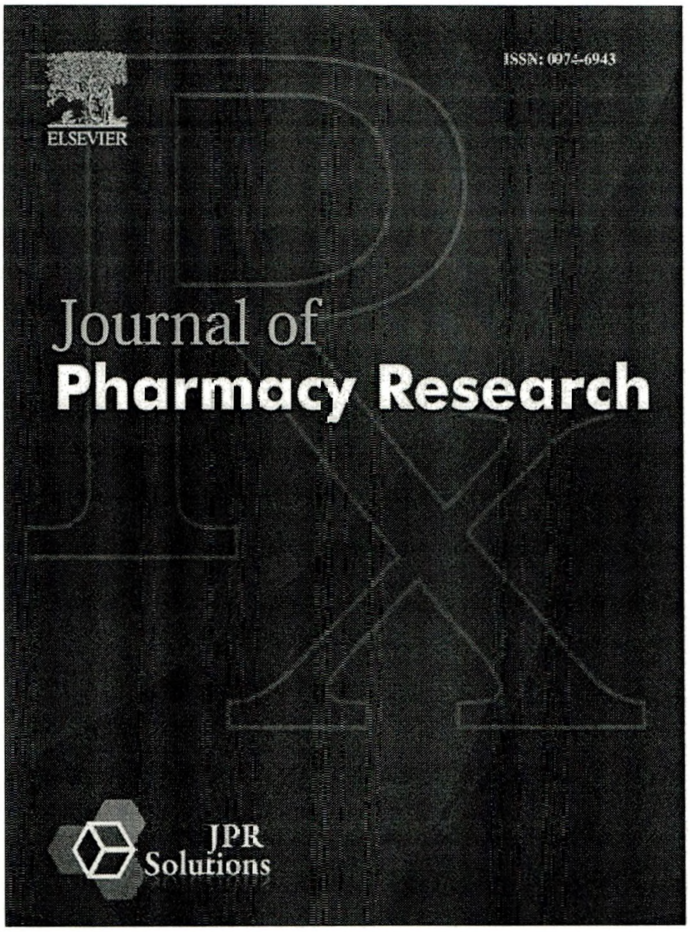
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Original Article

Antioxidant potential of the flowers of *Caesalpinia pulcherrima*, Swartz in an *in vitro* system subjected to oxidative stress

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ABSTRACT

Background: Oxidative stress leads to various pathological conditions including cancer. Antioxidant enzymes such as superoxide dismutase and catalase represent the cell defense mechanism for preventing oxidative damage. Recently many studies have focused on finding natural antioxidants, especially of plant origin for the treatment of oxidative stress associated diseases. The pharmacological and therapeutic properties of plants are attributed to the ability of antioxidants in them to scavenge free radicals.

Objective: In the present study, goat liver was selected as an *in vitro* model to determine the antioxidant effects of the three flowers (orange, pink and yellow) of *Caesalpinia pulcherrima* both in the presence and the absence of a standard oxidant (H_2O_2). The enzymic antioxidants (catalase, peroxidase, superoxide dismutase, glutathione reductase and glutathione S-transferase) and the non-enzymic antioxidants (vitamins A, C, E and reduced glutathione) were analysed.

Results: Treatment with hydrogen peroxide reduced the antioxidant levels in goat liver slices which were improved on co-treatment with the flower extracts, which proved the antioxidant efficacy of the flowers.

Conclusion: Our findings showed that the methanolic extract of the flowers of *C. pulcherrima* exhibits significant antioxidant activity against H_2O_2-induced oxidative stress in goat liver model.

1. Introduction

Free radicals are considered as the products of normal metabolic processes in the human body, but when produced in excess, they cause damage to biomolecules.[1] Oxygen radicals generated during reduction of oxygen can attack DNA bases or deoxyribose residues to produce damaged bases or strand breaks.[2] Such biomolecular damage by free radicals leads to many pathological diseases such as cancer, inflammation, and atherosclerosis.[3]

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Antioxidants from various sources, especially those of plant origin, reduce the adverse effects of free radicals. They act as scavengers by donating one of their own electrons in order to replace the stolen electron from free radicals.[4] Plant-derived bioactive compounds known as phytochemicals are rich in antioxidant and free radical scavenging properties.[5] Many research studies have been carried out to identify plants with significant antioxidant and anticancer potential by analysing their cytotoxic, antiproliferative, apoptotic and radical scavenging activities using both *in vitro* and *in vivo* systems.[6]

