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Modulator Efficacy of Dietary Inclusion of Ocimum sanctum Leaves Against Gentamicin-Induced Hepatotoxicity in Rats

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ABSTRACT

The oxidative stress and free radicals associated with Gentamicin proposed as the foremost cause for hepatotoxicity. Flavonoids are widely distributed in the *Ocimum sanctum* leaves, and exhibited various pharmacological activities including hepatoprotective activity. The present study was commenced to investigate the modulator efficacy of dietary inclusion of *Ocimum sanctum* leaves against Gentamicin-induced hepatotoxicity in rats. The aqueous extract was prepared from leaves of *Ocimum sanctum*, and screened for phytochemical analysis. The basal diet supplemented with 2% w/w and 4% w/w powdered leaves of *Ocimum sanctum* were prepared and evaluated hepatoprotective effect against hepatotoxicity induced by Gentamicin in rats. The preliminary phytochemical investigation showed the presence of carbohydrates, glycosides, alkaloids, saponins, flavonoids, tannins gums and mucilage in aqueous extracts. The pretreatment with basal diet supplemented with powdered leaves of *Ocimum sanctum* (2% w/w and 4% w/w) preserved the changes in biochemical parameters and validates the hepatoprotective effect of diets. The administration of diet supplemented with powdered leaves of *Ocimum sanctum* recovered the level of SOD, GSH, LPO and Catalase and protects the liver from oxidative stress. It was assumed that the effect of a diet supplemented with powdered leaves of *Ocimum sanctum* on liver protection is related to free radical suppressing activity.

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Introduction

The liver played a vibrant role in the cleansing of injurious substances. It has a regulatory effect on the several significant metabolic functions. It is also responsible for preserving homeostasis of the body[1]. Aminoglycoside antibiotics are commonly used for the treatment of severe Gram-negative bacterial infections by inhibiting bacterial protein synthesis, therefore the most widely used drugs in this category is gentamicin. Hepatotoxicity induced by gentamicin in experimental animals was reported. Gentamicin enhances the oxidative stress and generation of free radicals and causes inhibition of the antioxidant defense system in the liver. It suppresses the non-enzymatic and enzymatic antioxidants, which leads to an over production of Reactive Oxygen Species (ROS). This will cause damage to membrane lipids, proteins and nucleic acids, which leads to liver toxicity, dysfunction and injury[2, 3].

Hepatoprotective produces a protective effect to the liver against hepatotoxicity. The flavonoids, lignans, xanthines, coumarins, glycosides and polyphenols constituents present in the plants impart hepatoprotective activity. The above constituents are mostly found in medicinal plants and therefore a large number of plants is used as a hepatoprotective[4].

Ocimum sanctum, is a well known medicinal plant, which grows wild as well as in households and temples in India. It has been traditionally regarded as possessing rejuvenating, tonic and vitalizing properties that contribute to longevity and a healthy life. Leaves of *Ocimum sanctum* possess expectorant, diaphoretic, antiseptic, spasmolytic, stimulant and anticatarrhal properties and are used as cold and cough remedies, for fever, pain, gastrointestinal disorders (like dyspepsia, vomiting), worm infestations, skin diseases, snakebite and scorpion sting[5-9].

The significant hepatoprotective activity of *Ocimum sanctum* was reported earlier and in view of its hepatoprotective properties it is worthwhile to investigate and establish the hepatoprotective potential of the dietary containing Ocimum sanctum against Gentamycin -induced hepatotoxicity in rats.

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2 Material and Methods

Plant Material

The leaves of ***Ocimum sanctum*** **was** selected for the proposed study.

Preparation of Aqueous Extracts

1 Kilogram of powdered drug of ***Ocimum sanctum*** was extracted with distilled water. The extract was filtered while hot, and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The aqueous extracts were stored in the refrigerator for further experimental work.

Phytochemical analysis

Qualitative chemical tests were performed to determine the presence of alkaloids, carbohydrates, cardiac glycosides, polyphenols, saponins, tannins and terpenoids[10, 11].

