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RESEARCH ARTICLE

HEPATOPROTECTION BY PHENOLIC CONTENT OF *CAESALPINIA BONDUCELLA* SEED KERNEL EXTRACT AGAINST MASTITIS INDUCED OXIDATIVE DAMAGE IN ALBINO RATS

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ABSTRACT

The present study aimed to investigate the biochemical, antioxidant and histopathological factors & consequences which alter the defensive role of 50% hydro-alcoholic *Caesalpinia bonducella* seed kernel extract (CBSKE) (100 & 200 mg/kg b. wt.) of total Phenolic content on the experimentally induced mastitis by *Staphylococcus aureus* in the late lactating rats. Thirty lactating rats were randomly divided into five groups (n=6); physiological saline treatment (control) group-I, *S. aureus* induced (diseased) group-II, *S. aureus* + CBSKE treated (extract treatments) groups- III & IV and standard antibiotic Ciprofloxacin treated group-V. The concentration of *S. aureus* was adjusted to 2×10^7CFU/ML and inoculated into the abdominal fourth right and left (R4 & L4) mammary glands via the teat duct on the 14th day of lactation. After inoculation, simultaneous treatments were started orally. Dosage has been given per 6h time interval. Rats were euthanized at 72h of post treatment in each group. The blood and liver tissue were collected for biochemical, antioxidant and histological evaluation. The CBSKE treated groups exhibited more relevance to the antibiotic treated group more than the *S. aureus* treated group. Serum total urea, urea nitrogen, creatinine, uric acid, total protein, albumin globulin, AST, ALT, ALP, GGT, amylase and creatinine kinase in level of significance and antioxidant enzymes revealed a significant increase in the lipid peroxidation. Other antioxidants are significantly decreased in the activity levels of superoxide dismutase, catalase, glutathione peroxidase, glutathione-s-transferase, glutathione reductase and reduced glutathione. Further, histological alterations also suggest that the central vein and hepatocytes indicate recovery in treatment groups compared to the diseased group. The phenolic fraction of CBSKE exhibited more potential as an anti-mastitis therapeutics in a rat model.

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INTRODUCTION

Bovine mastitis is a most prevalence causing disease to dairy farms (Ruegg 2003). This pathology is basically caused by microbial intramammary infection. Among those different types of bacteria, causing organisms like contagiously pathogens of *S. aureus* are those which are implicated on the byproduct of dairy herds have acquired diseases (Franklin and Lowy 1998). These organisms are anaerobic opportunistic gram positive pathogens capable of causing pathology effectively in every tissue of the host *S. aureus* is generally considered as non-invasive extracellular pathogen that dama-ges the host cells, at least in part in two ways, firstly either by adhering to the extracellular matrix of the cells and or secondly by inflowing and persevering in the cells thereby, disturbing signaling mechanisms. The persevering nature of the *S. aureus* suggests that it has ability to overcome host defense mechanisms and also, colonization properties of bacteria could explain its chronic effects including osteomyelitis and mastitis (Greer and Rosenberg 1993). The liver is the foremost organ in the vertebrate body and is the major site of xenobiotic metabolism. Liver injury can be caused by toxic chemicals, drugs, bacteria and virus infiltration from ingestion or infection. Incidence of antibiotic resistant strains even becomes an massive threat to human and animal health (Weichhart *et al*., 2003). Numerous changes indicated that *S. aureus* upsets almost all mammalian hosts and sources a kind of diseases comprising skin infection, nasal colonization, sepsis, renal failure and arthritis (Larkin *et al*., 2009). Liver injury is a

