Effect of weight loss on mechanisms of hyperglycemia in obse non-insulin-dependent diabetes mellitus. Diabetes 35(9): 990 – 998.

Jain PG, Patil SD, Haswani NG, Girase MV, Surana SJ (2010) Hypolipidemic activity of *Moringa oleifera* Lam., Moringaceae, on high fat diet induced hyperlipidemia in albino rats. Revista Brasileira de Farmacognosia 20(6): 969–973.

Jenkins AL, Jenkins DJA, Zdravkovic U, Wursch P, Vuksan V (2002) Depression of the glycaemic index by high levels of β-glucan fibre in two functional foods tested in type 2 diabetes. Eur J Clin Nutr 56: 622 – 628.

Liu R (2007) Whole grain phytochemicals and health. J Cereal Sci 46(3): 207–219.

Martin JHJ, Crotty S, Warren P, Nelson PN (2007) Does an apple a day keep the doctor away because a phytoestrogen a day keeps the virus at bay? A review of the anti-viral properties of phytoestrogens. Phytochemistry 68(3): 266-274.

Mokbel MS, Hashinaga F (2005) Antibacterial and antioxidant activities of banana (Musa, AAA cv. Cavendish) fruits peel. Am J Biochem Biotechnol 1(3): 125-131.

Nadro MS, Arungbemi RM, Dahiru D (2006) Evaluation of *Moringa oleifera* leaf extract on alcohol-induced hepatotoxicity. Trop J Pharm Res 5(1): 539-544

Pagila DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidise. J Lab Clin Med 70(1): 158-69.

Rajasekaran S, Sivagnanam K, Subramanian S (2005) Modulatory effects of *Aloe vera* leaf gel extract on oxidative stress in rats treated with streptozotocin. J Pharm Pharmacol 57: 241-641.

Reitman S, Frankel S (1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28(1): 56 – 63.

Saritha V, Anilakumar KR (2010) Toxicological evaluation of methanol extract of *Aloe vera* in rats. Int J Pharmacol Biomed Res 1:142-149.

Shena Y, Songe X, Chena Y, Li L, Sunb J, Huanga C, Ou S, Zhang H (2017) Effects of sorghum, purple rice and rhubarb rice on lipids status and antioxidant capacity in mice fed a high-fat diet J Functional Foods 39: 103 – 111.

Singh M, Hanalakshini ED, Rao M (2000) Chemomodulatory action of aloe vera on the profiles of enzymes associated with carcinogen metabolism and antioxidant status regulation in mice. Phytomedicine 7: 209-219.

Snedecor GW, Cochran WG (1994) Statistical methods. 8th Edn. The Iowa State University Press Ames, Iowa, USA.

Vekiari SA, Oreopoulou V, Tzia C, Thomopoulos CD, (1993) Oregano flavonoids as lipid antioxidants. J Am Oil Chem Soc 70(5): 483 – 487.

Wolfe K, Wu X, Liu RH (2003) Antioxidant activity of apple peels. J Agric Food Chem 51: 609 – 614.