The Protective Effect Of Dietary Inclusion Against Gentamicin Induced Hepatotoxicity.

The basal diet (50% skimmed milk, 36% corn starch, 10% groundnut oil and 4% mineral and vitamin premix) was prepared and fed to normal and control group animals. The basal diet supplemented with 2% and 4% *Ocimum sanctum* and fed to Gentamicin-induced hepatotoxicity animals.

All the animals were divided into the five groups; each group consisted of 6 animals and they received the treatment as follows:

Group I: Normal and received basal diet

Group II: Received basal diet + 4% w/w ***Ocimum sanctum*** fruit for 30 days

Group III: Control group received Basal diet + Gentamicin (100 mg/kg i.p.) for 3 days

Group IV: 2% w/w ***Ocimum sanctum*** for 27 days + Gentamicin (100 mg/kg i.p.) for 3 days

Group V: 4% w/w ***Ocimum sanctum*** for 27 days + Gentamicin (100 mg/kg i.p.) for 3 days

The protocols were ended after 30 days. The animals were decapitated by cervical dislocation. The blood was withdrawn from animals by direct heart puncture, and kept in

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bottles containing EDTA. The plasma were separate out from blood by centrifuged at 3000 r/min for 10 min. Further the plasma was used for the determination of various biochemical parameters. Correspondingly, the liver was isolated, rinsed in cold saline and homogenized in phosphate buffer (pH 6.9). The clear supernatant was obtained after centrifuging the homogenates at 7500 r/min for 10 min. The *in vivo* antioxidant activity was estimated from clear supernatant[12-14].

Biochemical Parameters

Serum separated by centrifugation was used to determine serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum acid phosphatase (ACP) and total bilirubin[15, 16].

Analysis Of Antioxidant Enzymes Of Liver Tissue

The antioxidant activities in the rat kidney homogenate were assayed for superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH) and activity lipid peroxidation (LPO). The activity of SOD was evaluated by Nitro blue tetrazolium (NBT) reduction process. The H_2O_2 technique was employed for the assessment of CAT activity. The substrate 5, 5'- Dithio.bis (2 – nitrobenzoic acid) was used for the estimation of GSH level. The lipid peroxidation level was estimated by measuring the concentrations of malondialdehyde[17, 18].

Data analysis

Results were analyzed using one way analysis of variance (ANOVA) followed by the Tukey's test by using statistical software packages, Graph Pad Prism; version 5.03. Values were expressed as mean ± SEM and the $p < 0.05$ were considered as statistically significant.

3 Results And Discussions

In the present study, *Ocimum sanctum* leaves were selected for the study of protective efficacy of dietary inclusion against gentamicin-induced hepatotoxicity in experimental animals.

Phytochemical Screening

Preliminary phytochemical investigations of the aqueous extracts of leaves of *Ocimum sanctum* revealed the presence of flavonoids, tannins, phenolic compounds, glycosides and carbohydrates. The details are presented in table 1.

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The aqueous extracts of *Ocimum sanctum* was selected for further *in vitro* antioxidant activity evaluation as this extract revealed the presence of flavonoids and phenolic compounds. Therefore, in *vivo* activities were performed with basal diet supplemented with 2% and 4% *Ocimum sanctum* and fed to gentamicin-induced hepatotoxicity animals.

Hepatoprotective Activity

Table 2 showed the effect of various treatments on serum SGOT, SGPT, ALP, ACP and bilirubin. The animals treated with Gentamicin, developed significantly liver damage, were observed from the alteration in the activities of serum enzyme (SGOT, SGPT, ALP and ACP) and bilirubin in serum (Table 2). The level of values of the SGOT, SGPT, ALP, ACP and bilirubin was significantly increased in the Gentamicin treated rats compared to normal animals fed with basal diet. The animals treated with diets containing 2% w/w and 4% w/w *Ocimum sanctum* significantly reduced the serum level. The serum level of animals received diets containing only 4% w/w *Ocimum sanctum* (Group II) were normal, and it indicates that liver functions were not affected.