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collective feature of bacterial toxemia during sepsis condition which leads to the development of severe shock and multiple organ failure, which suggests that liver injury caused by *S. aureus* mediates Fas-ligand, activated by reactive oxygen intermediates. Earlier it has been suggested that *S. aureus* has the ability to induce free radicals in Swiss albino mice (Klintman *et al.,* 2004). Oxidative stress is caused due to an imbalance between the production of reactive oxygen species and the biological system's ability to detoxify them. Nevertheless, it has been suggested that reactive oxygen species is also one of the factors associated with apoptosis of hepatocytes (Chakraborty *et al*., 2001). Studies related to the *S. aureus* infection provide antioxidant status in the liver tissue is poorly understood. Administration of medicinal plants extract and their derivatives are in advance importance day-by-day. It has been suggested that natural antioxidants obtained from plants reduce oxidative stress thereby, protecting the vital organs. *C. bonducella* seed kernel extract, a caesalpiannace family member commonly known as bonduc, is distributed throughout the tropical areas of India. *C. bonducella* is traditional plant which possess a noticeable *In- vitro*-antibacterial, antineoplastic, antioxidant, antidiarrheals and antihyperlipidemic activity (Shukla *et al*., 2009, Hidayat *et al*., 2011). Reactive oxygen species and liver enzymes also play a vital role in liver injury (Chakraborty *et al*., 2001). *S. aureus* induced pathogenesis also mediates alterations in the oxidant-antioxidant and liver enzymatic status (Larkin *et al*., 2009). *C. bonducella* is well known for its antibacterial properties (Hidayat *et al*., 2011) and also antioxidant properties (Almaraz Abrarco *et al*., 2007). Infecting of foreign microorganism activate inflammatory cells to release oxidants such as superoxide anion, hydrogen peroxide and hydroxyl radicals among other free radicals (Oyarzún *et al*., 2005, Rom 2011). Hence, the present study was aimed to investigate the administration of *C. bonducella* seed kernel extract phenolic fraction to the *S. aureus* induced mastitis in late lactating period of rats by recovery of oxidative stress and enzymatic actions in liver tissue and liver profile parameters of the blood plasma of rats.

MATERIALS AND METHODS

Materials and chemicals

The seed samples were collected from the local market of Dharwad, Karnataka, India. Antioxidants and fine chemicals such as β-nicotinamide adenine dinucleotide phosphate, butylate hydroxyl toluene one reductase, sodium chloride 1,1,3,3- tetramethoxypropane. Thiobarbutaric acid, 2,4 dinitroflurobenzene, Sodium bicarbonate, and kits of aspartate aminotransferase alanine aminotransferase and lactate dehydrogenase were purchased from Sigma chemicals company, solvents were purchased from Sd-fine chemicals.

Preparation of plant extract

The endocarp of the seeds was used for the present study. They were washed thoroughly, separated into the kernel, shade dried, powdered by electrical grinder and stored in an airtight container and used for the present investigation. Further, taxonomic identification was conducted by Dr. G. S. Mulgund, Department of studies in Botany, Karnatak University, Dharwad. Herbarium voucher specimen number Bot/H/484 was deposited in the Department of Studies and Research in Botany, Karnatak University, Dharwad. The seed extract was prepared by 20 g powder of *C. bonducella* seed endocarp (kernel). Powder was extracted with 200 ml of 1:1 ratio of ethanol and distilled water by using a magnetic stirrer for 24 h. The crude extract obtained was filtered through Whatman paper-1 and the filtrate was evaporated to dryness on rotary flash evaporator 45º C. The yield of the extract was 3% (w/v). *C. bonducella* seed kernel extract (henceforth referred to as CBSKE) obtained was preserved in a sterile glass air tight container at 4°C for further use. These crude extracts were separated on the basis of intensifying of the bands appearing in the alumina, silica plate method of thin layer chromatography (TLC). Total soluble phenolics in the extracts were determined with Folin-Ciocalteau reagent according to the method using gallic acid as a standard phenolic compound and determined absorbance at 760 nm (Slinkard and Singleton 1977, Kim *et al*., 2003).

Experimental protocol

The animal facility is available in the Department of Zoology, Karnatak University, Dharwad. Thirty animals were used for this study. They animals were fed a standard pellet diet (Purchased from Krish Scientist's Shopee, Bangalore) and water provided *ad-libitum*. All the animals were maintained under well controlled conditions (12 h light: dark cycle, at the temperature of 25 ± 2º C) and housed in polypropylene cage (1per cage) containing sterilized paddy husk as bedding material. The animals were allowed to acclimatize for ten days before the experiment began. Animals were maintained in accordance with the guidelines of the study approved by the Institutional Animal Ethical Committee, Department of Zoology, Karnatak University, Dharwad, India. CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Animal Registration No. 639/02/a. CPCSEA guidelines were followed for maintenance, use and disposal of the experimental animals.

