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Antioxidant and Hepatoprotective Activity of Purelignan from Flaxseed (*Linumusitatissimum* L.) Onacetaminophen Induced Toxicity in Male Rabbit

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Abstract: *The present study aimed to assessment the antioxidant activity of flaxseed lignan in male rabbits with administrated orally 200 mg/kg paracetamol to induce hepato-nephrotoxicity. Biochemical studies show that there is significant increases ($p < 0.05$) in the levels of serum MDA with decrease in the levels of GSH, GPx, CAT, SOD, Zinc and Manganese in paracetamol treated group compared to the control group. These values were retrieved significantly by treatment with lignan extracts at two different doses (25 and 50 mg/ kg). The antioxidant study showed significant decrease in MDA while the levels of GSH, GPx, CAT, SOD, Zinc and Manganese were significantly increased in paracetamol with lignan treated groups. The treatment with lignan (hepato-nephrocurative) has recorded a decline in levels of the blood MDA with upturn the levels of GSH, GPx, CAT, SOD, Zinc and Manganese to normal values. From the results we can be concluded that the compound of pure lignan was better than partial pure lignan and can prevent liver and renal damage from paracetamol induced hepato-nephrotoxicity in rabbits. Therefore, the potential use of flaxseed lignan as a therapeutically useful hepatonephroprotectiveagent deserves further studies.*

Keywords: *flaxseedpure lignan, antioxidant activity, hepato-nephrotoxicity, hepato-nephroprotective.*

1. INTRODUCTION

Paracetamol is a drug of para-aminophenol group which is considered one of the commonly used and safe over the counter antipyretic and analgesic drugs, when administered at recommended doses (Ozkaya *et al.*, 2010). The main problem with this medication remains its misuse through intentional or unintentional ingestion of supra- therapeutic dosages which usually lead to hepatic necrosis (Plaa, 2010).Oxidative stress is reported to constitute a major mechanism in the pathogenesis of Paracetamol induced liver and renal damage in experimental animals. Because toxic overdoses of Paracetamol were reported to have life-threatening impacts on the liver and kidney, e.g. hepatic necrosis and renal failure in both human and experimental animals, early protection from Paracetamol induced nephrotoxicity has life-saving importance. Therefore, supplementation with antioxidants is very crucial to delay, prevent or remove oxidative damage (Demirbag*et al*., 2010).

Flaxseed (*Linumusitatissimum* L.) is a rich source of different types of phenolics such as lignans, phenolic acids, flavonoids, phenylpropanoids and tannins (Kasote, 2013). Plant lignans are the biologically important class of phenolic compounds. They belong to a group of phenols which are characterized by coupling of two phenylpropanoid units (Willfor*et al*., 2006). The levels of lignans in food vary widely, the richest source is flaxseed. The prevailing lignan in the flaxseed is secoisolariciresinoldiglucoside (SDG) (Cardoso Carraro *et al*., 2012). SDG is converted into mammalian lignans, enterodiol (ED) and enterolactone (EL) by colon bacteria (Wang *et al*., 2000).

Due to the over dose of paracetamol is the important cause to hepatotoxicity, the present study aimed to induce hepatotoxicity in male albino rabbits and determine the role of lignan as antioxidant and as hepatoprotective and hepatocurative.

2. MATERIALS AND METHODS

Flaxseeds were collected from the local market, identified as (*Linumusitatissimum L*.) by the botanist Prof. Dr. Ali Hussein AL-Musawi in the College of Science/Baghdad University. The Lignan was extracted according to [Al-Jumaily and Al-Azawi, 2014] by solvents which gave the best results were

ethanol: 1, 4 dioxane (1:1, v: v).SDG release after alkaline hydrolysis by using a methanolic NaOH, 20mM, pH=8 at 50ºC followed by using following chromatographic techniques: Liquid-liquid (that called partial pure lignan), Sephadex LH-20 column chromatography (that called pure lignan).

2.1. Preparation of Concentrations of Pure and Partial Pure Lignan

This process included the preparation of concentrations of pure lignan 25 and 50 mg/ml and partial pure lignan 25 mg/ml. This was done by dissolving 0.025, 0.05 g respectively by distil water Then the volumes were completed into 1ml which were chosen depending on animal experiments [Prasad. 2009].

2.2. Experimental Animals

Twenty four (24) adult male rabbits belonging to the New Zealand White species weighing between 1.800 - 2.000 kg were obtained from the College of Veterinary Medicine. The rabbits were kept in standard cages in the animal house at the Genetic Engineering and Biotechnology Institute, University of Baghdad, under standard laboratory condition (25oC, 12hr. light/dark cycle) with pellated food and tap water and libitum during experimental period.