Effects of Dietary Supplement On Liver Antioxidant

Table 3 exhibited animals treated with Gentamicin at a dose of 100mg/kg body weight leads to significant reduction in the activities of the antioxidant enzymes, namely SOD, GSH and CAT compared to the normal group, while increasing in LPO level. Conversely, feeding the Gentamicin treated rats with diets containing either 2% w/w and 4% w/w *Ocimum sanctum* leaves inclusions (Groups IV and V, respectively) caused a marked reversal in the depleted antioxidant enzyme level.

Liver imparts chief role in metabolism, detoxification, and protein synthesis. Drug-induced hepatotoxicity is one of the major causes of human mortality all over the world. The researchers are working for protection of liver against Gentamicin-induced toxicity.

The clinical uses of gentamicin has been narrow due to its associated side effects. It has been observed most patients taking gentamicin encountered liver inefficiency problem. Consequently, taking these medications face limitations due to the fact that one of the major side effects of Gentamicin is creating

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hepaotoxicity. The Gentamicin induces the production of free radicals, which can be seen after the use of gentamicin in cells, is effective in inducing toxic impacts of this drug on the structure and function of tissues[19, 20].

To evaluate liver injury, biochemical markers (SGOT, SGPT, ALP and ACP activity and serum bilirubin) levels were measured. The findings demonstrated the hepatotoxicity due to Gentamicin was confirmed by elevated levels of biochemical parameters like SGOT, SGPT, ALP, ACP and total serum bilirubin. The impairment of hepatic cell or membrane leads to discharge enzyme into circulation, and it has been observed in studies. Higher level of SGOT indicates the liver damage, due to oxidative stress produced from Gentamicin during metabolism by hepatic microsomes which in turn cause peroxidation of lipid of cellular membranes. SGPT catalyses the conversion of alanine to private and glutamate and is released in a similar manner. Therefore SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury[137]. The increased level of SGOT and SGPT in Gentamicin-induced liver injury is an indicator of cellular leakage and loss of membrane integrity of liver cells[21]. The animal fed with diets containing *Ocimum sanctum* leaves reversed the increased levels of SGOT and SGPT as a result of the stabilization of plasma membrane and the repair of hepatic cell damage induced by Gentamicin.

Serum ALP, ACP and bilirubin levels on other hand related to the function of hepatic cells. Increase in serum level of ALP and ACP is due to increased synthesis, in the presence of increasing biliary pressure. Hyperbilirubinemia was due to the excessive heme destruction and block of bile duct within the liver. Accordingly, there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged hepatocytes[22, 23]. The findings exhibited that the animals treated with diets containing *Ocimum sanctum* leaves significantly inhibit the generation of ALP, ACP and bilirubin. Effective control of bilirubin level and alkaline phosphatase activity point towards on early improvement in the secretory mechanism of the hepatic cell.

The LPO level in the liver tissue was markedly increased in response to Gentamicin intoxication, indicating oxidative damage of the liver. Gentamicin administration also

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reduced the levels of SOD, GSH and CAT in the liver tissue compared to the normal rates. The elevated LPO in liver indicates failure of antioxidant defense mechanisms. The oxidative stress plays an important role for unbalance between the production of reactive oxygen species and antioxidant defenses and it implies that cell damage of the liver. The marked declined in the leakage of liver enzymes into the serum also confirmed the inhibitory effect of a diet containing *Ocimum sanctum* leaves against lipid peroxidation[24]. In contrast, the significant increased in SOD, GSH and CAT levels compared to control group. A variation of these antioxidant defenses clearly contributed to the antioxidant and hepatoprotective activity of diet containing *Ocimum sanctum* leaves.

4 Conclusion

The present study was undertaken to explore the modulator efficacy of dietary inclusion of *Ocimum sanctum* leaves against Gentamicin-induced hepatotoxicity in rats. In conclusion, the study demonstrated that administration of gentamicin induces liver damage in rats. However, *Ocimum sanctum* supplemented diets ameliorate this gentamicin-induced hepatotoxicity through improvement in the rats' antioxidant status and modulating oxidative stress. Consequently, dietary inclusion of *Ocimum sanctum* may be a cheap management strategy in the management of acute hepatotoxicity or gentamicin-induced renal damage.