Thirty healthy adult female and male rats (body weight of 200±40 g; 90 days) of Wistar strain with 1:2 of male and female were caged. Pregnancy was confirmed by checking of vaginal smear on next morning. After Fourteenth day of parturition, 30 rats were randomly divided into five groups in each group having six animals. Six lactating female rats infused with 100µl of pyrogen-free physiological saline (pH 7.4) i.e. group-I (control) and 24 lactating female rats infused with 100µl of *S. aureus* culture was adjusted to 2×10^7CFU/ML in physiological buffer saline and inoculums into the left 4(L4) and right 4 (R4) mammary glands the offspring were weaned 2h prior to experimental inoculation. Intramammary inoculation was according to the method of Chandler *et al*., (1970). The infused animals were divided into four groups; group-II treated as *S. aureus* (diseased), simultaneous treatments were started orally. Dosages has been given per 6h time interval of group-III and IV treatment of CBSKE 100 and 200 mg/Kg body weight, group-V treated with standard antibiotic of Ciprofloxacin 20mg/kg body. Briefly, after 72h of inoculation and treatments blood samples were collected by

cardiac puncture technique under sodium pentaborbitone anesthesia (40 mg/kg) dissect with microbial free equipments of whole liver and kidney tissues were collected for relative organ weight and some liver immersed in phosphate buffer saline kept at -80 for tissue antioxidant studies. Other tissue was fixed in 10% neutral buffered formalin solution histological studies.

Histopathological study

Liver tissues were fixed at 10% buffered formaldehyde and processed in a graded series of alcohol and dehydration in the benzene embedded in the molten paraffin wax for histological examination by conventional methods and stained with Mayer's Hematoxylin and Eosin (H&E). The liver pathology was scored as described by French *et al*., (1988) as follows: 0 = no visible cell damage; 1 = focal hepatocytes damage on <25% of tissue; 2 = focal hepatocyte damage on <25–50% of the tissue; 3 = extensive, but focal, hepatocytes lesion; 4 = global hepatocytes necrosis. The morphology of any lesions observed was classified and registered (Gray 1964). The histopathological examinations were carried out.

Serum biochemical assays

Collected blood was placed at room temperature for 1h, and then centrifuged at 3000 g for 10 min to obtain serum. Serum biochemical parameters Alkaline phasphatase (ALP), Aspartate amino transferase (AST), Alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), Amylase, Creatinine kinase, Urea, Urea nitrogen, Uric acid, Creatinine, Globulin and Total protein were assayed by the method of Reitman and Frankel (1957) using commercially available kits.

Measurement of lipid peroxidation products

Liver tissues were homogenized in chilled 20 mm Tris–HCl (pH 7.4) (1:10, w/v). The homogenate was centrifuged at 2500g for 30 min at 4°C. Aliquots of the homogenate were collected and stored at -80° C for the studies. The determination of MDA by thiobarbituric acid (TBA) was used as an index of the amount of lipid peroxidation according to the methods of Buege and Aust. The supernatant of liver tissue homogenate (1 ml) was mixed with 1 ml of 7.5% (w/v) cold trichloroacetic acid (TCA) to precipitate proteins and then centrifuged at 1500 RPM. The supernatant was reacted with 1 ml of 0.8% (w/v) TBA in a boiling water bath for 45 min. After cooling, the lipid peroxidation product (MDA) was assayed according to an improved thiobarbituric acid reactive substance (TBARS) fluorometric method after excitation at 555 nm and emission at 515 nm using 1,1,3,3-tetraethoxypropane (TEP) as the standard. The results were expressed as MDA formation per milligram of protein.

Assays of glutathione (GSH)

Liver tissues were homogenized in ice-cold potassium phosphate buffer (pH = 7.4). The homogenate was centrifuged at 2500 g for 30 min at 4°C. Aliquots of the homogenate were collected and stored at -80°C for the studies. The content of reduced GSH was determined by modifying the method of Van- Dam *et al*. Liver homogenate and a 5% TCA mixture was pre-incubated for 5 min at 4° C and then centrifuged at 8000g for 10 min at 4° C. Aliquots of the homogenate were collected to which 50-dithio-bis-(2-nitrobenzoic acid) (DTNB) was immediately added and incubated for 5 min at 4 C°. The absorbance was measured at 412 nm, and the concentration of GSH was calculated using the absorbance of 1 M of product with E412 = 13600 M^{-1} cm^{-1}.

