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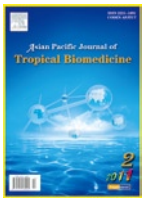

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In vivo simulated *in vitro* model of *Jasminum sambac* (Linn.) using mammalian liver slice technique

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ABSTRACT

Objective: To evaluate the antioxidant status of *Jasminum sambac* (*J. sambac*) using mammalian liver slice technique in *in vivo* simulated *in vitro* model. **Methods:** Antioxidant activity of *J. sambac* was studied against H_2O_2 induced free radicals in goat liver. **Results:** Administration of H_2O_2 showed significant decline in the levels of antioxidant enzymes in liver homogenate. Pretreatment with *J. sambac* had significant protection in those levels within normal range. Also the plant normalized the lipid peroxidation which evidently showed that the methanolic extract of *J. sambac* had a potent antilipid peroxidative effect. **Conclusions:** The present study suggests that *J. sambac* has a potent antioxidant effect and it can be used to treat various diseases caused by free radicals.

1. Introduction

Liver is an organ of paramount importance, which plays a pivotal role in regulating various physiological processes in the body, such as metabolism, secretion and storage. It has great capacity to detoxify toxic substances and synthesize useful principles. The damage to the liver caused by hepatotoxic agents is of grave consequences. In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are in rise. Jaundice and hepatitis are two major hepatic disorders that account for the high death rate[1]. Oxidative stress plays an important role in many diseases including liver diseases. However, the over production of oxidative stress can lead to damage in DNA, cell membrane, protein and cellular membranes and consequently induces degeneration, destruction and toxicity of various molecules and causes muscular dystrophy, cancer as well as liver diseases[2].

The production of oxidative stress can be controlled by the antioxidant systems in living organisms. The medicinal value of plants has assumed a more important dimension in the past few decades owing largely to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potential[3]. At present a variety of medicinal plants are available to alleviate diseases like liver diseases, cancer, respiratory disorders and much more.

Jasminum sambac (*J. sambac*) is largely used in folk medicine to prevent and treat breast cancer, and also has hepatoprotective, antileprotic and antiulcerative effects. But there is no scientific evidence for its hepatoprotective activity. Hence the present study was undertaken to explore the key behind the use of *J. sambac* as a hepatoprotective against hydrogen peroxide induced hepatotoxicity in goat liver model.

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2. Materials and methods

2.1. Preparation of extract

The fresh flowers of *J. sambac* were collected from Coimbatore, Tamil Nadu, India. The specimen sample was authenticated by Professor GVS Moorthy, Botanical Survey of India, Tamilnadu Agricultural University, Coimbatore, India. The voucher specimen (No.BSI/SRC/5/23/09-10/Tech-972) was also maintained in the herbarium cabinet. The flowers were washed thrice with distilled water to remove the contaminants and air dried in shade. Coarsely powdered sample (450 g) was extracted with methanol. This extract was then filtered through filter papers and filtrates were evaporated under reduced pressure at 40 ℃ using a rotary evaporator to get 5.5 g *J. sambac*.

2.2. Preparation of mammalian liver slices

The goat liver was selected as the mammalian tissue to determine the antioxidant effect of methanolic extract in the presence and absence of the standard oxidizing compound (H_2O_2). The dose of H_2O_2 used was the same as the level used *in vivo* studies by intraperitonial administration (2 mL/kg tissue). The liver was collected fresh from local slaughter house immediately after the sacrifice of the animal. The tissue was quickly plunged into cold sterile Hanks balanced salt solution (HBSS) buffer and maintained at 40 ℃. Very thin (≈1 mm) slices of the tissues were cut by using the sterile scalpel and tissue (250 mg) was taken in 1.0 mL of sterile HBSS, in broad, flat bottomed flasks. The necessary compounds (H_2O_2 and methanolic extract) were added and incubated at 37 ℃ for one hour with mild shaking. Appropriate control groups were also set up. The standard oxidant H_2O_2 was used at a concentration of 2 mL/kg tissue.

