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Cassia tora L. Cream Inhibit Ultraviolet B Induced Oxidative Stress in Rats

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Abstract: The aim of this study was to determine the *in vivo* antioxidant activity of newly formulated Oil in Water (O/W) creams of methanolic extract of *Cassia tora* L. leaves. O/W creams of methanolic extract of *Cassia tora* L. leaves was prepared, evaluated and tested for acute dermal toxicity study. The different O/W creams showed good physical characteristics and pass the sensitivity, irritation, grittiness and bleeding test. Exposure of Ultraviolet-B (UV-B) light decreased the reduced Glutathione (GSH), Catalase (CAT) and Superoxide Dismutase (SOD) activities and increased the Lipid Peroxidation (LPO). The oxidative stress induced by exposure of UV-B light in rats was minimized by single dose topical application of different concentrations of O/W creams and *Cassia tora* Methanolic extract (CTM). Researchers concluded that topical O/W creams and methanolic extract of *Cassia tora* leaves has potent antioxidant activity.

Key words: Oxidative stress, O/W creams, glutathione, lipid peroxidation, antioxidant

INTRODUCTION

Free radical stress leads to tissue injury and progression of disease such as cancer, aging, ischemia, liver injury, arthritis and Parkinson's syndrome. Safer antioxidants suitable for long term use are needed to prevent or stop the progression of free radical mediated disorders (Sumanth and Rana, 2006). Many plants possess antioxidant ingredients that provided efficacy by additive or synergistic activities. Some studies showed that a number of plant products including polyphenolic substances and herb extracts exert potent antioxidant action. Some traditional natural antioxidants are already exploited commercially either as antioxidant additives or as nutritional supplements (Krishnaraju *et al*., 2009). Exposure of UV-B light leads to oxidative stress, cellular changes, DNA damage and also damage the skin. UV-B exposure decreased the GSH, CAT and SOD activities while it increased the LPO (Gupta and Sharma, 2006; Goettsch *et al*., 1999).

Cassia tora L. (Fabaceae) also known as Charota has been reported to possess a significant *in vitro* antioxidant activity using DPPH assay (Zhang *et al*., 2007). However, it is not available as topical cream so it has been prepared different concentrations of O/W creams of methanolic extract of *C. tora* and evaluated its antioxidant activity *in vivo* using UV-B induced oxidative stress in rats.

MATERIALS AND METHODS

Plant material: Leaves of *Cassia tora* L. were collected from Dabhoi, Vadodara, Gujarat, India and authenticated by Prof. P.S. Nagar, Department of Botany, Maharaja Sayaji Rao University of Baroda, Vadodara, Gujarat, India. The voucher specimen (03PG768, Niraj) has been deposited in the herbarium section of the Botany Department. The leaves was dried in shade and crushed in the grinder, coarse powder used for extraction.

Preparation of extracts: The methanolic extract was prepared by cold maceration method by taking 200 g of powdered leaves and extracting with 600 mL of methanol for 4 days. Extract was filtered; filtrate was evaporated using a rotary evaporator under reduced pressure to dryness. The extract was used to prepare different concentration of O/W creams (0.05, 0.1 and 0.2%). Creams were evaluated for sensitivity, grittiness, irritation and bleeding test (Singhal and Kansara, 2012).

Acute dermal toxicity study: The acute dermal toxicity test of cream was determined according to the OECD 402. Adult Wistar rats (250-300 g) of either sex were used. Approximately 24 h before the test, fur should be removed from the dorsal area of the trunk of the test animals by clipping of shaving. Not <10% of the body surface area should be clear for the application of the test substance. Starting dose of 2000 mg kg^{-1} (topically) of cream was given to three groups (n = 6) each. Cream should be held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 24 h exposure period. The treated animals were monitored for 14 days for changes in fur, eyes, behavior and toxic reactions.