Assays of anti-oxidant enzymes

The glutathione peroxidase (GPO) activity was determined spectrophotometrically, according to the method of Mohandas *et al*., (1984). The following solutions were pipetted into a cuvette: 0.1 ml of homogenate and 0.8 ml of 100 mM potassium phosphate buffer, p^H 7.0, containing 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 unit/ml GSH reductase, and 1 mM GSH. The mixture was pre-incubated for 5 min at room temperature. Thereafter, the overall reaction was initiated by adding 0.1 ml of 2.5 mM H_2O_2. Enzyme activity was calculated by the change of the absorbance at 340 nm for 5 min. GPO activity could be expressed as nanomoles of NADPH per minute per milligram of protein. The glutathione reductase (RDG) assay was performed through monitoring the consumption of NADPH for reducing glutathione disulfide (Bellomo *et al*., 1987). The following solutions were pipetted into a 1 cm spectrophotometric cuvette: 0.1 ml of homogenate and 0.9 ml of 0.10M phosphate buffer, p^H 7.0, containing 1 mM $MgCl_2$–$6H_2O$, 50 mM GSSG, and 0.1 mM NADPH. This mixture was pre-incubated for 5 min at room temperature. RDG activity was calculated by the change of the absorbance at 340 nm for 5 min; RDG activity was expressed as nanomoles of NADPH per minute per milligram of protein. The catalase (CAT) activity was determined following the method of Cohen *et al*., (1970). A mixture of 50 mM phosphate buffer (p^H 7.0), 20 mM H_2O_2 and cell lysate in a final volume of 3 ml was incubated at room temperature for 2 min. The change in absorbance at 240 nm in 2 min was calculated. The catalase activity was expressed as µmole/min/mg protein. Superoxide dismutase (SOD) was analysed based on the principle in which xanthine reacts with oxidase to gerate superoxide radicals which react with 2- (4-iodo-phenol) -3- (4-nitrophenol) -5-phenyltetrazoliumchlioride to form red formazan dye. The SOD was determined by the method of Antolovich *et al*., (2002).

Statistical analysis

All data are expressed as mean ± SE. ANOVA was used to evaluate the difference between multiple groups. If significance was observed between the groups, the Tukey's test was used to compare the means of two specific groups. $p<$ 0.05 was considered to be significant.

RESULTS

Effect of plant extract on the tissue weights of rats

Table 2 shows the liver and kidney weights of rats in each group. Compared with controls, there was no significant difference of the kidney weight between *S. aureus*-induced rats

and the control group. It was observed that *S. aureus*-induced rats and low dose of CBSKE (100 mg) showed a significant increase ($p < 0.05$) in the relative liver weight when compared with the control group. However, treated at high doses of CBSKE (200 g/kg b wt) and ciprofloxin combined with *S. aureus* for six days could significantly decrease ($p < 0.05$) the relative liver weight when compared with *S. aureus* induced group.

Histopathology of liver

Figure- 3 shows that *S aureus*-induced liver injury caused the concave liver surface and lymphocytic infiltration in the central vein. The hepatic cells were found to be fatty degeneration, necrosis and cytoplasmic vacuolization in *S. aureus*-induced group. Table 1 summarizes the data relating to liver damage induced by *S. aureus* in pathological histology. The level of fatty degeneration, necrosis and vacuole formation were obvious after acute *S. aureus* treatment. The presence of hepatocytes was assessed as an index of liver proliferative capacity in response to phagocytes-induced injury (Sigala *et al*., 2006). Histological examination showed a preventive effect of the seed kernel on *S. aureus*-induced hepatoxicity.

Effect of CBSKE on *S. aureus* induced hepatic Cellular biomarkers

Subsequent of the main biomarkers of liver metabolic enzymes, we analyzed whether the supplementation of CBSKE could protect rats against *S. aureus* induced acute liver destruction. The therapeutic roles of co-administration with

The therapeutic roles of co-administration with CBSKE on *S. aureus* induced elevation of biomarkers are shown in figure 1 (a, b & c). Co-administration of lactating *S. aureus* induced

CBSKE on *S. aureus* induced elevation of biomarkers are shown in figure-1 (a, b & c). Co-administration of lactating *S. aureus* induced animals with CBSKE could significantly ($p<0.05$) reduced the serum biochemical parameters like ALP, AST, ALT, GGT, Amylase, Creatinine kinase, Urea, Urea nitrogen, Uric acid, Creatinine, Globulin and Total protein.

