Possible therapeutic effects of Lacprodan® alpha-10 (whey protein product) against lipopolysaccharide-induced hepatotoxicity in rats

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Abstract: The aim of this study was to study the effects of the food supplement Lacprodan® alpha-10 on oxidative stress induced by Escherichia coli endotoxin (LPS) in rat liver. Thirty six Sprague Dawley rats were assigned to six equal groups. Groups 1-3 received saline, Lacprodan® alpha-10 (100mg/kg and 200 mg/kg), respectively. Groups 4-6 were injected with LPS (4 mg/kg, intraperitoneal) as a single dose 24 hrs prior to administration of Lacprodan® alpha-10 (at two dose levels) for 15 days. Results revealed that Lacprodan® alpha-10 treatment enhanced glutathione (GSH) and superoxidismutase (SOD) activities in liver homogenate in a dose response manner and decreased lipid peroxidation (MDA) and nitric oxide (NO) levels as well as the serum enzymes alkaline phosphatase (ALP),alanine and aspartate aminotransferase (ALT & AST) compared to LPS- treated group. Histopathological studies revealed that Lacprodan® alpha-10 (100 mg/kg) normalized the architecture of liver more than the higher dose (200 mg/kg) in rats treated with LPS. Conclusion: Lacprodan® alpha-10 proved a therapeutic value against hepatotoxicity induced by LPS and should be considered when over-consuming this food supplement in endotoxemic settings.

Key words: Lacprodan-alpha-10; lipopolysaccharide; hepatotoxicity; antioxidant; histopathology; rat

INTRODUCTION

The liver plays a key role in the clearing of gut-derived lipopolysaccharide (LPS), a major component of the outer membrane of all Gram-negative bacteria that can trigger the synthesis and release of proinflammatory cytokines and inducible nitric oxide synthase (iNOS) (Su et al., 2000; Zhang et al.,2000). A major mechanism of LPS-mediated effects is the activation of NF-kB through TLR (Zhang and Ghosh, 2000), although NADPH oxidase–induced reactive oxygen species (ROS) also has been reported to contribute to endotoxin-mediated liver injury (Gujral et al.,2004). Endotoxemia frequently occurs in patients with liver cirrhosis, with the extent of endotoxia correlating with the degree of liver failure. Moreover, endotoxia is believed to participate in the pathogenesis of liver diseases, including alcoholic liver disease and non alcoholic steatohepatitis (NASH), in which tissue necrosing factor (TNF) plays a central role (Crespo et al., 2001, Iimuro et al., 1997). Consistent with the involvement of oxidative stress after LPS exposure, GSH has been shown to play an important role in the susceptibility to LPS-induced liver injury (Payabvash et al., 2006). For instance, in experimental models, LPS has been reported to deplete GSH stores in the liver, and mice deficient in GSHPx exhibited enhanced susceptibility to LPS-mediated liver damage (Jaeschke et al., 1999). Consistent with these findings, the exogenous administration of GSH has been shown to decrease LPS induced systemic inflammatory response and mortality (Sun et al., 2006).

Steroids, vaccines, and antiviral drugs, have been used as therapies for liver pathologies, have potential adverse side-effects, especially if administered chronically or sub-chronically (McHutchison and Patel 2002 ). Therefore, natural products with better effectiveness and safe profiles are needed as a substitute for chemical therapeutics. As oxidative stress plays a central role in liver pathologies and their progression, the use of antioxidants have been proposed as therapeutic agents, as well as drug co-adjuvants, to counteract liver damage (Kruzel et al., 2010).

Whey protein (WP) is typically a mixture of beta-lactoglobulin (~65%), alpha-lactalbumin (~25%), and serum albumin (~8%), which are soluble in their native culture forms (Horton, 1995).The biological components of whey demonstrate a range of immune-enhancing properties (Low et al., 2003). In addition, it has the ability to act as an antioxidant (Brown et al., 2004, Nada, 2009) antihypertensive (Saito, 2008), antitumor (Bounous and Gold, 1991), hypolipidemic (Marshall, 2004), antiviral (Neurath et al., 2006), antibacterial (Shah, 2000) and chelating agent (Hurrell et al., 1989).

