Effects of *Nigella sativa* Seeds & *Plantago ovata* Husk on Fat Rich Diet Induced Inflammatory Responses in *Rattus norvegicus*

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**ABSTRACT**

Consumption of herbal plants as a remedy to relieve the side effects of fat rich diets is on rise currently. The present study was conducted to scrutinize the impact of fat and fat reducing herbal agents on the serum proteins of *Rattus norvegicus*. Four groups of adult (A) & weaning (W) *R. norvegicus* one control and three experimental groups were designated as 0, I, II and III and were fed on rat chow, fat rich diet (FRD), FRD + 5% *Nigella sativa* seeds and FRD + 5% *Plantago ovata* husk respectively. The total serum protein concentrations in adult groups A-I & A-II were significantly elevated (P<0.01) while significantly lower concentrations were found in group A-III (P<0.001). The comparative serum profile of weaning groups revealed protein fractions of 201KDa in W-III while among the adult rat groups the protein fraction of 86 KDa in A-II group. The protein fractions of 295 KDa, 246 KDa, 133 KDa & 110 KDa were absent in group A-III as compared to other adult groups. It can be concluded that fat rich diets can alter the serum protein levels and seeds of *N. sativa* could preserve serum protein profile in adult *R. norvegicus* contrary to *P. ovata husk* which caused hypoproteinemia. 

**Key words:** Hypoproteinemia, High fat diet, *P. ovata* husk, *N. ovata* seeds, Protein profile, *Rattus norvegicus*

**INTRODUCTION**

Liver is a central player in the whole body homeostasis by its ability to metabolize glucose and fatty acids. When consumption of energy far exceeds the combustion of calories, the unburnt energy is conserved in the form of TG (triglycerides) in adipose tissue, leading to obesity (Evans, et al., 2004; Hamaguchi, et al.,2005). Liver is inflamed and termed as fatty due to fat retention within hepatocytes. Clinically fatty liver disease (FLD) is now broadly categorized into two types, alcoholic FLD (AFLD) and non-alcoholic FLD (NAFLD) (Browning & Horton, 2004; Adams & Angulo, 2005). Inflammation is a part of the non-specific immune response that occurs in reaction to any type of injury. In some disorders this process, which under normal conditions is self-limiting, becomes continuous and chronic inflammatory diseases develop subsequently (Ferrero-Miliani et al., 2007).

The complex series of reactions initiated in response to infection, physical trauma, or malignancy is called the acute-phase response (APR) (Sheikh et al., 2007; Malik et al., 2011). APR is characterized by leukocytosis, fever, alterations in the metabolism of many organs as well as changes in the plasma concentrations of various acute-phase proteins (APPs) (Hack et al., 1997; Gabay & Kushner, 1999). APPs have been defined as any protein whose plasma concentrations increases (positive acute-phase proteins; fibrinogen, serum amyloid A, albumin, C-reactive protein) or decreases (negative acute-phase proteins; albumin, transferrin, insulin growth factor - I) by at least 25 percent during an inflammatory disorder (Morley & Kushner, 1982). These inflammatory responses define the treatment directions. Various allopathic medicines are available to treat the FLD but due to the side effects associated with their long term usage, scientists are now studying the natural herbs for the potential capabilities of controlling diseases.

*Nigella sativa* (Kalonji), is a herb and distributed throughout India (CSIP, 1996; Rifat-Uz-Zaman, 2004). Avicenna referred *N. sativa* seeds as body energy stimulator and a potential helper against fatigue. It is also included in the list of naturally occurring drugs of Tibbe-Nabavi/ Medicines of the Prophet Muhammad (PBUH) owing to the traditional recommendation for healing all diseases (Bukhari, 1985). In "Unani medicines system", *N. sativa* is also a preferred therapy against numerous diseases. Its therapeutic treatments include piles, jaundice, cough, ascites, hydrophobia, dyspepsia, fever, paralysis, skin diseases, flatulence, diarrhea, abdominal disorders, intrinsic hemorrhage, dysentery and amenorrhea. *"N. sativa"* seeds are used in a variety of

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pharmacological and nutritional analysis. Laboratory and human subject studies conducted on its "seeds and oil" have reported their effectiveness to motivate immune response, cure diabetes, rheumatism, local anesthesia, inflammatory diseases and cancer (Haq et al., 1999; MHFS., 1989; Ramadan & Morsed, 2002; Khanam, M., 2007; Warrier et al., 2004).