Caesalpinia pulcherrima is one such candidate plant which blooms in three different colours (orange, pink and yellow) with unique long stamens. It is commonly known as peacock flower or "Barbados pride" in English and as "Mayil kondrai" in Tamil and belongs to the family Fabaceae. The aerial parts of the plants have been used traditionally for the treatment of various diseases including asthma, bronchitis, cholera, diarrhoea, dysentery and malarial infection.[7] The flowers of *C. pulcherrima* have been reported to possess antiviral activity.[8]

In recent years, the use of animals in research, teaching and testing has become an important ethical and political issue. Alternative scientific tests are being developed, which are more efficient and reliable than animal tests. Several non-animal tests have been developed that are cost-effective, practical, and expedient.[9]

The major advantage of using organ slices as *in vitro* model is that they represent the multicellular, structural and functional features of *in vivo* tissue. Organ slices have been used extensively as a promising model for elucidating the mechanism of drug induced organ injury and for characterizing species susceptibilities.[10] Precision-cut liver slices are widely used to elucidate the pharmacological metabolism and to investigate the toxicology and efficacy of novel substances on primary material under standardized conditions.[11] They also mimic the *in vivo* situation of the liver due to the presence of the physiological extracellular matrix.[12] Hence in the present study, the goat liver slices were selected as an *in vitro* model to determine the antioxidant potential of the methanolic extract of the three different flowers of *C. pulcherrima* (yellow, pink and orange) against H_2O_2 induced oxidative stress.

2. Materials and methods

2.1. *Plant material*

Fresh flowers of *C. pulcherrima* (Fig. 1) were collected from the local areas of Coimbatore. The three different flowers namely yellow, pink and orange were procured. The plant was identified and certified by the Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore. The voucher specimen was collected and maintained.

2.2. *Preparation of the plant extract*

The methanolic extract of the three different flowers were prepared using cold extraction method. After evaporation, the yields of the extracts were calculated and the residues were re-dissolved in dimethyl sulfoxide (DMSO) [20 mg flower extract per 5 μl DMSO]. The concentration of the flower extract used for each antioxidant assay was 100 μg.

2.3. *In vitro model system used*

Fresh goat liver was obtained from the local slaughterhouse and transported on ice to the laboratory. The liver was quickly plunged in ice-cold PBS and maintained at 4 °C till use. Thin slices (1 mM thickness) of the liver were cut using a sterile scalpel and the slices were taken in PBS at a proportion of 0.25 g in 1 ml, in broad, flat bottomed flasks. H_2O_2 was used as the oxidising agent to induce oxidative stress at a final concentration of 200 μM. The liver slices were treated with H_2O_2 both in the presence and the absence of the flower extracts (yellow, pink and orange) and incubated at room temperature for 1 h with mild shaking. After incubation, the mixture was homogenized using a Teflon homogenizer followed by centrifugation and the supernatant was used for the analysis.

2.4. *The treatment groups*

The treatment groups set up for the study included the untreated control containing the liver slices alone, the positive control in which the liver slices were treated with H_2O_2 and the test groups in which the liver slices were treated with respective flower extracts in the presence and absence of the oxidant H_2O_2. Appropriate controls treated with the flower extracts in the absence of the oxidant were also set up.

2.5. *Analysis of enzymic antioxidant activity*

The SOD activity estimated by the method of Misra and Fridovich (1972).[13] Catalase activity was estimated by the method of Luck (1974).[14] The peroxidase activity was assayed using the method proposed by Reddy et al (1985).[15] GST activity was determined by the method of Habig et al (1974).[16] Glutathione reductase activity was assayed as per the method of David and Richard (1983).[17]

2.6. *Analysis of the levels of non-enzymic antioxidants*

Ascorbic acid levels were estimated based on the method of Roe and Keuther (1943).[18] The tocopherol level was estimated by the method of Rosenberg (1992).[19] The GSH level was estimated by the method of Moron et al (1979).[20] Vitamin A content was measured by the method proposed by Bayfield and Cole (1980).[21]

2.7. *Statistical analysis*

The parameters analysed were expressed as Mean ± SD and the statistical analysis was done using SigmaStat (Version 3.1). Statistical significance was determined by one way ANOVA with $P < 0.05$ considered to be significant.

3. Results and discussion

3.1. *Effect on enzymic antioxidants*

The levels of both enzymic and non-enzymic antioxidants were assessed in the liver slices subjected to oxidative stress

Yellow Pink Orange

Fig. 1 – *Caesalpinia pulcherrima* flowers.

in the presence and the absence of the flower extracts. The activities of enzymic antioxidants in the liver slices treated with H_2O_2 and/or flower extract are represented in Table 1. The activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) decreased significantly on treatment with H_2O_2 compared to that of untreated control. Treatment with the flower extracts alone showed no significant changes in the SOD activity. The co-treatment with methanolic extract of the three flowers (yellow, pink and orange) significantly improved the SOD activity compared to that of untreated control. A similar trend was found in peroxidase activity.