Several reports have indicated that whey protein has potential antioxidant activity due to its ability to elevate cellular glutathione (GSH) levels and SOD activity, and inhibit MDA production (Bayrama et al., 2008; Nada, 2009). Most whey proteins are cysteine rich, including alpha-lactalbumin, beta-lactoglobulin, and bovine serum albumin (Morr and Ha, 1993).
The amino acid profile of alpha-lactalbumin is rich in lysine, leucine, threonine, tryptophan, cystine, and calcium metalloprotein. Alpha-lactalbumin has been found to have immunomodulation (Montagne, 2000), anticancer (Svensson et al., 1999 and 2000), antimicrobial activity (Pihlanto-Leppala et al., 1999 and Pellegrini et al., 1999). Lacprodan® alpha-10 is a product from whey protein with high concentration of alpha-lactalbumin (43%). It is a food supplement produced by Arla Foods Ingredients amba, Denmark.

This study was performed to investigate the therapeutic effect of Lacprodan® alpha-10 in treatment of hepatotoxicity-induced by LPS, and its possible effect on the antioxidant status in hepatotoxic rats.

MATERIALS AND METHODS

Materials:
Animals
Sprague Dawley rats of both sexes weighing 180–200 gm were used throughout the experiments. Animals were housed under standard environmental conditions (23 ± 1 °C, 55 ± 5% humidity and a 12-h light: 12-h dark cycle) and maintained with free access to water and a standard laboratory diet ad libitum. Animal care and the experimental protocols were approved by the National Research Centre Animal Care and Use Committee and were in accordance with the guidelines of the International Association for the Study of Pain Committee for Research and Ethical Issues (Zimmermann, 1983).

Lipopolysacchaide (LPS) from Escherichia coli 055:B5 was purchased from sigma aldrich (USA), and Lacprodan® alpha-10 was obtained as a gift from Arla food amba (Denmark)

Experimental design:
1. Effect of Lacprodan® alpha-10 in normal rats:
   Three groups of rats (n = 6 per group) were treated with the vehicle only (saline) (group 1) Lacprodan® alpha-10 (100 and 200 mg/kg) (groups 2 and 3) respectively, daily orally for 15 days.
2. Effect of Lacprodan® alpha-10 on LPS-induced hepatic damage in rat:
   Three equal groups of rats (6 rats each). Animals were injected with LPS (4mg/Kg, i.p.) as a single dose according to Sebai et al (2010)- 24 hr prior to saline (+ve control) (group 4) or Lacprodan® alpha-10 administration [at 100 & 200 mg/kg (groups 5&6)] for 15 days.

Biochemical analysis:
At the end of experimental period, blood samples were collected from retro-orbital venus plexus under ether anesthesia from all animals in plain test tubes. Serum was prepared for biochemical analysis of aspartate and alanine aminotransferase (ALT and AST) activities according to the method of Reitman and Frankel (1957) using biomerieux diagnostic kits, France. Alkaline phosphatase (ALP) activity was determined according to the method of Belfield and Goldberg (1971); and total protein (TP) according to the method of Gornal et al.(1949). All animals were sacrificed by decapitation under ether anesthesia, then livers were removed and a part from the liver was homogenated and used for determination of : Lipid peroxidation (MDA) content [Begona Ruiz-Larrea et al.,(1994)]; reduced glutathione (GSH) content [Beutler et al. (1963)]; nitric oxide (NO) content [Montgomery and Dymock (1961)]; and superoxide dismutase (SOD) content (Marklund and Marklund, 1974).

Histopathological studies:
A part from the rat livers from all groups was removed and immediately fixed in 10% neutral buffered formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene and embedded in paraffin. 4–5 µm thick sections were prepared and stained with hematoxilen and Eosin (H&E) for photomicroscopic observation (Drury and Wallington, 1980).

Statistical analysis:
Data were analyzed by ANOVA single factor using EXCEL Microsoft office 2007, and presented as mean ± S.E.

Results:
Effect of Lacprodan® alpha-10 on liver enzymes activities in serum of normal rats:
Results are shown in table 1.
In those given Lacprodan® alpha-10, AST showed non-significant decrease within gr 3 and gr 4 when compared with normal control value. At the same time there was significant ALT decrease in the rats treated
with 200 mg /Kg Lacprodan® alpha-10 when compared either with the control or with the lower dose (100 mg/Kg) of Lacprodan® alpha-10.

Rats treated with 100 and 200 mg/Kg Lacprodan® alpha-10 showed similar significant decrease in ALP activity compared to the control group. Total proteins (TP) values exhibited significant increase after treatment with the higher dose of Lacprodan® alpha-10 when compared with the other groups. The lower dose of Lacprodan® alpha-10 didn’t increase total protein(TP) in serum (Table 1).