After the incubation period, the tissues were homogenized in the same aliquot of the HBSS buffer using a Teflon homogenizer and centrifuged to remove the debris. The supernatant was then used for the estimation of various parameters to assess the antioxidant potential.

2.3. In vivo simulated in vitro model in H_2O_2 induced free radicals

The following groups were set up for antioxidant assay. Group 1 served as normal control, group 2 corresponded to H_2O_2 induced free radicals, group 3 were treated with *J. sambac* at 20 mg (20 μL) per mL of HBSS, group 4 represented as positive control (rutin at 70 mg/kg tissue) and group 5 were treated with *J. sambac* alone. After the incubation time homogenized tissues were used to analyze the antioxidant enzymes.

2.4. Analysis of antioxidant status

The homogenized liver tissues were used for the analysis of antioxidant enzymes such as super oxide dismutase (SOD)[4], catalase (CAT)[5], glutathione peroxidase (GPX)[6], glutathione transferase (GST)[7], glutathione reductase (GR)[8], glucose-6-phosphate dehydrogenase (G6PD)[9], glutathione (GSH)[10], vitamin A (Vit-A)[11], vitamin C (Vit-C)[12], vitamin E (Vit-E)[13], lipid peroxidation (LPO)[14], protein[15], polyphenol oxidase (PPO)[16].

2.5. Statistical analysis

Values were expressed as mean±SD. Statistical difference in mean was analyzed using one way ANOVA and followed by least square mean deviation comparison tests (LSD). $P<0.05$ was considered statistically significant.

3. Results

The results of the present study showed that, the levels of enzymic and non enzymic antioxidants were significantly decreased in H_2O_2 induced group. Treatment with *J. sambac* at 20 mg/mL of HBSS caused significant increase in those

Table 1
Effect of *J. sambac* flower extract on the antioxidant status in H_2O_2 induced free radicals in goat liver (mean±SD).

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
SOD	11.73±0.04	4.46±0.26[a]	8.78±0.13[b]	10.49±0.18[b]	11.53±0.04
CAT	20.17±0.38	17.04±1.10[a]	19.82±1.54[b]	23.12±1.48[b]	20.04±1.40
GPX	4.56±0.43	2.97±0.48[a]	4.40±0.13[b]	5.44±0.19[b]	4.54±0.44
GST	0.41±0.01	0.15±0.02[a]	0.22±0.02[b]	0.30±0.01[b]	0.39±0.01
GR	6.65±0.20	3.75±0.21[a]	5.80±0.23[b]	6.26±0.09[b]	6.57±0.24
G6PD	1.63±0.10	1.05±0.03[a]	1.05±0.03[b]	1.30±0.06[b]	1.56±0.08
GSH	24.29±0.01	12.65±0.04[a]	20.14±0.02[b]	21.00±0.50[b]	24.18±1.73
Vit-A	0.03±0.01	0.00±0.01[a]	0.04±0.01[b]	0.06±1.01[b]	0.02±0.00
Vit-C	1.31±0.02	0.69±0.05[a]	1.03±0.01[b]	1.24±0.01[b]	1.33±0.02
Vit-E	14.01±1.32	9.93±0.95[a]	11.42±0.80[b]	13.31±0.95[b]	13.81±0.80
LPO	44.42±0.66	83.45±0.67[a]	57.29±1.19[b]	52.47±0.14[b]	44.40±0.57
Protein	8.68±1.03	3.08±0.87[a]	3.43±0.96[b]	5.97±0.81[b]	8.56±0.97
PPO	205.20±1.20	35.60±0.55[a]	148.10±0.55[b]	183.20±0.22[b]	200.00±1.50

[a]: $P<0.05$ compared with normal control; [b]: $P<0.05$ compared with H_2O_2 induced group.

values when compared with toxic group. Its antioxidant effect was also compared to that of rutin, the known standard antioxidant. *J. sambac* alone treated group showed no significant changes in the levels of antioxidants when compared with group 1 at $P<0.05$.