The twenty four rabbits were randomly divided into six groups of four animals each. Animals in first group were received regular standard diet, tap water and severed as control (C).

Rabbits in the 2nd group were received with daily oral dose of Paracetamol (200 mg/kg) for nine weeks. A rabbit was considered to be positive control for paracetamol. Rabbits in the 3rd group were received daily oral dose of paracetamol (200 mg/kg) + partial pure lignan (25 mg/kg) for six weeks and the treatment was done by administrated partial pure lignan (25 mg/kg) for three weeks. Rabbits in the 4th group were received with daily oral dose of Paracetamol (200 mg/kg) + pure lignan (25 mg/kg) for six weeks and the treatment was done by administrated pure lignan (25 mg/kg) for three weeks. Rabbits in the 5th group were received with daily oral dose of Paracetamol (200 mg/kg) + pure lignan (50 mg/kg) for six weeks and the treatment was done by administrated pure lignan (50 mg/kg) for three weeks. While animals in the 6 group were received with daily oral dose of Paracetamol (200 mg/kg) + Vitamin C (50 mg/kg) for six weeks and the treatment was done by administrated Vitamin C (50 mg/kg) for three weeks. The experimental protocol was approved by the institutional animal ethics committee of NRI Medical College and General Hospital in accordance with CPCSEA (Committee for the purpose and control and supervision on Experiments on Animals guidelines).

2.3. Methods of Biochemical Analysis of Blood Samples

2.3.1. Collection of Blood

Blood samples were collected continuously at the end of every week to the end of the ninth week. At the end of the first week the blood was collected intravenously through the large veins at the back of the rabbit's ear. Prior to blood collection the ear was sterilized by dabbing with cotton wool soaked in ethanol 70%. Blood collection was carried out using sterile needles and syringes, 5-6 ml of blood were collected in test tubes from all rabbit. The blood sample was rocked slightly and centrifuged at 3000 rpm for 5 minutes. The supernatant was then stored in the freezer at -21^{o}C until analyzed [Onoagbe, et al., 1999].

2.3.2. Biochemical Estimation

The serum malondialdehyde MDA [Young, 2000] and Mn and Zn concentration was estimated according to [Young,2000]; serum albumin [Young,2000]; Serum Glutathione (GSH) activity [Young,2000]; Serum Glutathione peroxidase (Gpx) [Young,2000] ; Serum Superoxidase dismutase (SOD) [Kind and King ,1954];Total serum bilirubin [Pearlman and Lee ,1974]; Serum urea [Drupt,1974] .

2.3.3. Statistical Analysis

Completely randomized design (CRC) program [SAS, 2010] was used to test the effect of the treatment on traits involved in this study. The least significant difference (LSD) test was also used to compare significance between the means [Steel and Torrie, 1980].

3. RESULTS AND DISCUSSION

3.1. Effect of Partial Purified and Pure Lignan on Serum Glutathione (GSH) Level

Table (1) shows that the serum glutathione (GSH) concentrations were significantly decreased ($p < 0.05$) in the paracetamol treated group (group 2) of rabbits for 6 weeks (3.45 ± 0.08 U/ml) compared to the control negative group (9.55 ± 0.29 U/ml) indicating the induction of severe hepatotoxicity. Glutathione is an important non-enzymic antioxidant that protects the liver against paracetamol induced damage (Prescott, 2005). The depletion of cellular GSH level in the hepatic cells is known to play a key role in paracetamol toxicity (Rosa *et al.,* 2012).Treatment with partial pure lignan (Group 3) (6.17 ± 0.07 U/ml) for six weeks showed significant ($p < 0.05$) increase in concentrations of serum (GSH) compared to the paracetamol treated group. Furthermore the levels of (GSH) significantly increased ($p<0.05$) in the pure lignan treated groups (Group 4 and 5) (7.77 ± 0.22 and 9.42 ± 0.07 U/ml) respectively, but the best effective was (Group 5) for six weeks when compared to the paracetamol treated group. The effect of non-enzymic antioxidants vitamin- C (Group 6) showed significantly increased ($p<0.05$) in serum (GSH) (9.61 ± 0.22 U/ml) for six weeks, this shows the effective role of lignan as hepatoprotective. Palani *et al.* (2011) observed that the ethanol extract of *Salaciaoblonga* enhanced the activities of antioxidant enzymes SOD, CAT & GPx and restored GSH levels against paracetamol induced nephrotoxicity. Furthermore Makoshi *et al.* (2013) showed the aqueous extract of *P. niruri*proved to be capable of providing hepatoprotection against paracetamol induced hepatotoxicity. Phenolic acids such as *p*-coumaric acid and ferulic acid glucosides were accumulated at high concentrations in the flaxseed found to possess antioxidant properties (Yuan *et al*., 2008).