**Effect of Lacprodan® alpha-10 on liver enzyme activities in LPS-treated rats:**

The LPS- hepatotoxicated group showed significant elevation in ALT, AST, and ALP and significant decrease in TP compared with the control group (or with other treatment groups) (Table 1).

ALT values were significantly decreased in groups treated with Lacprodan® alpha-10 versus control or with LPS-alone. The level of AST did not change in groups treated with Lacprodan® alpha-10 alone ( gr 3 & gr4) or even after LPS-treatment (gr 5 & gr 6) on comparable to the control value (table 1). However, ALP activity decreased significantly in groups treated with Lacprodan® alpha-10 when compared with LPS- treated group, in which 200 mg/Kg Lacprodan® alpha-10 decreased ALP value significantly more than the lower dose (100 mg/Kg) as shown in Table (1).

It was observed that, the higher dose of Lacprodan® alpha-10 (200 mg /Kg) significantly increased total protein (TP) level when administered alone or after LPS- injection (gr 4and gr 6). The administration of 100 mg /Kg Lacprodan® alpha-10 did not change total protein value when given to the normal or LPS- hepatotoxic rats (gr 3 and gr 5).

**Table 1:** Therapeutic effect of Lacprodan® alpha-10 (at 100 and 200 mg/kg, orally ) on serum levels of transaminases (ALT and AST), Alkaline phosphatase (ALP) and total proteins (TP) in saline- or LPS- treated rats. Rats were treated with test substance for 15 days of daily treatment.  (n= 6 rat / group ; Means ± S.E.).

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control</th>
<th>LPS</th>
<th>LAC100</th>
<th>LAC200</th>
<th>LAC 100+LPS</th>
<th>LAC 200+LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT  IU/L</td>
<td>A 21.57</td>
<td>B 25.55</td>
<td>A 20.33</td>
<td>C 10.58</td>
<td>C 12.64</td>
<td>C 12.81</td>
</tr>
<tr>
<td>AST  IU/L</td>
<td>A 32.82</td>
<td>B 44.25</td>
<td>A 34.68</td>
<td>C 31.19</td>
<td>C 34.50</td>
<td>C 32.03</td>
</tr>
<tr>
<td>ALP  IU/mL</td>
<td>A 77.47</td>
<td>B 108.91</td>
<td>A 70.17</td>
<td>C 67.00</td>
<td>C 79.17</td>
<td>C 86.41</td>
</tr>
<tr>
<td>TP  g/dl</td>
<td>A 7.617</td>
<td>B 5.017</td>
<td>A 8.167</td>
<td>C 8.917</td>
<td>C 7.817</td>
<td>C 8.683</td>
</tr>
</tbody>
</table>

One way ANOVA
Means in the same row with different letters are significantly different at P<0.05.

**Effect of Lacprodan® alpha-10 on antioxidant activities in liver homogenate:**

Results are shown in table 2. LPS- treatment caused significant depletion in GSH value and SOD as well as significant elevation in MDA and NO values (Table 2).

In-significant changes were observed in GSH and MDA in normal groups treated with Lacprodan® alpha-10 (100 and 200 mg /Kg) when compared to control group . However, Lacprodan® alpha-10 treatment caused significant elevation in SOD value in the higher dose only, while the lower dose Lacprodan® alpha-10 exerted non-significant changes observed when compared with the control group or with the higher dose treatment group.

Nitric oxide level increased significantly in the normal treatment group treated with Lacprodan® alpha-10. (Table 2). The administration of Lacprodan® alpha-10 treatment to LPS- treated groups, revealed that the lower dose normalized GSH level and the higher dose significantly increased GSH when compared with LPS- control group.

There was significant reduction in MDA values in LPS- toxic group of animals treated with Lacprodan® alpha-10 (100 and 200 mg/Kg) compared with all other tested groups. Moreover, the higher dose of Lacprodan® alpha-10 (200 mg /kg) normalized MDA value when compared to LPS- control group.

SOD activity increased significantly in both LPS- hepatotoxic groups when compared with LPS- alone; while their SOD values still subnormal on comparing with control group. It was noticed that Lacprodan® alpha-10 (100mg /Kg, gr 5) significantly increased SOD level on comparison with the higher dose by treatment LPS-hepatotoxic rat (gr 6).
Lacprodan® alpha-10 treatment (100 and 200 mg/Kg) resulted in significant reduction in NO levels when compared with LPS-control group.