The catalase activity in the liver slices reduced significantly compared to that of the untreated group. On treatment with the orange flower extract alone, the enzyme activity was increased compared to that of untreated control and no significant changes were found in the yellow and pink flower extract treated groups. All the three flowers of *C. pulcherrima* significantly elevated the catalase activity ($P < 0.05$) in the presence of the oxidant.

A similar trend was observed in a study where pretreatment with chloroform and ethanolic extract of *Vitis vinifera* L. stem bark showed significant antidiabetic activity by improving the SOD, catalase and peroxidase levels in diabetes induced group of rats.[22] The concentration of SOD, CAT and GSH was significantly decreased in the liver of in Wistar rats after treatment with doxorubicin which was reversed on co-treatment with *Punica granatum* Linn. (Punicaceae) extract.[23]

The effect of *C. pulcherrima* flower extracts on GST and GR activities of liver slices exposed to H_2O_2 is also shown in Table 1. H_2O_2 significantly reduced the activities of GST and GR compared to untreated control. The liver slices treated with the three flower extracts alone showed a significant increase

Table 1 – Effect of *C. pulcherrima* flower extracts on enzymic antioxidant activities in goat liver slices exposed *in vitro* to H_2O_2.

Groups	Enzymic antioxidants				
	SOD[a] (Units/g tissue)	CAT[b] (Units/g tissue)	POD[c] (Units/g tissue)	GST[d] (Units/g tissue)	GR[e] (Units/g tissue)
Liver slices	15.44 ± 3.85	276.06 ± 12.58	30.12 ± 3.50	0.042 ± 0.001	2.53 ± 0.14
Liver slices + H_2O_2	10.42 ± 2.87[f]	151.11 ± 16.35[f]	18.88 ± 0.33[f]	0.015 ± 0.002[f]	1.49 ± 0.28[f]
Liver slices + CPY	13.62 ± 2.47	276.06 ± 12.58	31.70 ± 2.91	0.030 ± 0.004[f]	2.30 ± 0.20
Liver slices + CPY + H_2O_2	11.61 ± 2.77[f]	208.33 ± 7.21[f,g,h]	18.50 ± 1.27[g,h]	0.021 ± 0.004[f,h]	2.16 ± 0.14[f,g]
Liver slices + CPP	14.25 ± 3.13	262.49 ± 20.38	30.58 ± 2.97	0.026 ± 0.002[f]	2.12 ± 0.08[f]
Liver slices + CPP + H_2O_2	11.52 ± 3.46[f,h]	200.46 ± 11.81[f,g,h]	22.70 ± 0.08[f,h]	0.020 ± 0.002[f]	1.97 ± 0.06[f,g]
Liver slices + CPO	13.33 ± 3.23	291.91 ± 14.87[f]	32.14 ± 0.87	0.026 ± 0.004[f]	2.38 ± 0.13
Liver slices + CPO + H_2O_2	11.14 ± 4.70[f,g,h]	227.34 ± 15.18[f,g,h]	28.12 ± 0.08[g]	0.020 ± 0.002[f]	2.17 ± 0.12[f,g]

Values are expressed as Mean ± S.D of triplicates, enzyme activity was expressed as Units/g liver tissue.
CPY – yellow flower of *C. pulcherrima*.
CPP – pink flower of *C. pulcherrima*.
CPO – orange flower of *C. pulcherrima*.
a One unit is defined as the amount of enzyme that gives 50% inhibition of NBT reduction in 1 min.
b One unit is defined as the amount of enzyme required to decrease the absorbance at 240 nm.
c One unit is defined as the change in absorbance at 430 nm/min.
d One unit is defined as the millimoles of NADPH oxidized/minute.
e One unit is defined as the nanomoles of CDNB conjugated/minute.
f Statistically significant ($p < 0.05$) compared to untreated control.
g Statistically significant ($p < 0.05$) compared to H_2O_2 control.
h Statistically significant ($p < 0.05$) compared to the respective plant control.

Table 2 – Effect of *C. pulcherrima* flower extracts on non-enzymic antioxidant levels in goat liver slices exposed *in vitro* to H_2O_2.