Table1. *Effect of lignan as Hepatoprotective and Hepatocurativeon serum GSH (U/ml)*

Group	**Mean ± SE**		**Probability ≤ (P)**
	1-6 Week	7-9 Week	
1	9.39 ± 0.41 a	9.73 ± 0.48 a	NS
2	4.17 ± 0.09 e	3.12 ± 0.02 d	P<0.05*
3	5.89 ± 0.20 d	7.70 ± 0.19 c	P<0.05*
4	7.16 ± 0.05 c	8.73 ± 0.07 b	P<0.05*
5	8.16 ± 0.08 b	10.05 ± 0.12 a	P<0.05*
6	8.60 ± 0.02 ab	10.05 ± 0.07 a	P<0.05*

Different letters represent significant ($p \leq 0.05$) different between means of column.

**P value significant.*

3.2. Effect of Partial Purified and Pure Lignan on Serum Glutathione Peroxidase (GPX) Level

Table (2) shows that the serum (GPX) levels were significantly decreased ($p < 0.05$) in paracetamol treated rabbits (group 2) after 6 weeks (17.61 ± 0.82 U/ml) compared to the control negative group (38.57 ± 1.89 U/ml) indicating the induction of severe hepatotoxicity. Kuriakose and Kurup (2010b) showed decline in the level of antioxidant enzyme like GPX observed in paracetamol treated rat is a clear manifestation of excessive formation of free radicals. Treatment with partial pure lignan (Group 3) (30.08 ± 0.08 U/ml) for six weeks showed significant ($p < 0.05$) increase in concentrations of serum (GPX) compared to the paracetamol treated group. Furthermore the levels of (GPX) significantly increased ($p<0.05$) in the pure lignan treated groups (Group 4 and 5) (31.97 ± 0.04 and 36.22 ± 0.41 U/ml) respectively, but the best effective was (Group 5) for six weeks when compared to the paracetamol treated group. The effect of non-enzymic antioxidants vitamin- C (Group 6) showed no significant difference ($p>0.05$) in serum (GPX) (36.46 ± 1.36U/ml) for six weeks, this shows the effective role of lignan as hepatoprotective. The results showed that treatment with lignan produced dose-dependent elevation in serum (GPX) levels. Both doses of pure lignan (25 and 50 mg/kg) reversed these changes and were more effective than partial pure lignan (25 mg/kg), but pure lignan (50 mg/kg) was more effective than partial pure lignan (25 mg/kg) and pure lignan (25 mg/kg), and approach with vitamin C (50 mg/kg). Kasote, (2013) reported that the increase in the serum level of this enzyme (GPX) might be due to the presence of various phenolic and flavonoid compounds in the pure extract of lignan that enhanced the liver's regeneration ability.

Table2. *Effect of lignan as Hepatoprotective and Hepatocurativeon serum GPX (U/ml).*

Group	Mean ± SE		Probability ≤ (P)
	1-6 Week	7-9 Week	
1	38.26 ± 1.34 a	38.54 ± 1.45 a	NS
2	19.65 ± 0.38 d	16.89 ± 0.29 c	P<0.05*
3	30.18 ± 0.54 c	33.18 ± 0.81 b	P<0.05*
4	32.09 ± 0.39 c	35.20 ± 0.63 b	P<0.05*
5	35.25 ± 0.16 b	38.17 ± 0.49 a	P<0.05*
6	36.20 ± 0.87 ab	38.07 ± 0.27 a	P<0.05*

Different letters represent significant (p ≤ 0.05) different between means of column.

**P value significant (NS) = not significant*

3.3. Effect of Partial Purified and Pure Lignan on Serum Catalase (CAT) Level

Table (3) shows that the serum Catalase (CAT) concentrations were significantly decreased (p <0.05) in the paracetamol treated group (group 2) of rabbits for 6 weeks (20.70 ± 1.19 U/ml) compared to the control group rabbits (group 1) (50.33 ± 1.39 U/ml) indicating the induction of severe hepatotoxicity.