Table 2: Therapeutic effect of Lacprodan® alpha-10 (at 100 and 200 mg/kg, orally ) on glutathione (GSH) , malonaldehyde (MDA) , superoxidisedismutase (SOD) and nitric oxide (NO) levels in liver homogenate in saline- or LPS- treated rats. Rats were treated with test substance for 15 days of daily treatment. (n= 6 rat / group ; Means± S.E.).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>LPS</th>
<th>LAC100</th>
<th>LAC200</th>
<th>LAC 100+LPS</th>
<th>LAC 200+LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH mg/gm tissue</td>
<td>A 1.46</td>
<td>B 1.16</td>
<td>A 1.51</td>
<td>1.74</td>
<td>1.45</td>
<td>1.39</td>
</tr>
<tr>
<td>MDA nmol/g tissue</td>
<td>AB 35.10</td>
<td>C 39.85</td>
<td>A 37.45</td>
<td>36.91</td>
<td>33.01</td>
<td>36.54</td>
</tr>
<tr>
<td>SOD† % inhibition of pyrogallol</td>
<td>A 63.67</td>
<td>0.00</td>
<td>AB 69.55</td>
<td>BC 73.00</td>
<td>52.10</td>
<td>36.77</td>
</tr>
<tr>
<td>NO nmol/g tissue</td>
<td>A 314.62</td>
<td>B 487.45</td>
<td>C 394.14</td>
<td>419.38</td>
<td>424.47</td>
<td>439.33</td>
</tr>
</tbody>
</table>

One way ANOVA
Means in the same row with different letters are significantly different at P<0.05.
†SOD values were calculated versus LPS value (% inhibition of pyrogallol auto-oxidation ;10 unit SOD causes 50% inhibition).

Histopathological results:
In control animals liver sections showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and central vein (Fig. A).

The liver of LPS-intoxicated rats showed loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization and congestion of sinusoids and hemorrhage and mononuclear cellular infiltration of lymphocytes (Fig B).

No pathological changes could be noticed the liver of rats treated with doses 100 and 200 mg/kg compared with control group. The hepatic cells appeared normal (Fig C and D).

In LPS treated rats, treatment with Lacprodan® alpha-10 (100 mg/kg) resulted in a remarkable improvement compared to LPS control. The rat liver of this group revealed congested central vein and hepatic cords were slightly distorted (Fig. E).

The group treated with Lacprodan® alpha-10 (200 mg/kg ) showed moderate to weak activity in protecting the liver cells from LPS-injury. The rat liver of this group revealed hydropic degeneration and binucleation in some hepatocytes with enlargement of sinusoids and erythrocyte accumulation (Fig. F).

Discussion:
The mechanism of the harmful effects of LPS are in part due to its ability to induce an oxidative stress status characterized by depletion of endogenous antioxidant enzyme activities such as SOD and glutathione. The prooxidant action of the endotoxin is due to its ability to induce excessive ROS and RNS accumulation leading to cellular injury by impairment of vital macromolecules as protein and lipid (Mallis et al., 2001) resulting in altered membrane fluidity and mitochondrial function (Cadenas and Cadenas, 2002). However, several naturally occurring antioxidant compounds were largely used to protect against liver diseases both in experimental and clinical situations. The best of our knowledge, our report is the first one to deal with the therapeutic effect of Lacprodan® alpha-10 on LPS-induced oxidative stress in rat liver, using two dose levels (100 mg/kg and 200 mg/kg).

When LPS is released from gram-negative bacteria and enters the bloodstream, the liver tightly regulates the entry and processing of LPS by virtue of its ability to clear LPS and respond to LPS (Su, 2002). In animals, LPS is cleared from the circulation within a few minutes after intravenous injection (Mathison and Ulevitch, 1979; Zlydaszyk and Moon, 1976). In addition to its ability to clear LPS, the liver also responds to LPS and produces cytokines. LPS directly causes liver injury by mechanisms involving inflammatory cells such as Kupffer cells and chemical mediators such as superoxide, nitric oxide, TNF and other cytokines (Bykov et al, 2003; Enomoto et al, 2002; Hewett et al, 1993; Suzuki et al, 1996; Tsukada et al, 2003; Wang et al, 1995).
Fig. (A): Photomicrograph of section of liver from control rats showing normal structure of liver, central vein and hepatic cords of hepatocytes with prominent nucleus separated with blood sinusoids. (H & E X 400)

Fig. (B): Section of liver of rats treated with LPS showing abnormal architecture of liver tissue and remarkable fatty degeneration with necrotic cells (arrow head). (H & E X 200)

Fig. (C): Section of liver of rats treated with at dose (100 mg/kg b.wt.) showing normal structure (H & E X 400)

Fig. (D): Section of liver of rats treated with at dose (200 mg/kg b.wt.) showing normal structure (H & E X 1000)

Fig. (E): Section of liver of rats treated with at dose (100 mg/kg b.wt.) and LPS showing congested of central vein and hepatic cords were slightly distorted (H & E X 1000)

Fig. (F): Section of liver of rats treated with Lacprodan® alpha-10 (200 mg/kg b.wt.) and LPS showing hydrophic degeneration and enlargement of sinusoids and erythrocyte accumulation (H & E X 1000).

