

# Counter effects of *N. Sativa* L. and *P. ovata* L. on indicative markers of non alcoholic fatty liver disease

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**Abstract:** The broad spectrum of non-alcoholic fatty liver disease (NAFLD) diseases ranges from simple liver inflammation to steatosis, leading to fibrosis and cirrhosis. Four groups of weaning (30g) *Rattus norvegicus* were designated as W-0, W-I, W-II and W-III. For sixteen weeks group W-0 was given standard pallet diet, group I consumed diet "A" (20% fat Sucrose + 33% tea whitener + 34% ground pallet diet +13% water), group W-II was fed on diet "B" (50g *Nigella sativa* seeds/kg of A) and group W-III was provided with diet "C" (50g *Plantago ovata* husks /Kg of A). The analysis of CBC, LFTs, and Lipid profile revealed that there were highly significant changes ( $P<0.001$ ) in the MCV, PLT, Hb, MCH, MCHC, RBC, RDW%, WBC, MPV, Triglycerides, cholesterol, LDL and the significant alterations ( $P<0.01$ ) in albumin, AST, bilirubin, AST/ALT, HDL and cholesterol/HDL were observed in the experimental groups when compared with control by using one way ANOVA. We concluded that high-energy diet can alter the blood profile. Moreover fat plummeting agents have counter impact on the hematology as well as serology of diet induced NAFLD in *R. norvegicus*.

**Keywords:** Thrombocytopenia, NAFLD, hypertriglyceridemia, hypercholesterolemia, *R. norvegicus*, hyperbilirubinaemia

## INTRODUCTION

Liver is the central player of whole energy homeostasis, when the consumption of energy far exceeds the combustion of energy the excessive energy is stored in adipose tissues in form of triglycerides (TG) and reused on demand. Liver is the vital organ for fat metabolism; under normal circumstances hepatocytes have a limit of 5-10% by weight. Any agent causing the defective fat metabolism may lead to excessive fat deposition in hepatocytes and development of fatty liver disease (FLD). On the basis of contributing agents, it is categorized into AFLD (Alcoholic fatty liver disease) and NAFLD (Nonalcoholic fatty liver disease). AFLD is developed due to excessive alcoholism while the factors involved in NAFLD include metabolic syndromes, diabetes, insulin resistance, toxic agents, environmental and genetic factors and the most important is obesity (Barisio D'Angelo *et al.*, 2009; Ferrer *et al.*, 2008; Tagle, 2003). It has a wide spectrum of diseases ranging from simple inflammation to steatosis and ultimately to hepatocellular carcinoma (Raszeja-Wyszomirska *et al.*, 2008). NAFLD is the most prevalent hepatic disorder of western countries (Gronbaek *et al.*, 2003) and emerging in the Asian countries like Pakistan due to diet patterns in adults as well as children.

The medicinal use of plants dates from the earliest time of man's evolution (Dattner 2003; Fong 2002). In the last few decades increase in the use of herbal products as an alternative to conventional treatment to heal and cure various diseases is on rise due to the concerns about the

reported side effects of orthodox medicines (Javed *et al.*, 2012). *N. sativa* is miraculously among the promising medicinal herbs with rich religious and historical background (Salem, 2005). Its pharmacological properties hold anti-histaminic, anti-fertility, anti-diabetic, uricosuric, anti-inflammatory, anti-oxidant, anti-hypotensive, anti-nociceptive, immune modulatory, anti-microbial and anti-tumor (Ali and Blunden, 2003; Salem, 2005).

Substantial health benefits of fiber rich diets have been reported (Galisteo *et al.*, 2005). In individuals with mild to moderate hypercholesterolemia soluble fibers have shown the cholesterol lowering effects of low fat diets (Jayaram *et al.*, 2007). *P. ovata* (Psyllium husks) is water soluble and have hypolipidemic and hypoglycemic effects in individuals with type 2 diabetics, hypercholesterolemia and hypersensitive experimental models (Anderson *et al.*, 1999; Anderson *et al.*, 2000; Obata *et al.*, 1998; Romero *et al.*, 2002).