According to Isik *et al.* (2006) paracetamol treated rats had decreased activities of catalase. In another study conducted by Kuriakose and Kurup (2010b) showed decline in the level of antioxidant enzyme catalase observed in paracetamol treated rat is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation. Treatment with partial pure lignan (Group 3) (39.37 ± 0.67 U/ml) for six weeks showed significant (p < 0.05) increase in concentrations of serum (CAT) compared to the paracetamol treated group. Furthermore the levels of (CAT) significantly increased (p<0.05) in the pure lignan treated groups(Group 4 and 5) (43.94 ± 1.34 and 47.04 ± 0.03 U/ml) respectively, but the best effect was (Group 5) for six weeks when compared to the paracetamol treated group. The effect of non-enzymic antioxidants vitamin-C (Group 6) showed significantly increased (p<0.05) in serum (CAT) (47.83 ± 0.12U/ml) for six weeks, this shows the effective role of lignan as hepatoprotective. Palani *et al.,* (2011) observed that the ethanol extract of *Salacia oblonga* enhanced the activities of antioxidantenzyme CAT and restored GSH levels against paracetamol induced nephrotoxicity. Last three weeks (7, 8 and 9) paracetamol stopped to administrate treatment with lignan will be continued, the results show significant increase in (CAT) concentration (50.75 ± 0.55 U/ml) in the 9 week (group 5) and become approach with normal value, this shows the effective role of lignan as hepatocurative. Kraushofer and Sontagm, (2002) established that flaxseed have a considerable amount of phenolic compounds**.** And the intake of natural antioxidants has been associated with reduced risks of organ toxicities (Jainand Agrawal, 2008). In another study Fakurazi *et al*. (2012) suggested that *Moringaoleifera* has a potential role in therapeutic action via inhibiting oxidative stress due to presence of phenolic compounds and its antioxidant nature.

Table3. *Effect of lignan as Hepatoprotective and Hepatocurativeon serum Catalase (U/ml)*

Group	Mean ± SE		Probability ≤ (P)
	1-6 Week	7-9 Week	
1	50.54 ± 1.16 a	50.49 ± 1.19 a	0.92 NS
2	25.62 ± 0.35 e	20.06 ± 0.53 c	P<0.05*
3	37.57 ± 0.48 d	44.89 ± 1.11 b	P<0.05*
4	41.23 ± 0.29 c	48.25 ± 0.37 a	P<0.05*
5	45.60 ± 0.47 b	50.41 ± 0.42 a	P<0.05*
6	47.54 ± 0.33 b	50.48 ± 0.19 a	P<0.05*

Different letters represent significant (p ≤ 0.05) different between means of column.

**P value significant, (NS) = not significant*

3.4. Effect of Partial Purified and Pure Lignan on Serum Superoxide Dismutase (SOD) Level

Table (4) shows that the serum superoxide dismutase (SOD) concentrations were significantly decreased (p <0.05) in the paracetamol treated group (group 2) of rabbits for 6 weeks (11.26 ± 0.25 U/ml) compared to the control negative group (group 1) (25.69 ± 0.49 U/ml) indicating the induction of severe hepatotoxicity. Reactive oxygen species (ROS) are generated in the body mostly during chemical assault and are scavenged by SOD. However, exhaustion can occur during paracetamolassault, resulting in the depletion of these enzymes (James *et al.*, 2003).

Treatment with partial pure lignan (Group 3) (18.97 ± 0.43 U/ml) for six weeks showed significant (p < 0.05) increase in concentrations of serum (SOD) compared to the paracetamol treated group.

Furthermore the levels of (SOD) significantly increased ($p<0.05$) in the pure lignan treated groups (Group 4 and 5) (21.34 ± 0.23 and 22.42 ± 0.31 U/ml) respectively for six weeks when compared to the paracetamol treated group. The effect of non enzymic antioxidants vitamin-C (Group 6) showed significantly increased ($p<0.05$) in serum (SOD) (22.93 ± 0.53 U/ml) for six weeks, this shows the effective role of lignan as hepatoprotective. Ansari and Rashid, (2012) confirmed that paracetamol caused changes in the levels of several liver components. The extract of *Tabernaemontanadivaricata* when given orally was able to target the liver enzymes, and found to improve liver enzyme contents in paracetamol-induced wistar rats.

Last three weeks (7, 8 and 9) paracetamol stopped to administrate, treatment with lignan will be continued, the results show significant increase in (SOD) concentration (24.84 ± 0.67 U/ml) in the 9 week and become approach with normal value in group 5, this shows the effective role of lignan as hepatocurative.