Effect of CBSKE on hepatic phase II detoxifying and antioxidant enzymes

Figure 2 (a & b) shows the effects of CBSKE on hepatic GSH related enzymes in *S. aureus*-induced damage in rats. After induction of *S. aureus*, the activities of GPO and RDG were significantly decreased as compared with the control group. Plant extract treated animals with two doses of CBSKE (100-200 mg/kg) separately for 6 days significantly elevated the expression of GPO as compared with the group of *S. aureus*-treated alone ($p < 0.05$). The activity of RDG was found to be increased with administration of high doses of seed kernel and ciprofloxacin compared to the group of CBSKE-treated alone. In contrast, neither GST nor CAT was influenced by the treatment of *S. aureus*, ciprofloxacin, or with seed kernel. However, high dose of seed kernel could restore the antioxidant capacity exhausted by *S. aureus*.

Table 1. Histological injury recovery score of liver under comparing the *S. aureus* induced group with different doses of CBSKE, Ciprofloxacin and control rats

Groups	Injury of score		
	Fatty Degeneration	Necrosis	Vacuole Formation
Control	0	0	1
S. aureus	4	3	3
S. aureus + CBSKE (100mg/Kg.b.wt.)	3	2	4
S. aureus + CBSKE (200mg/Kg.b.wt.)	2	1	1
S. aureus + Ciprofloxacin	1	1	1

Liver tissues were scored for hepatic injury via light microscopy with score 0 = no visible cell damage; 1 = focal hepatocytes damage on less than 25% of the tissue; 2 = focal hepatocytes damage on 25-50% of the tissue; 3 extensive, but focal, hepatocytes lesions; 4 = global.

Table 2. Body weight and relative organ weights are comparing of different treatment and control group versus diseased group. The data were represented as mean ± SE

Groups	Body Weight (g)	Relative organ weight (g/100 g BW) Liver	Kidney
Control	227±17	3.4 ±0.08	0.60±0.00
S.aureus	221±18	3.9±0.05*	0.62±0.01
S.aureus + CBSKE (100mg/Kg. b.Wt)	217±29	3.9±0.16*	0.63±0.01
S.aureus + CBSKE (200mg/Kg. b.Wt)	219±23	3.2±0.05**	0.56±0.00
S.aureus + Ciprofloxacin (20mg/Kg. b.Wt)	223±31	3.1±0.07**	0.55±0.00

* *S. aureus* induced and 100 mg/kg b.Wt. of CBSKE significant different from the control group.
** Significantly different from the group treated with 200 mg /kg b.Wt. of CBSKE and Ciprofloxacin treated groups.

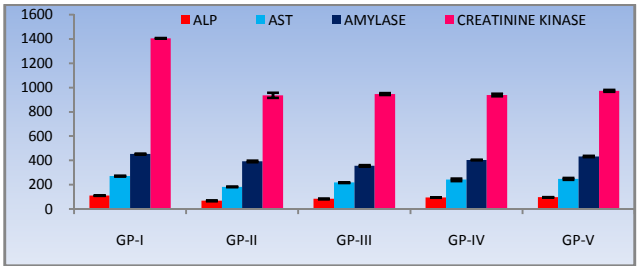
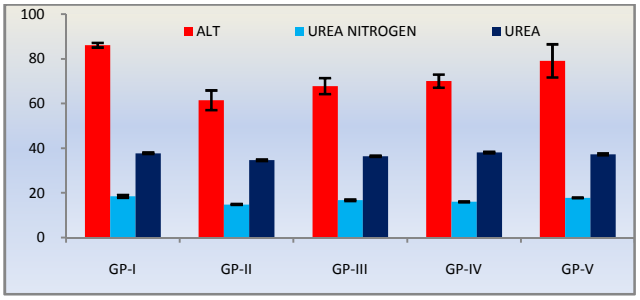


Fig. 1(a)