In spite of large number of pharmacological studies conducted throughout the world on *N. sativa* seeds and *P. ovata* husks still there is much to investigate. Therefore, the objective of the present study was to analyze the possible prospective effects of these herbs against diet induced NAFLD and associated changes in hematological indices and biochemistry of *R. norvegicus*.

## MATERIALS AND METHODS

Weaning rats of 30g were divided into four groups (n=10). The control (W-0) group was given standard

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laboratory pallet diet while experimental groups were fed on high-energy diet composition (Naderali *et al.*, 2001) with slight modifications. Groups W-I was provided with diet "A" (20% fat Sucrose + 33% tea whitener + 34% ground pallet diet +13% water). The group W-II consumed the diet B ("A" + 5% *N. sativa* seeds) and group W-III was given diet C ("A" + 5% *P. ovata* husk). Animals were kept in 12h dark and light cycle and had *ad libitum* access to water and feed for sixteen weeks in the animal house of Department of Zoology, University of the Punjab, Lahore. Animals were given anesthesia Norcuron 150µl/100g (of body weight), and sacrificed. Blood was drawn directly from heart, and organs were excised, washed with 0.9% saline and stored in 10% formalin for preservation. About 2ml of blood was taken in EDTA coated tubes and the rest of the blood was centrifuged at 5000 rpm for 20 minutes. Complete blood cells count and LFTs were performed in a regular bioclinical lab with the help of commercially available methods. Data were expressed as Mean ± SEM. One way ANOVA with post host Tukey's test was performed by using the GraphPad Prism version 5.01 for windows, Graphpad software, San Diego California USA, www.graphpad.com". The level of significance was set at  $P \leq 0.05$ .

## RESULTS

### **Complete blood count**

#### **Hematocrit percentage (HCT %)**

A statistically significant increase of (HCT) % in experimental group W-I ( $P < 0.01$ ), W-II ( $P < 0.01$ ) and W-III ( $P < 0.05$ ) were observed in comparison with W-0 group (fig. 1A).

#### **Hemoglobin (Hb)**

The blood Hb level was significantly higher in experimental group W-I, W-III ( $P < 0.01$ ) and W-II ( $P < 0.05$ ) when compared with W-0 group (fig. 1B).

#### **Mean corpuscular hemoglobin (MCH)**

A significantly higher value of (MCH) ( $P < 0.05$ ) was observed in the experimental group W-II in comparison with W-0 group (fig. 1C).

#### **Mean corpuscular hemoglobin concentration (MCHC)**

The concentration of (MCH) was significantly lower ( $P < 0.001$ ) in experimental group W-I ( $P < 0.05$ ) and W-II ( $P < 0.001$ ) in comparison with W-0 group. However the MCHC ( $P < 0.05$ ) was higher significantly in group W-III in comparison with group W-II (fig. 1D).

#### **Mean corpuscular volume (MCV)**

MCV showed significantly higher value ( $P < 0.01$ ) in experimental group W-I, ( $P < 0.001$ ) in W-II and ( $P < 0.05$ ) in group W-III when comparison was made with W-0 group. In case of inter group comparison there was a significantly lower value of MCV ( $P < 0.05$ ) in-group W-III as compared to group W-II (fig. 1E).

### **Mean platelet volume (MPV)**

In comparison with W-0 group the MPV was significantly lower in experimental group W-I ( $P < 0.001$ ), W-II ( $P < 0.01$ ) and W-III ( $P < 0.001$ ) in-group W-II. In the intergroup comparison the MPV were significantly higher in group W-II than group W-I ( $P < 0.001$ ) and group W-III ( $P < 0.01$ ) (fig. 1F).

### **Platelets (PLT)**

PLT showed significantly lower count in experimental group W-I ( $P < 0.05$ ) and group W-III ( $P < 0.001$ ) in comparison with W-0 group. However there was a significantly higher count of PLT in-group W-II ( $P < 0.001$ ) as compared to the group W-I and group W-III (fig. 1G).