Groups	Non-enzymic antioxidants			
	Vitamin C (mg/g tissue)	Vitamin E (μg/g tissue)	Vitamin A (μg/g tissue)	Reduced glutathione (nmol/g tissue)
Liver slices	0.14 ± 0.02	4.17 ± 0.47	95.95 ± 0.77	2.16 ± 0.2
Liver slices + H_2O_2	0.10 ± 0.002	0.80 ± 0.03^{a}	58.70 ± 2.96^{a}	1.30 ± 0.03^{a}
Liver slices + CPY	0.17 ± 0.01^{a}	2.84 ± 0.24^{a}	84.50 ± 2.54^{a}	1.97 ± 0.18
Liver slices + CPY + H_2O_2	0.12 ± 0.001^{c}	1.88 ± 0.30^{a,b,c}	71.85 ± 2.05^{a,b,c}	1.63 ± 0.02^{a}
Liver slices + CPP	0.16 ± 0.005	3.09 ± 0.59^{a}	85.05 ± 4.31^{a}	1.95 ± 0.04
Liver slices + CPP + H_2O_2	0.013 ± 0.01^{b,c}	1.92 ± 0.11^{a,b,c}	70.40 ± 0.70^{a,b,c}	1.58 ± 0.01^{a}
Liver slices + CPO	0.16 ± 0.003	3.01 ± 0.23^{a}	88.90 ± 1.55^{a}	2.03 ± 0.30
Liver slices + CPO + H_2O_2	0.12 ± 0.001^{c}	1.94 ± 0.14^{a,b,c}	75.05 ± 1.20^{a,b,c}	1.72 ± 0.19^{a,b}

Values are expressed as Mean ± S.D of triplicates.
a Statistically significant ($p < 0.05$) compared to untreated control.
b Statistically significant ($p < 0.05$) compared to H_2O_2 control.
c Statistically significant ($p < 0.05$) compared to the respective plant control.

in GST and GR activities than the untreated control. The toxic effect of H_2O_2 was counteracted upon co-treatment with the three flower extracts. A significant reduction in GR activity was observed in the H_2O_2 treated group compared to the untreated control. Co-treatment of liver slices with *C. pulcherrima* flower extracts significantly elevated the GR activity compared to that of the H_2O_2 treated group.

A recent study on the management of nephrolithiasis using natural products has reported that the supplementation with ethanolic extract of *Saccharum spontaneum* restored the levels of GST, GR, SOD, CAT and GPx in liver and kidney homogenate thereby exhibited antiurolithiatic activity against ethylene glycol induced nephrolithiasis in male Wistar albino rats.[24] The above findings also correlated with another study where *n*-hexane extract of *Podophyllum hexandrum* rhizome protected the rat liver tissue against CCl_4 induced oxidative stress by significantly increasing the levels of GSH, GPx, GR, SOD and GST in a dose dependant manner.[25]

Treatment with the extract of *Nyctanthes arbortristis* leaves[26] and *Curcuma amada*[27] (both leaves and rhizome) significantly improved the enzymic antioxidant status of goat liver slices subjected to oxidative stress. In another study, administration of *Alternanthera sessilis* leaf extract also increased the antioxidant status of rat liver exposed to the oxidant.[28]

3.2. *Effect on non-enzymic antioxidants*

Apart from enzymic antioxidants, non-enzymic antioxidants are also found in biological systems and are found to play an important role in defence mechanisms against oxidative stress. The effect of the methanolic extract of the three different flowers of *C. pulcherrima* on non-enzymic antioxidant levels in liver slices exposed to oxidative stress was analysed and the results are shown in Table 2. H_2O_2 significantly decreased the levels of ascorbic acid, tocopherol, GSH and vitamin A, which were improved on co-treatment with the flower extracts.

These findings correlated with a study in which the supplementation of the protein deficient diet (PDD) diet with six locally consumed plants in Nigeria for nutritionally stressed male albino rats resulted in significantly higher ($P < 0.05$) levels of vitamin E and vitamin C in liver and kidney tissues.[29] Similarly, treatment with *Moringa oleifera* leaf extract increased the levels of non-enzymic antioxidants and glutathione content in CCl_4-treated goat liver slices.[30] Our results also correlated with another study in which a significant increase ($P < 0.01$) in the levels of vitamins C, E, A and GSH was observed in goat liver slices exposed to H_2O_2 after treatment with the leaf extract of *Zea mays*.[31]

4. Conclusion

In the present study, precision-cut goat liver slices were chosen as an *in vitro* model and was maintained and treated in an environment that simulates the conditions *in vivo*. All the three flowers (yellow, pink and orange) of *C. pulcherrima* significantly improved the antioxidant status of the goat liver slices challenged with oxidative stress *in vitro*. The above findings showed that the three flowers of *C. pulcherrima* flowers possess significant antioxidant potential, which may be rendered by the secondary metabolites and active molecules present in the flowers.

Conflicts of interest

All authors have none to declare.

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