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Table 1: Phytochemicals present in leaves of *Ocimum sanctum* extracts

Phytoconstituent		Aqueous
Alkaloids	Dragendorff's test	-
	Hager's test	-
	Mayers	-
	Wagners	-
Glycosides	Legal's test	+
	Keller killiani test	+
	Baljet test	-
	Keller-Killiani test	-
	Borntrager's test	+
Carbohydrates	Molish test	+
	Benedict's test	+
	Fehling's test	-
Tannins and Phenolic compound	5%$Fecl_3$ solution	+
	Lead acetate solution	+
	Bromine water	-
	Potassium ferric cyanide and ammonia solution	+
Flavonoids	Shinoda test	+
Steroid test	Liebermann burchard test	-
	Salkowski test	-
Protein	Biuret test	-
	Ninhydrin test	-
Fat and oil test	Saponification test	-
	Spot Test	-

+ = Present, – = Absent

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Table 2: Effect of diets supplemented with *Ocimum sanctum* on liver function test for different parameters in animals treated with Gentamicin.

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	ACP (U/L)	Bilirubin (mg/100 ml of blood)	
					Direct (mg/dl)	Total (mg/dl)
Normal rats (Basal diets) (Group-I)	72.1 ± 3.14	75.4 ± 2.65	124.7 ± 5.24	135.2 ± 4.47	0.18 ± 0.52	0.43 ± 0.24
Ocimum sanctum (4% w/w) + Normal rats (Group-II)	65.3 ± 4.62	76.8 ± 3.17	119.5 ± 2.38	142.4 ± 5.23	0.23 ± 0.38	0.49 ± 0.91
Control rats Gentamicin (100 mg/kg) + Basal diets (Group-III)	186.7* ± 2.71	195.2* ± 2.92	263.5* ± 4.86	211.6* ± 3.54	1.21* ± 0.17	2.05* ± 0.44
Ocimum sanctum (2% w/w) + Gentamicin (100 mg/kg) (Group-IV)	89.2[a] ± 3.18	105.9[a] ± 4.35	192.5[a] ± 3.24	156.4[a] ± 2.95	0.59 ± 0.32	0.85 ± 0.19
Ocimum sanctum (4% w/w) + Gentamicin (100 mg/kg) (Group-V)	70.6[a] ± 1.76	72.6[a] ± 3.82	119.2[a] ± 2.64	126.9[a] ± 1.69	0.25[a] ± 0.41	0.39[a] ± 0.54

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Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 when compared with normal Group I and

Ocimum sanctum (4% w/w) Group II, [a]P<0.05 when compared with Gentamicin (100 mg/kg) treated Group

III considered as statistically significant.

Table 3: Effect of diets supplemented with leaves of *Ocimum sanctum* on oxidative stress induced by Gentamicin in the liver of experimental animals.

Treatment	Enzymes involved in oxidative stress in the liver			
	LPO (Mole/gm)	SOD (U/gm)	GSH (µMole/gm)	Catalase (U/mg)
Normal rats (Basal diets) (Group-I)	65.9±3.53	73.1±2.84	4.2±1.23	8.6±2.17
Ocimum sanctum (4% w/w) + Normal rats (Group-II)	68.3±1.86	64.1±5.42	4.6±1.47	7.9±1.32
Control rats Gentamicin (100 mg/kg) + Basal diets (Group-III)	171.5±4.21*	9.2±2.17*	0.32±1.02*	0.95±2.11*
Ocimum sanctum (2% w/w) + Gentamicin (100 mg/kg) (Group-IV)	98.6±4.82[a]	43.1±5.24[a]	3.2±1.35[a]	5.8±1.26[a]
Ocimum sanctum (4% w/w) + Gentamicin (100 mg/kg) (Group-V)	70.1±2.69[a]	67.4±3.72[a]	4.3±2.49[a]	7.4±0.84[a]