### **Red blood cells (RBC)**

Significantly higher RBC were observed in group W-I ( $P < 0.001$ ), W-II ( $P < 0.05$ ) and W-III ( $P < 0.01$ ) as compared to W-0 group, however in intergroup comparison there was a significant decrease in group W-II in comparison with group W-I ( $P < 0.01$ ) and group W-III ( $P < 0.001$ ) (fig. 1H).

### **RDW%**

There were highly significant increase of RDW% ( $P < 0.001$ ) in W-I and W-II when compared with W-0 group, while in intergroup comparison group W-III had significantly lower values ( $P < 0.001$ ) than W-I and W-II (fig. 1I).

### **White blood cells (WBC)**

White blood cells (WBC) showed significantly lower values in experimental group W-I ( $P < 0.001$ ) and W-II ( $P < 0.01$ ) when compared with W-0 group. In an intergroup comparison group W-II had significantly higher WBC than W-I ( $P < 0.001$ ) and group W-III ( $P < 0.01$ ) (fig. 1J).

### **Liver function test**

#### **Serum Alkaline Phosphatase (ALP)**

The changes in the serum activity of ALP was significantly higher in experimental group W-II ( $P < 0.05$ ) when compared with W-0 (fig. 2A).

#### **Serum aspartate aminotransferase (AST)**

The serum AST activity was statistically significantly higher in-group W-II ( $P < 0.05$ ) in comparison with group W-0 and W-I (fig. 2B).

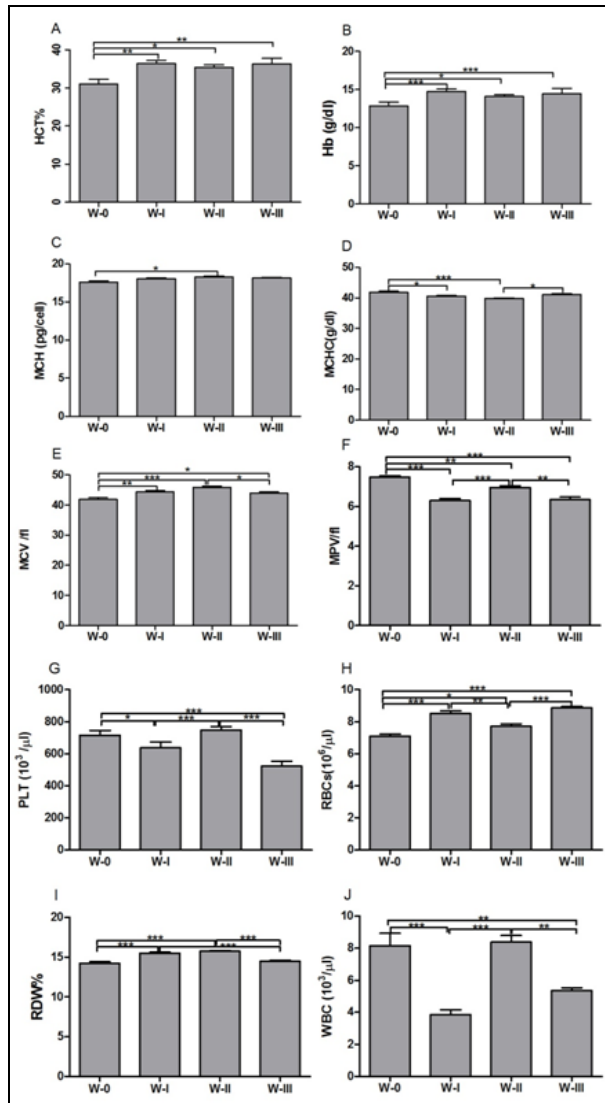
#### **Serum alanine aminotransferase (ALT)**

Over all non-significant changes were observed in ALT activity of serum of all experimental groups when compared with group W-0 when analyzed by using one way ANOVA (fig. 2C).