Results of the present study revealed that a single intraperitoneal toxic dose of LPS (4 mg/kg), administered to albino rats, elicited a significant depletion in hepatic glutathione (GSH) level from that of normal control animals. This result is in accordance with the findings of other investigators who found that administration of a high dose of LPS approaching our dose, caused severe hepatocellular injury as indicated by the depletion of hepatic GSH (Lu et al., 2005; Sebai et al., 2010). This depletion was explained on the basis that LPS, upon entrance in the liver, releases ROS which is conjugated by glutathione S-transferase (GST) to GSH to detoxify it and facilitate its excretion.
The present results also showed that LPS significantly increased hepatic level of malondialdehyde (MDA) which is a biomarker of lipid peroxidation process. This result strongly suggested an oxidative stress-mediated hepatotoxicity, following the depletion of hepatic GSH and loss of intracellular antioxidant enzymes (Kunimoto et al., 1987). LPS-reactive metabolites formed in the liver and LPS-induced depletion of GSH was accompanied by a high level of lipid peroxides (Engin et al., 2011). This in turn, triggers secondary events such as mitochondrial dysfunction with associated energy imbalance and altered intracellular calcium level, which all are signs of fibrosis (Lu et al., 2005; Kheir-Eldin et al., 2001; Sebai et al., 2010).

Moreover, our results showed a remarkable increase in hepatic nitric oxide (NO) level, after LPS-administration, which strongly suggested the presence of cellular injury, partially mediated by reactive oxygen species (ROS). ROS mediated injury decreases the availability of NO, as superoxide anion (O−2) reacts with NO to yield peroxynitrite (ONOO−) a reactive nitrosative molecule which has a powerful oxidant activity that adds to the process of oxidative damage. (Iwakiri and Groszmann, 2007). Also, Mansour et al (2006) reported that NO is a significant mediator of radiation-induced acute tissue damage, although NO has well-known vasodilating and signal transduction functions, inflammation in both lung and liver can lead to increased expression of iNOS and enhanced NO production (Carbonell et al., 2000). Treatment with Lacprodan® alpha-10 (100 mg/kg and 200 mg/kg) elicited a decrease in hepatic SOD levels in LPS-treatment groups when compared to administration of Lacprodan® alpha-10 (100 mg/kg and 200 mg/kg) alone. The later treatment groups showed an increase when compared with the control group. Whey proteins (WP and alpha-lactalbumin; the major component of Lacprodan® alpha-10) are rich in cysteine, glutamate and methionine (cysteine donor), these three amino acids are precursors to the tripeptide glutathione. The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage (Sanmugapriya and Venkataraman 2006). This is accomplished by a set of endogenous antioxidant enzymes such as SOD and glutathione. These enzymes constitute a mutually supportive team of defense against ROS (Venukumar and Latha, 2002).

Regarding the results of the present study, LPS has resulted in significant elevations in liver serum aminotransferases enzymes (ALT and AST) activities. This is due to underlying conditions of oxidative stress, whereby polyunsaturated fatty acids in membranes are subjected to ROS-induced oxidation, membrane integrity is lost, and there is leakage of enzymes to extracellular, then to the circulation (Lu et al, 2005).

As regards to the effect of Lacprodan® alpha-10 on LPS-induced reduction in hepatic GSH, the drug administered alone ,in a dose of 100 mg/kg and 200 mg/kg, produced a significant improvement in GSH level compared to the LPS toxicated group, almost similar to the saline control group. This result is consistent with many investigators who confirmed the ability of whey protein to increase the level of GSH in liver of rats (Bounous, 2000). The influence of Lacprodan® alpha-10 on the cellular status of GSH depends on the fact that WP and alpha lactalbumin have a high content of cysteine and methionine (Balbis et al., 2009), which are important antioxidants and are necessary for the glutathione synthesis that directly participates in the fight against inflammatory diseases (Tseng et al., 2006). Cysteine further influences the production of cytokines, which are immune-regulating hormones. Even in healthy humans, plasma cysteine decreases under stress (Mercier et al., 2004).