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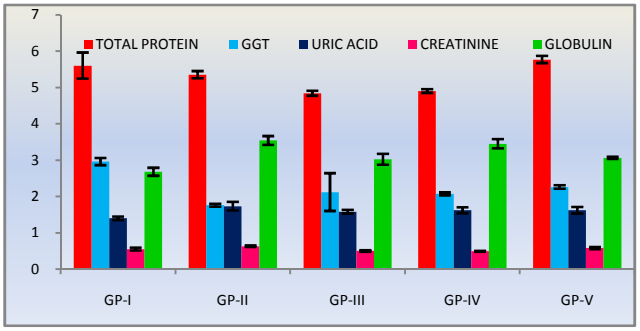

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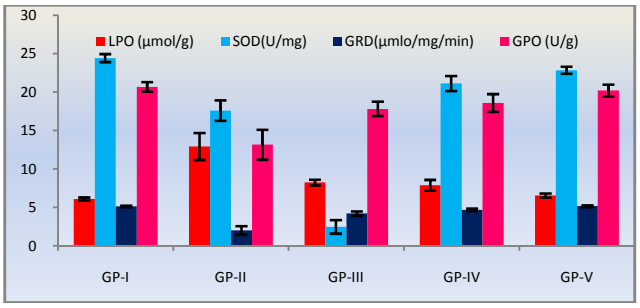
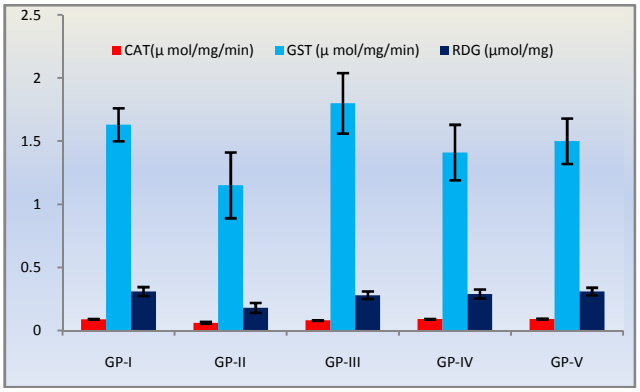


Fig. 2 (a)



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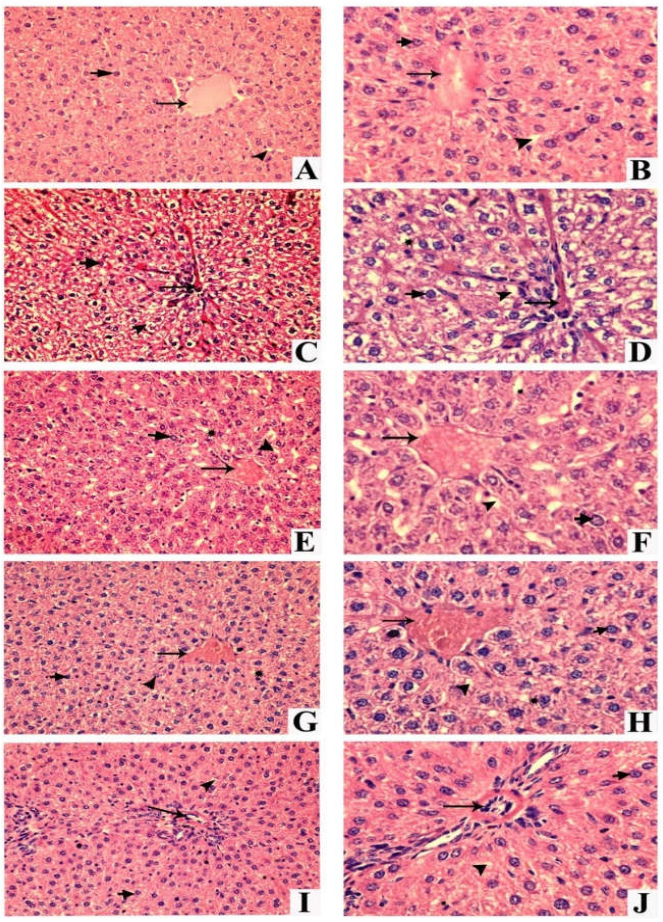

Fig. 3 Photomicrographs of liver sections of control group of (A and B) (×200 and ×400); C and D shows *S.aureus* infection injury on congestion of central vein, fatty-degeneration (aster), necrosis (small arrow) occurring on the hepatocytes and vacuoles (arrow head) are raised in larger numbers; E and F shows lower dose of CBSKE therapeutic recovery on *S.aureus* infection injury; G and H indicates the higher doses of CBSKE therapeutic recovery on *S.aureus* infection injury and I and J shows the Standard antibiotic of Ciprofloxacin treated sections. [(×200 and ×400); H&E stained]