### **AST/ALT**

The ratio of AST and ALT was  $< 1.0$ , and statistically significantly higher in experimental group W-I ( $P < 0.05$ ),

W-II ( $P<0.01$ ) and W-III ( $P<0.01$ ) in comparison with W-0 group (fig. 2D).



**Fig. 1:** Complete blood cell count of the experimental groups as compared with control group. Results indicate Mean  $\pm$  SEM. (\*\*\* $P<0.001$ , \*\* $P<0.01$ , \* $P<0.05$ )

**Albumin**

The serum albumin level was significantly lower in experimental group W-I ( $P<0.05$ ) than W-0 group and group W-II (fig. 2E).

**Bilirubin**

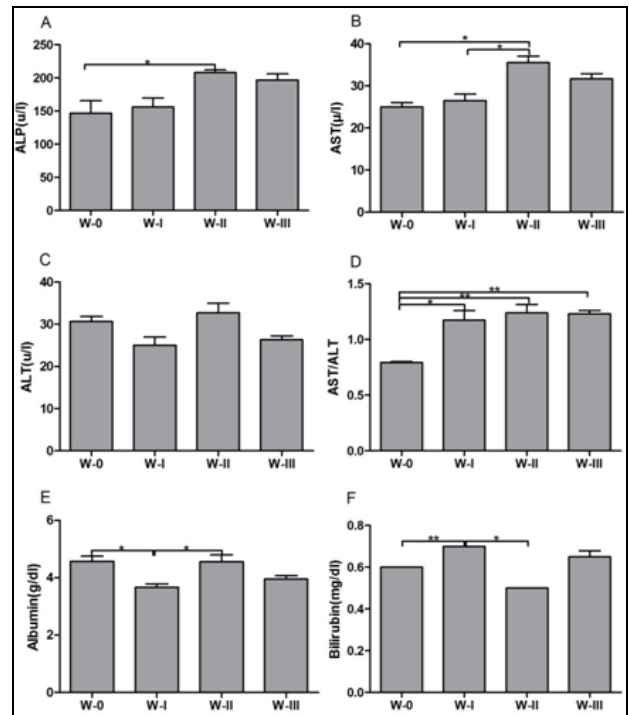
Non-significant increase was observed in-group W-I in comparison with W-0 group. In intergroup experiment bilirubin in experimental group W-II was significantly lower than group W-I ( $P<0.01$ ) and group W-III ( $P<0.05$ ) (fig. 2F).

**Lipid Profile**

**Serum Cholesterol level**

There was a significantly higher level of cholesterol in the serum of group W-I ( $P<0.01$ ), W-II ( $P<0.05$ ) and W-III

( $P<0.001$ ) in comparison with W-0 group, While in intergroup comparison group W-I ( $P<0.05$ ) and W-II ( $P<0.01$ ) had statistically significantly lower serum cholesterol as compared to W-III (fig. 3A).



**Fig. 2:** Liver function tests of the experimental groups as compared with control group. Results indicate Mean  $\pm$  SEM. (\*\*\* $P<0.001$ , \*\* $P<0.01$ , \* $P<0.05$ )

**Triglycerides (TG)**

The serum TG was significantly higher in-group W-I in comparison with W-0, ( $P<0.001$ ) W-II ( $P<0.01$ ) and W-III ( $P<0.001$ ). While there was a higher serum level of TG in group W-II ( $P<0.05$ ) as compared to W-III (fig. 3B).

**Low density lipid (LDL)**

The LDL in the serum of experimental group W-I ( $P<0.001$ ), W-II ( $P<0.01$ ) and W-III ( $P<0.01$ ) was observed to be significantly higher when compared with W-0 group. In intergroup comparison experimental group W-I was higher than W-II ( $P<0.05$ ) and W-III ( $P<0.01$ ) (fig. 3C).