Dietary fiber cuts down gastro-intestinal-transit time and augments stool weight (Devroede, 1993). The "husk" of "Plantago ovata" commonly called psyllium, which is extensively in use as a supplement dietary fiber to treat constipation. Its husk is acquired by refining the seeds to eliminate the hulls. Its husk has a high percentage of hemicellulose, rhamnose, made up of a "xylan backbone" connected with arabinose, & "galacturonic-acid units" (arabinoxylans). In some researches, seed is used as an alternative to the husk, and is commercially accessible. The seed comprises of “35% soluble” and “65% insoluble” polysaccharides (lignin, hemicellulose, and cellulose). Psyllium is categorized as a “mucilaginous fiber" owing to its potential of gel formation like compound in water because of being the endosperm of the seed, where it retains water to prevent the seed from desiccation. It also has "hypo-cholesterolemic" effects, even though the precise mechanism through which psyllium husk carries out a drop in cholesterol is not clear. (Matheson et al., 1995). Soluble fiber enhancement of the usual diet leads to reductions in "low density lipid cholesterol" (LDL-C) and triglycerides to "high density lipid cholesterol" (HDL-C) ratio (TG/HDL-C) compared to interventions focused on fat reduction (Reid et al., 2002).

Instead of numerous studies conducted on the fat reducing role of P. ovata husks and N. sativa seeds, there is no substantial work reported on fat induced inflammation and the role of these herbs against this inflammatory response. In the current study our main emphasis was on the inflammatory responses of the body after consumption of fat rich diet as well as the impact of the above mentioned fat reducing agents.

MATERIALS & METHODS

Colonies of Rattus norvegicus

Colonies of Rattus norvegicus (Wistar rats) were reared in the “Animal House” of Department of the Zoology, University of the Punjab, Lahore, Pakistan". Animals were divided into two groups depending upon the body weight i.e., weaning (W) rats of 30±10g and adult (A) of 200±15g body weight. Each group was sub-divided into four groups (n=10) designated as group 0, I, II and III on the basis of the different diet compositions (Abbas et al., 2014). The groups 0 and I served the negative and positive control, respectively.

Induction of Inflammation in Rattus norvegicus

Out of the four groups, the group 0 was selected as negative control and was fed on regular rat chow throughout the experimental study, group I served as positive control and received a fat rich diet (FRD) with composition of 33% tea whitener + 20% Sucrose +13% water + 34% ground rat chow (Abbas et al.,2014) . Groups II and III were treated groups and fed on FRD supplemented with 50g N. sativa seeds/P. ovata husk. Animals had ad libitum access to water and food for sixteen weeks, and were kept under 12 hour dark and light cycle.

Sacrificing & sample collection

After feeding for sixteen weeks the animals were fasted overnight and anesthetized by Intraperitoneal (IP) injection of Narcuron (1.5 ml/kg body weight). After dissection the blood was drawn through cardiac puncture and centrifuged to separate serum. To estimate the protein concentration an accurate and rapid Bradford Assay (Bradford, 1976) was performed. Bench Mark protein ladder (Cat. # 10747012) by Life technologies was used for protein profiling employing Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The densitometry analysis for electrophoretically resolved “protein fractions” was carried out by TotalLab Quant v11.5. It provided the data based on “molecular density” of each protein fraction.

RESULTS

Total Protein

The variations in total serum protein concentrations in weaning groups were observed to be non-significant (Figure 1a) while in adult groups A-I and A-II significantly elevated (P<0.01) serum total protein level was observed as compared to the negative control group rats (A-0). However, significantly lower protein concentrations were found in group A-III (P<0.001) in comparison with groups A-I and All (Figure 1b).

Protein Profiling by SDS PAGE

The comparative study of the serum profile of weaning groups against protein ladder of range 10-220 KDa revealed the protein fractions from 54KDa to 280 KDa. The protein fractions of 280 KDa, 134 KDa, 110 KDa, were found in W-I, W-II.
and W-III as compared to the W-0. However, 201 KDa protein fraction was present only in W-III (Figure 2 and 4a). The serum protein fractions of adult rat groups resolved on the 8% SDS-PAGE scrutinized the protein bands from 54 KDa to 295 KDa against protein marker. The protein fractions of 295 KDa, 246 KDa, 133 KDa and 110 KDa were absents in group A-III, while, protein fraction of 86 kDa was present only in A-II group rat sera (Figure 3 and 4b).

**DISCUSSION**

Inflammation performs a fundamental role in the host defense against insidious pathogens, wound and tissue repair (Barton, 2008; Chen & Nunez, 2010). Liver has immediate access to dietary fat and its retention within hepatocytes induces inflammation of the liver resulting in inflammatory response in the form of acute phase proteins (Browning & Horton, 2004; Adams & Angulo, 2005; Galisteo et al., 2005).