Furthermore the results from the present study revealed a therapeutic effectiveness for Lacprodan® alpha-10 100 mg/kg and 200 mg/kg against LPS as evidenced by significant increase in GSH level when compared to that LPS-toxicated group, this may be due to the strong antioxidant activity of both WP and alpha-lactalbumin (Lacprodan® alpha-10 components) through preservation of more reduced glutathione in the liver. It is remarkable that LPS- treatment groups with Lacprodan® alpha-10 (100 mg/kg and 200 mg/kg) significantly reduced the elevated MDA values caused by LPS injection. The significant decrement in MDA level caused by Lacprodan® alpha-10 in rats receiving LPS may be explained on the basis that Lacprodan® alpha-10 contributes to the synthesis of sulfur-containing amino acids such as methionine and cysteine which are considered potent antioxidants (Abdel- Wahhab et al., 1999). Accordingly Lacprodan® alpha-10 can defend against a number of reactive oxygen species (ROS) such as hydroxyl radical, superoxide anion, and alkoxyl radicals, also it is able to regenerate other oxidized antioxidants such as recycling vitamin E peroxylradical (tocopheroxyl radical), oxidized vitamin C (dehydrascorbate) and hence prevents the symptoms of both vitamin C and vitamin E deficiency in rats.

According to our results, Lacprodan® alpha-10 (100 mg/kg and 200 mg/kg) increased hepatic nitric oxide (NO) level in normal rats. NO is mediated through the reaction of NO with a number of targets such as haem groups, cysteine residues and iron and zinc clusters. As well as, NO is synthesized in response to increases in intracellular calcium levels or in response to stimuli such as shear stress (Villanueva and Giulivi, 2010). In combination of LPS with Lacprodan® alpha-10 (100 mg/kg and 200 mg/kg) a significant reduction in the level of NO was noticed in all groups (in lesser extend) when compared to LPS toxicated group.

The ALT and AST are enzymes that are located in the liver cells and leak out and make their way into the general circulation when the liver cells are injured (Johnston, 1999). LPS significantly increased serum ALT and AST activities. Treatment with Lacprodan® alpha-10 at the dose level 100 mg/kg attenuated the increased levels of the serum enzymes, produced by LPS and caused a subsequent recovery towards normalization almost like
that of normal control group. Furthermore, the higher dose of Lacprodan® alpha-10 (200 mg/kg) had lower therapeutic effect against LPS-hepatotoxicity. The therapeutic effect of Lacprodan® alpha-10 and its ability to reverse the LPS elevated levels of ALT and AST may be due to its rich content of sulphydryl peptides (-SH group), in which, whey proteins are rich in cysteine, glutamate and methionine (cysteine donor), these two amino acids are precursors to the tripeptide glutathione. The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD and glutathione. These enzymes constitute a mutually supportive team of defense against ROS (Sanmugapriya and Venkataraman, 2006; Venukumar and Latha, 2002).

Because of the hepatocellular injury caused by LPS, it was expected that serum ALP activity will increase in response to liver injury, on the light of our results, LPS significantly increased serum ALP. Treatment with Lacprodan® alpha-10 at the dose level 200 mg/kg attenuated the increased levels of the serum ALP, produced by LPS and caused a subsequent recovery towards normalization almost like that of normal control group. Furthermore, the lower dose of Lacprodan® alpha-10 (100 mg/kg) had effective but lower therapeutic effect against LPS-hepatotoxicity.

In affected liver, ALP levels will be increased. The ALP values indicate to the biliary system, either within the liver or in the larger bile channels outside the liver. ALP is elevated in a large number of disorders that affect the drainage of bile, such as gallstone or tumor blocking the common bile duct. It serves as an indicator of liver damage when there is cholestasis or lack of bile flow (Johnston, 1999).

ALP enters the general circulation when the liver cells are injured (Johnston, 1999). the ALP is regarded to be a more specific indicator of liver inflammation, since AST may be elevated in diseases of other organs such as heart or muscle disease.

Albumin (as a main component of the proteins) is produced mainly in the liver and its estimated is a test of liver function (Johnston, 1999), and this means that it improved the liver function and counteracted the LPS hepatotoxicity.

Conclusion: Lacprodan® alpha-10 (100 mg/kg) is significantly effective agent against liver injury induced by lipopolysaccharide which is a well-known hepatotoxicant .But it is better recommended to be used on the long term.

REFERENCES