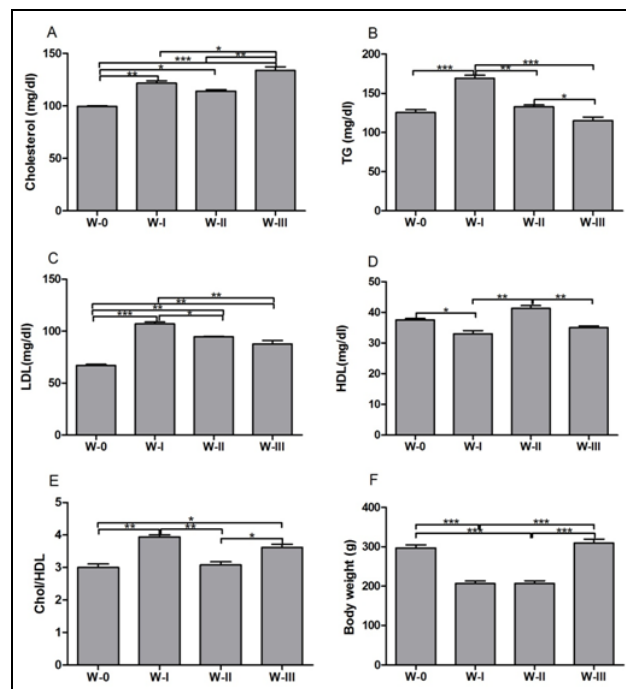
**High density lipid (HDL)**

A decrease in the serum level of HDL was observed in experimental group W-I as compared to group W-0 ( $P<0.05$ ) and W-II ( $P<0.01$ ). However group W-II ( $P<0.01$ ) had significantly higher level of HDL than group W-III (fig. 3D).

**Cholesterol/HDL**

In experimental group W-I ( $P<0.01$ ) and W-III ( $P<0.05$ ) significantly higher cholesterol to HDL ratio was observed as compared to W-0. Group W-II had

statistically significantly lower ratio in comparison with group W-I ( $P < 0.01$ ) and W-III ( $P < 0.05$ ) (fig. 3E).



**Fig. 3:** Lipid profile (A-E) and body weight (F) of the experimental groups as compared with control group. Results indicate Mean  $\pm$  SEM. (\*\* $P < 0.001$ , \* $P < 0.01$ , \* $P < 0.05$ )

### Body weight

There was significant decline ( $P < 0.001$ ) in body weight of rats of groups W-I and W-II as compared to W-0. However, significant elevation ( $P < 0.001$ ) of body weight was noticed in-group W-III rats as compared to W-I and W-II (fig. 3F).

## DISCUSSION

In the diagnosis of various diseases and pathological conditions induced by drugs, heavy metals, dyes, industrial compounds, pesticides and several other components the hematological indices have widely been used (Morgan *et al.*, 1980; Shakoori *et al.*, 1988). The present study was conducted to analyze the effects of fat rich diet as well as supplements of fat reducing agents (*N. sativa* seeds and *P. ovata* husks) in the body on the clinical biochemistry and blood profile.

The results revealed by the study exhibited significant increase in HCT%, RBC, Hb MCV and RDW% while a significant decrease in PLT count (Thrombocytopenia), WBC, MCHC and MPV in the experimental group W-I fed on high fat diet. The effect of this diet was nullified to some extent in experimental group W-II fed on *N. sativa* supplemented diet to bring near the baseline values of PLT, WBC and RBC. While the experimental group W-III

supplemented with *P. ovata* husks had normal values of MCHC and RDW%. It has been documented that many biochemical and hematological abnormalities occur in chronic liver diseases (Ogasawara *et al.*, 2005). A vast data on chronic ALD has reported the significant decreases in HCT, lymphocytes, RBC count, PLT, Hb concentration, while significant increases in MCV, MCH and RDW% MCH (Colman and Herbert, 1980; Costa *et al.*, 2007; Gheno *et al.*, 1981; Ogasawara *et al.*, 2005; Shakoori *et al.*, 1988). Chronic alcoholism is associated with inflammation and haematotoxic effects, while NAFLD has limited effect on hematological parameters (Das *et al.*, 2011). Some studies on NAFLD have reported its asymptomatic nature (Diehl *et al.*, 1988; Powell *et al.*, 1990).