H_2O_2 intoxicated group showed significant elevation in the level of LPO. Effect of *J. sambac* on lipid peroxidation showed very potent inhibition in both plant and standard drug treatment group when compared with toxic group. However, the protein content in liver tissues showed a significant decline in group 2. Pretreatment with *J. sambac* and rutin, offered significant increase in the protein level as compared with group 2 (Table 1).

4. Discussion

Free radical is an atom or a molecule with one or more unpaired electrons in its outermost orbits. Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing caused by reactive oxygen species. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent diseases[17].

Hydrogen peroxide can easily cross the cell membrane and attack different sites by converting into water. It can cause DNA damage in the form of both single and double strand breaks which is believed to be the initial step in the induction of cancer. In the present study, induction of H_2O_2 produced free radicals in *in vitro* model was used to investigate whether the plant extract of *J. sambac* could decrease efficiently the toxicity produced by these toxic substances.

The present results indicated that pretreatment of liver homogenate in *in vitro* model with *J. sambac* at 20 mg/mL concentration resulted in significant elevation of antioxidant enzymes. The recovery towards normalization of these enzymes caused by plant treatment was almost similar to that caused by rutin in the present study.

Rajan *et al*[18-22] reported the decreased activity of SOD, CAT and GPX in CCl_4 induced rats. On treatment with *Cassia tora* these levels attained near normal levels. Decreased activity of these enzymes is due to the effect of free radical metabolites on the liver detoxificant enzymes like CAT, SOD and peroxidase. The reduced enzymes activity is due to enzyme inactivation during catalytic cycle[23].

CAT is an enzymatic antioxidant widely distributed in all animal tissues including red blood cell and liver. CAT decomposes hydrogen peroxide and helps protect the tissues from highly reactive hydroxyl radicals. Treatment with plant extract showed significant improvement in hepatic CAT levels when compared to toxic group[24].

GST level was reduced in toxic group. After treatment with *J. sambac* the levels recovered to near normal. The decline in the levels of GST is due to the excessive formation of free radicals and activation of lipid peroxidation systems which leads to tissue damage[25]. Anilkumar *et al*[26] reported that *Emblica officinalis* Gaertn treated animals showed a significant increase in the levels of G6PD.

GSH is a tripeptide. It is an antioxidant and helps protect cells from reactive oxygen species such as free radicals and peroxides. It is a potent inhibitor of the neoplastic process, and plays an important role in the endogenous anti-oxidant system. It is found in particularly high concentration in the liver and is known to have a key function in the protective process[27]. The present investigation also falls in previous study of Dahiru *et al*[28-32] showing the levels of glutathione, vitamin E and vitamin C were elevated in treated groups when compared with control and induced groups. Non enzymic antioxidants have been reported to inhibit iron induced lipid peroxidation and thus reduce levels of free circulation iron. Thus the free radical scavenging property of *J. sambac* could maintain the near normal level of non-enzymic antioxidants[33].

J. sambac showed very potent inhibition of goat liver lipid peroxidation *in vitro* at 20 mg/mL when compared to toxic group. Malondialdehyde (MDA) is the major oxidation product of peroxidized poly-unsaturated fatty acids and the increased MDA content is an important indicator of lipid peroxidation. LPO is easily induced by free radical *via* the reaction of hydroxyl radical with unsaturated fatty acids. Lipid peroxidation, an autocatalytic free radical chain propagating reaction, is known to be associated with pathological conditions of a cell[34]. In the present study methanolic extract of *J. sambac* prevented the rise of lipid peroxides, showing their significant antilipid peroxidant effect which was also reported by Meera *et al*[35].

In conclusion, the methanolic extract of *J. sambac* showed the highest antioxidant capacities in H_2O_2 induced free radicals in goat liver model. This medicinal plant could be potentially rich sources of natural antioxidants and could be developed into functional food or drug for prevention and treatment of diseases caused by oxidative stress. In the future, the specific components with high antioxidant capacity in this medicinal plant could be isolated and explored for their health effects on oxidative stress.

Conflict of interest statement

We declare that we have no conflict of interest.

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