Table4. *Effect of lignan as Hepatoprotective and Hepatocurativeon serum SOD (U/ml)*

Group	Mean ± SE		Probability ≤ (P)
	1-6 Week	7-9 Week	
1	24.75 ± 0.84 a	24.70 ± 0.81 a	NS
2	13.20 ± 0.22 e	11.08 ± 0.01 c	P<0.05*
3	18.11 ± 0.04 d	21.39 ± 0.50 b	P<0.05*
4	20.14 ± 0.14 c	22.95 ± 0.60 b	P<0.05*
5	22.01 ± 0.22 b	24.22 ± 0.30 a	P<0.05*
6	22.52 ± 0.01 b	24.29 ± 0.05 a	P<0.05*

Different letters represent significant ($p \leq 0.05$) different between means of column.

**P value significant, (NS) = not significant*

Table (5) shows that the serum Malondialdehyde (MDA) concentrations were significantly increased ($p <0.05$) in the paracetamol treated group (group 2) of rabbits for 6 weeks (7.69 ± 0.08 U/ml) compared to the control negative group (1.61 ± 0.14 U/ml) indicating the induction of severe hepatotoxicity. Treatment with partial pure lignan (Group 3) (4.27 ± 0.03 U/ml) for six weeks showed significant ($p < 0.05$) decrease in concentrations of serum (MDA) compared to the paracetamol treated group. Furthermore the levels of (MDA) significantly decreased ($p<0.05$) in the pure lignan treated groups (Group 4 and 5) (2.46 ± 0.03 and 1.86 ± 0.02 U/ml) respectively, but the best effective was (Group 5) for six weeks when compared to the paracetamol treated group. The effect of non-enzymic antioxidants vitamin- C (Group 6) showed not significantly different ($p>0.05$) in serum (MDA) (1.76 ± 0.08 U/ml) for six weeks, this shows the effective role of lignan as hepatoprotective. This explanation was agreed with Sasidharan *et al.* (2010) show the *L. edodes* extract possesses antioxidant activity with Hepatoprotective properties.

Last three weeks (7, 8 and 9) paracetamol stopped to administrate, treatment with lignan will be continued, the results show gradual and significant decrease in (MDA) concentration (1.69 ± 0.07, 1.61 ± 0.02 and 1.51 ± 0.91 U/ml) respectively in group 5 and become best of normal value, this shows the effective role of lignan as hepatocurative. The decrease in the serum level of (MDA) might be due to the antioxidant activity of lignan from phenolic and flavonoid compounds in the pure extract that enhanced the liver's regeneration ability (Kasote, 2013).

Table5. *Effect of lignan as Hepatoprotective and Hepatocurativeon serum MDA (U/ml)*

Group	**Mean ± SE**		**Probability ≤ (P)**
	1-6 Week	7-9 Week	
1	1.54 ± 0.06 f	1.60 ± 0.03 d	NS
2	5.71 ± 0.11 a	7.82 ± 0.11 a	P<0.05*
3	4.08 ± 0.05 b	3.01 ± 0.06 b	P<0.05*
4	2.47 ± 0.02 c	1.94 ± 0.03 c	P<0.05*
5	1.92 ± 0.01 d	1.60 ± 0.03 d	P<0.05*
6	1.79 ± 0.01 e	1.59 ± 0.03 d	NS

Different letters represent significant ($p \leq 0.05$) different between means of column.

**P value significant, (NS) = not significant*

Table (6) shows that the serum zinc (Zn) concentrations were significantly decreased ($p < 0.05$) in the paracetamol treated group (group 2) of rabbits for 6 weeks (7.77 ± 0.06 mg/l) compared to the normal rabbits (group 1) (15.57 ± 0.52 mg/l). Treatment with partial pure lignan (Group 3) (11.82 ± 0.48 mg/l) for six weeks showed significant ($p < 0.05$) increase in concentrations of serum (Zn) compared to the paracetamol treated group. The best effective was (Group 5) for six weeks when compared to the paracetamol treated group. The effect of non-enzymic antioxidants vitamin- C (Group 6) showed significant increase ($p<0.05$) in serum (Zn) (14.16 ±0.39 mg/l) for six weeks, this shows the effective role of lignan as hepatoprotective.