The results of the LFTs of the current project included slight elevations in ALP, AST, ALT, bilirubin and lower albumin concentration in experimental group W-I. *N. sativa* was useful to bring the serum albumin within its normal range in W-II. AST/ALT ratio was greater than 1.0 in all the three experimental groups.

Compared to the control groups, the NAFLD patients have significantly higher HCT and thrombocytopenia (Das *et al.*, 2011). The thrombocytopenia is autonomously linked with significant baseline fibrosis or progression of fibrosis, in patients with chronic liver disease (Stepanova *et al.*, 2010). Mean platelet volume (MPV) is a marker of platelet activation, which is a determinant of atherosclerosis. MPV may be used as a follow-up marker in patients with NAFLD at the point of atherosclerosis (Arslan and Makay, 2010). We analyzed that there was a decrease in the MPV in the animals but according to literature patients with NAFLD have higher MPV (Ozhan *et al.*, 2010). The increased MCV in alcoholic liver disease also correlated with a decrease of erythrocyte deformability significantly (Shiraishi *et al.*, 1993).

The increase in HCT and RBC can be due to the increase in erythropoietin (EPO) and it is hepatic positive acute phase protein synthesized during inflammation (Ramadori *et al.*, 2010). Primarily higher RDW% indicates greater variations in RBC size (anisocytosis), usually caused by perturbation in maturation or degradation of erythrocytes (Evans and Jehle, 1991).

Hematological parameters are usually normal unless cirrhosis and portal hypertension lead to hypersplenism. Thrombocytopenia, with or without detectable splenomegaly, is a common clinical clue that cirrhosis (complicated by hypersplenism) has developed (Salt 2004).

The diagnosis of NAFLD is often recommended after the finding of mildly abnormal LFTs, although patients with normal alanine aminotransferase (ALT) values can have

the entire histological spectrum of NAFLD (Fracanzani *et al.*, 2008; Mofrad *et al.*, 2003). ALT is usually greater than aspartate aminotransferase (AST) and rarely more than thrice the upper limit of normal. The presence of more advanced disease is suggested when AST: ALT ratio is greater than 1.0 (Angulo *et al.*, 1999). In case of rare liver function test abnormality alkaline phosphatase can be slightly elevated (Pantsari and Harrison, 2006). Hyperbilirubinaemia and low albumin indicate advanced liver disease and are probably not features of NAFLD (Oh *et al.*, 2008).

The serum lipid profile of the experimental group W-I had hypercholesteremia, hypertriglyceridemia and elevated LDL level while a lower HDL level. The variations were to some extent reversed by the lipid lowering agents *N. sativa* seeds and *P. ovata* husks in experimental groups W-II and W-III respectively. The elevated serum cholesterol, TG and LDL with lower HDL observed in current project were reported likewise in the past studies (Duseja *et al.*, 2005; Shams *et al.*, 2011; Wanless and Lentz, 1990). Lipid plummeting effect of *N. sativa* and *P. ovata* exhibited in the current experimental project were in accordance with the previous studies (Le *et al.*, 2004; Moreno *et al.*, 2003). The NAFLD is mainly linked with obesity yet it is also reported in lean persons as evident by the lower body weights of the W-I group rats of our current study (Younossi *et al.*, 2012). The impact of *N. sativa* seeds on body weight reduction was similar to the studies of Qidawai *et al.*, 2009 while no significant decline in body weight with *P. ovata* husks supplementation was observed likewise the previously reported studies (Allen, *et al.*, 2004).

## CONCLUSION

The results obtained by the present study inferred that the seeds of *N. sativa* and *P. ovata* husks could protect blood from severe alteration induced by fatty diets. It can be said that *N. sativa* can be considered promising against hematotoxicity associated with diet induced NAFLD. Thrombocytopenia may be initial markers of NAFLD but disease may be asymptomatic because of the normal body weights and LFTs yet, AST/ALT ratio can be a successful marker to interpret the severity of the disease.

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