The comparative study of the serum protein profile of weaning groups revealed the protein fractions of 54 to 280 KDa. The protein fractions of 280, 134 and 110 KDa, were found in weaning rat groups W-I, W-II and W-III as compared to the W-0. However, protein fraction of 201 KDa was present only in group W-III. The serum protein profile of adult groups scrutinized the protein bands from 54 to 295 KDa. The protein fractions of 295, 246, 133 and 110 KDa were absent in group A-III. However, protein fraction of 86 KDa was present only in group A-II. Haptoglobin (86 KDa) is a "positive acute-phase" protein, its plasma level elevates during inflammation, infections, trauma, malignant proliferation and tissue damage, but declines as a result of severe haemolysis. Thus, varied concentrations of haptoglobin have clinical significance in the diagnosis (Javid, 1978; Bowman & Kurosky, 1982; Dobryszczka, 1997). Protein bands of 110KDa and 270KDa appeared in negative control group (A-0) disappeared in all adult groups except those treated with *P. ovata* husks supplemented diet.

These results indicated the inhibitory effects of fatty diet on sortilin (110 KDa) but also the recovery effects of *P. ovata* husk supplementation, while this fat reducing herbal agent drastically affected a protein band of 310KDa which appeared in all adult groups otherwise. Sortilin (110 KDa) in hepatocytes have the role in degradation of nascent very low density lipids (Strong et al., 2012). Reelin (310KDa) is an extracellular matrix protein, largely secreted by the liver (Botella-Lopez et al., 2008). Fibrinogen is an inflammatory marker protein (Libby et al., 2002). Fibrinogen with high molecular weight (340 KDa) on partial degradation results in lower molecular weights 305 KDa or 270 KDa (Holm & Godal, 1984; Holm et al., 1985; Nieuwenhuizen, 1995). Elevated fibrinogen level in plasma is linked with increased risk of cardiovascular diseases, including ischaemic heart disease (Kamath & Lip, 2003; Shi et al., 2010).

Response of albumin to herbal supplemented diets was more prominent in adult experimental groups as compared to weaning group. However, significantly higher levels of albumin were observed under effects of *N. sativa* seeds as compared to the *P. ovata* husks. The albumin level increased not only under the effects of *N. sativa* but was also found to be related to the age of the animal as reported earlier in mammals (Zanouny et al., 2013). Clinically, albumin (66KDa) is an indicator of the existence and progression of several diseases and is crucial regulator of colloidal osmotic-balance in the blood (Moshage, 1997; Obal et al., 1998;). Albumin is a “negative acute-phase” (APP). Hypoalbuminemia is consequence of the acute-phase conditions (Princen et al., 1981), associated with many diseases such as liver diseases, kidney diseases, cancer, severe burns, infections and some genetic abnormalities (Takeuchi & Takada, 1968; Grant et al., 1987). Liver has first-pass access to dietary nutrients and have a crucial role in their metabolism. During hepatotoxicity, this metabolism is perturbed and cirrhotic liver may lead to hypoproteinemia due to disrupted proteins (Schwartz et al., 1974). In accordance with this study the group provided with
HFD supplemented with the *P. ovata* husk exhibited hypoproteinemia.

**Fig., 2:** Densitometric comparison of electrophoretically resolved serum proteins of weaning rats groups against protein ladder. W=Weaning, Group 0=negative control, Group I=positive control fed on FRD, Group II=Experimental group given FRD+ 5% *N. sativa* seeds, Group III=Experimental group fed on FRD+5% *P. ovata*
Fig. 3: Densitometric comparison of electrophoretically resolved serum proteins of adult rat groups against protein ladder. A=Adult, Group 0=negative control, Group I=positive control fed on FRD, Group II=Experimental group given FRD+5% N. sativa seeds, Group III=Experimental group fed on FRD+5% P. ovata husks.
Fig. 4: Densitometric comparison of electrophoretically resolved serum proteins between weaning rat groups (a) and adult rats (b) with molecular weight (KDa) on x-axis and volume (mm²) on y-axis. 1-9 are the band positions.

It is reported that hepatic diseases are among the most prevalent diseases leading to death in spite of all the innovative research performed in this regard. As a consequence, traditional medicinal herbs are being recommended to cure liver ailments (Anbarasu et al., 2012). Taking together all findings of our current research, we conclude that among the two fat plummeting agents used in the present study to reverse inflammatory response induced by fat-rich diet, the therapeutic potential of *N. sativa* seeds was higher than *P. ovata* husk. Furthermore, the hypoproteinemic role of *P. ovata* husk is noticeable and there is need of further research in this regard.

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REFERENCES