Last three weeks (7, 8 and 9) paracetamol stopped to administrate, treatment with lignan will be continued, the results show gradual and significant increase in (Zn) concentrations (14.73 ± 0.21, 14.90 ± 0.17 and 15.24 ± 0.15 mg/l) respectively in group 5 and become approach with normal value, this shows the effective role of lignan as hepatocurative. (Cu/Zn) containing superoxide dismutase dependent superoxide dismutase is involved in the general defense system against natural or chemically induced production of reactive oxygen species (Halliwell, 1999). The increase in the (Zn) ratio indicates the stability of the antioxidant protection (Vasilyeva *et al.*, 2013).

Table6. *Effect of lignan as Hepatoprotective and Hepatocurativeon (Zn) (mg/l)*

Group	Mean ± SE		Probability ≤ (P)
	1-6 Week	7-9 Week	
1	15.55 ± 0.14 a	15.62 ± 0.16 a	NS
2	8.21 ± 0.03 e	7.27 ± 0.13 d	NS
3	10.02 ± 0.01 d	12.17 ± 0.08 c	P<0.05*
4	12.12 ± 0.16 c	14.17 ± 0.11 b	P<0.05*
5	13.74 ± 0.11 b	14.95 ± 0.15 b	P<0.05*
6	13.80 ± 0.16 b	14.97 ± 0.22 b	P<0.05*

Different letters represent significant ($p \leq 0.05$) different between means of column.

**P value significant, (NS) = not significant*

Table (7) shows that the serum manganese (Mn) concentrations were significantly decreased ($p <0.05$) in the paracetamol treated group (group 2) of rabbits for 6 weeks (6.83 ± 0.93 mg/l) compared to the control negative group (12.56 ± 0.67 mg/l). Mn-containing superoxide dismutase dependent superoxide dismutase is involved in the general defense system against natural or chemically induced production of reactive oxygen species (Halliwell, 1999). Rukgauer*et al.* (2001) found that the decrease in the (Mn) level is associated with the violation of the structure and functional activity of the SOD and its ability to remove the reactive oxygen. Treatment with partial pure lignan (Group 3) (9.75 ± 0.23 mg/l) for six weeks showed significant ($p < 0.05$) increase in concentrations of serum (Mn) compared to the paracetamol treated group. Furthermore the levels of (Mn) significantly increased ($p<0.05$) in the pure lignan treated groups (Group 4 and 5) (11.02 ± 0.12 and 11.84 ± 0.33 mg/l) respectively, but the best effect was in (Group 5) for six weeks when compared to the paracetamol treated group. The effect of non-enzymic antioxidants vitamin-C (Group 6) showed significantly increased ($p<0.05$) in serum (Mn) (11.96 ± 0.32 mg/l) for six weeks, this shows the effective role of lignan as hepatoprotective. Last three weeks (7, 8 and 9) paracetamol stopped to administrate, treatment with lignan will be continued, the results show gradual and significant increase in (Mn) concentrations (12.13 ± 0.08, 12.38 ± 0.11 and 12.52 ± 0.05 mg/l) respectively in group 5 and become approach with normal value, this shows the effective role of lignan as hepatocurative. The increase in the (Mn) ratio indicates the stability of the antioxidant protection (Vasilyeva *et al.*, 2013).

Generally, one phenol group on the lignans is oxidized, suggesting that the number of phenols per molecule may not predict radical scavenging antioxidant ability of lignans. Therefore, the antioxidant is highest with SECO and ED and lowest with SDG (Rajesha *et al.*, 2010). The antioxidant potential was assessed by measuring activities of hepatic marker enzymes. Results of this study clearly indicated that beneficial flaxseed antioxidant components help to restore the elevated activity of hepatic enzymes and renal markers at almost normal level.

Table7. *Effect of lignan as Hepatoprotective and Hepatocurativeon serum (Mn) (mg/l)*

Group	Mean ± SE		Probability ≤ (P)
	1-6 Week	7-9 Week	
1	12.58 ± 0.24 a	12.62 ± 0.27 a	0.478 NS
2	7.16 ± 0.02 d	6.62 ± 0.04 d	P<0.05*
3	8.85 ± 0.11 c	11.23 ± 0.05 c	P<0.05*
4	9.78 ± 4.25 c	11.82 ± 0.13 b	P<0.05*
5	11.08 ± 0.08 b	12.34 ± 0.02 a	P<0.05*
6	11.28 ± 0.06 b	12.37 ± 0.04 a	P<0.05*

Different letters represent significant ($p \leq 0.05$) different between means of column.

**P value significant, (NS) = not significant*

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