Effect of CBSKE on hepatic GSH content

Figure-2 (a & b) shows the effect of CBSKE on the content of GSH in *S. aureus*-induced hepatotoxicity in rats. The administration of a single dose of *S. aureus* to rats resulted in a decline of total GSH content in the liver homogenate. Both pre-treatment of ciprofloxacin and CBSKE significantly inhibited the depletion of GSH, compared to the group of *S. aureus* -treated rats alone ($p < 0.05$). Interestingly, could protect GSH content depletion induced by *S. aureus*.

DISCUSSION

The outcome of the present study indicates that Co-administration of 50% hydro-alcoholic CBSKE ameliorates antioxidant status in *S. aureus* induced oxidative stress in the liver of rats as evidenced by a) decrease in the lipid peroxidation products, b) increase in the activity levels of antioxidant enzymes and reduced glutathione levels, c) liver enzymatic status of blood plasma, and d) restoration of liver architecture. Liver sepsis is a serious constant problem all over the world and *S. aureus* is also one of the major contributors of

liver sepsis and as a result liver injury. It is well known that *S. aureus* produces a broad range of virulence determinants which may be structural components of the bacterial cell envelope and adhesions, or toxins and enzymes, which are secreted extracellularly (Larkin *et al*., 2009). These virulence determinants are believed to mediate the pathogenesis of bacteria, including sepsis (Chakraborty *et al*., 2001). Studies of Klintman *et al*., (2004) also reported that Fas ligand is one of the virulence factors responsible for liver injury caused by *S. aureus*. Further, studies of Weglarczyk *et al*., (2004) also suggested that *S. aureus* infection leads to release of reactive oxygen free radicals, which is important for the activation of CD95 (Fas) -Fas ligand interactions thereby lead to apoptosis of monocytes. Thus, it is apparent that *S. aureus* induced effects are complex and at least in part mediates oxidative stress.

In general stability exists between the generation of lipid peroxidation products *viz.,* Reactive oxygen species (ROS) and the level of endogenous antioxidants throughout physiological conditions which serve to protect tissue from oxidative damage. Disruption of this balance, either through increased production of ROS or decreased levels of antioxidants, results in a condition referred to as "oxidative stress". Thus, evaluation of lipid peroxidation and the antioxidant enzyme status and reduced glutathione content in biological tissue has been always used as markers for tissue injury and oxidative stress. Lipid peroxidation can cause changes in membrane fluidity and permeability and increase the rate of protein degradation, which eventually lead to cell lysis. It is well acknowledged that free radical scavengers such as SOD, CAT, GSH and metabolism regulatory enzymes such as GSH-PX, GR and GST can protect the cellular system from deleterious effect of free radicals (Weglarczyk *et al*., 2004). SOD, as a first line defense antioxidant enzyme plays an important role in the dismutation of superoxide and thereby, leads to hydrogen peroxide which is eventually neutralized by GPO and catalase (Blokhina *et al*., 2003).

In the present study, there was a significant increase in the lipid peroxidation products with a significant decrease in the activity levels of antioxidant enzymes such as SOD, catalase, GPO, GST, GR and antioxidant content, reduced glutathione in the liver of rats intoxicated with *S. aureus*. The decrease in the activity levels of SOD, catalase and GPO indicates accumulation of superoxide ions and also hydrogen peroxide ions, which might lead to an observed increase in the lipid peroxidation products in the liver of rats intoxicated with *S. aureus*. Further, alterations in the glutathione metabolism were observed in the *S. aureus* injected rats. Glutathione reductase is a known defense against oxidative stress, which in turn needs glutathione as cofactors. It is known that, GSH plays an important role in hepatocytes defense against ROS, free radicals and electrophilic metabolites (Whiteside *et al*., 2004**).** Hence, severe GSH depletion leaves cells more vulnerable to oxidative damage by radicals and increases protein thiolation or oxidation of SH groups. The decrease in the reduced glutathione content might reflect decreased levels of the activity of glutathione reductase and glutathione peroxides in the liver of *S. aureus* infected rats. Consistent with our results, earlier it has been suggested that *S. aureus* infection leads to enhanced levels of reactive oxygen intermediates with reduced content of glutathione (Castell *et al*., 1997). It is also evident that an increase in the free radicals changes the architecture of the vital organs. Consistently, there were histological alterations as evidenced by disorganization of the hepatocytes were observed accompanied by centrilobular hepatocytes hypertrophy. One of the important findings of the present study was co-administration of aqueous-alcoholic extracts of CBSKE significantly ameliorate the antioxidant status in the liver of *S. aureus* injected rats. Plant and their products are well known for their antioxidant properties. Phenolic compounds of many plants have immense beneficial effects on certain diseases through their potential to scavenge free radicals and neutralize the reactive oxygen species. CBSKE contained important amounts of flavanols, a type of phenolic compound. Flavanols play an important role in the boosting of endogenous antioxidant status, thereby mitigating the lipid peroxidation products (Whiteside et al., 2004).

The measurement of Cellular Marker activities in blood serum is frequently used as a diagnostic tool in human and animals for the assessment of damage to vital organs following induced to inflammatory agents (Yousef *et al*., 2002, Barse *et al*., 2006, Nassr-Allah and Abdelhamid 2007). During cellular damage, these enzymes are leaked into the serum and hence minimize the activities of these enzymes in serum is considered as a sensitive indicator of even minor cell damage (Bernet *et al*., 2001). Significant increase in the levels of both cellular markers in serum of animals exposed to CBSKE and standard antibiotic group without treatment suggests that the tissues might have been damaged by *S. aureus*. Decrease in levels of cellular marker an indication of liver damage. The increased is activities of these liver enzymes demonstrate perturbations in the metabolism of amino acids and cellular damage in these tissues. *S. aureus* induce cellular damage, protein reduction and increase the activities of these enzymes in serum by translocation (Agarwal *et al*., 2010, Pramanik and Biswas 2012). The toxicity of carbon tetrachloride on liver damage and eventual release of transaminases to the serum has been well documented (Salawu and Akindahunsi 2006, Edewor *et al*., 2007). Positive correlations obtained between these pollutants and increase in the activities of these enzymes in serum and Supplementation with 200 mg/kg CBSKE during exposure had significant effects on reduction in the release of ALP, AST, ALT, GGT, Amylase, Creatinine kinase, Urea, Urea nitrogen, Uric acid, Creatinine, Globulin and Total protein into the serum in kidney and liver while, less effect was observed for ALT in other tissues. Treatment after exposure with 200 mg/kg BW supplementation of feed with CBSKE did not have much impact on the leakage of AST and ALT into the serum. The protective ability of CBSKE against the effects of *S.aureus* could be due to the inherent antioxidative compounds and phytochemicals. It has also been reported to contain anti-inflammatory constituent which could assist in stopping the inflammation of tissues (Chinedu *et al*., 2011). This study showed that simultaneous supplementation with CBSKE during exposure to *S. aureus* provides better results than after treatment all of these compounds are established to possess antimicrobial activity. Thus, the beneficial role of CBSKE might be direct or indirect. In the present study, the increase in the antioxidant enzymes suggests the antioxidant properties of

the plant which in turn decreases the lipid peroxidation products, thereby restores the liver architecture and protects histo-architecture of the liver of CBSKE co-administered *S. aureus* induced rats. These reflect the direct actions of CBSKE whereas, indirectly it affects the colonization of *S. aureus* through its antioxidant properties. Thus, from the results it seems apparent that, the protective role of CBSKE might be due to its antioxidant properties and/ or antimicrobial properties against *S. aureus* induced oxidative stress in the liver damage in rats. Further, studies are in progress in our laboratory to know the interactions of purified products from the CBSKE against specific virulence determinants of *S. aureus*.

Conclusions

Staphylococcus aureus is a well known mastitis causing environmental pathogen. The mechanism (s) of pathogenicity of *S. aureus* is complex and at least in part mediates oxidative stress. The present study clearly demonstrates the protective role of phenolic content of CBSKE against *S. aureus* induced hepatotoxicity probably, by neutralizing the lipid peroxidation products, through the elevation of intrinsic cellular markers and antioxidant mechanisms. In depth studies related to natural products in the management of microbe-mediated diseases will be highly useful to develop new therapeutic formulations.

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