Ultraviolet-B induced oxidative stress in rats: The animals were treated with respective doses of different concentrations of O/W creams (Test 1: 0.05, Test 2: 0.1,

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Test 3: 0.2%), standard (Tretinoin-0.05%), cream base and crude extract apply topically (single dose) during whole treatment. The animals were divided into seven groups as follows:

- Group 1: Positive control
- Group 2: Standard (Tretinoin-0.05%) cream (Topical)
- Group 3: Test 1 (0.05%) cream (Topical)
- Group 4: Test 2 (0.1%) cream (Topical)
- Group 5: Test 3 (0.2%) cream (Topical)
- Group 6: Cream base (Topical)
- Group 7: Extract (Topical)

Select Wistar rats (Male, 300 g) and divided into seven groups. Hair on the dorsal skin was carefully shaved. Test creams were applied topically on the dorsal part of the skin where radiation exposed. An area (1.5-2.5 cm) on one side of the flank is irradiated for 15 min (1.5 J cm^{-2}) at a vertical distance of 20 cm with UV-B lamps. Biochemical parameters like LPO, SOD, CAT and GSH were assessed in the blood withdrawn from the retro-orbital plexus at the end of last treatment in the UV-B induced psoriasis (Nakaguma *et al*., 1995).

Statistical analysis: All the experimental results were expressed as mean±SEM. For statistical comparisons, explorative probabilities were obtained by the one-way ANOVA followed by Dunnett's multiple comparison tests and intergroup difference was considered significant when $p<0.05$.

RESULTS AND DISCUSSION

Evaluation of creams: Three different concentrations of o/w creams (Test 1: 0.05, Test 2: 0.1 and Test 3: 0.2%) were prepared to evaluate antioxidant activity. Physical evaluation revealed that creams having light green colour, characteristic odour, semisolid in nature and pH ranges from 6.5-7. They were passed the sensitivity test, irritation test. During stability study, no phase separation and liquefaction were observed.

Acute dermal toxicity study: The acute dermal toxicity test of creams was determined according to the OECD 402. The creams were safe up to the dose of 2000 mg kg^{-1}. There were no changes in fur, eyes and behavior of treated animals as well as no toxic reactions determined and from results suitable dose (250 mg (0.05%), 500 mg (0.1%) and 1000 mg (0.2%)) was chosen for each activity in each cream for further *in vivo* studies.

Evaluation of *in vivo* antioxidant activity: *Cassia tora* methanolic extract and their O/W cream were evaluated for their antioxidant activity using UV-B induced oxidative stress in rats. There was a marked depletion of SOD, CAT and GSH levels in positive control group. Treatment with the different O/W creams of methanolic extract of leaves of *Cassia tora* L. and CTM significantly increased their level (Table 1). There was a dose dependent inhibition of *in-vivo* lipid peroxidation by the different formulations of methanolic extract of leaves of *Cassia tora* L. cream and CTM (Table 2).

The present study has shown that exposure of UV-B light for 15 min, produced oxidative stress in rats. The literature has documented free radical generation and DNA damage occurs during the exposure of UV-B light (Goettsch *et al*., 1999). UV exposure, particularly UV-B rays, causes the generation of free radicals and related reactive oxygen species which contribute to carcinogenesis by directly damaging cellular macromolecules, including DNA (Katiyar *et al*., 2001). The level of the markers of oxidative stress, observed in UV-B induced rats, substantiate the possibility of extensive generation of free radicals. It is further observed that application of different concentrations of O/W creams and CTM, prevented the UV-B induced oxidative stress parameters and the effect was comparable to that of standard. Hence, the observed *in vivo* antioxidant activity of different concentrations of O/W creams and CTM are substantiation of its earlier reported activity in *in vitro* studies.

Table 1: Effect of *Cassia tora* creams on blood SOD, CAT and GSH in UV-B induced oxidative stress in rats

Groups	Increase of SOD (%)	Increase of CAT (%)	Increase of GSH (%)
Positive control	-	-	-
Test 1	79.04±0.01^{a}	52.85±0.01	56.42±0.25^{c}
Test 2	138.53±0.02^{c}	130.83±0.01^{c}	89.46±0.15^{c}
Test 3	91.04±0.02^{a}	76.76±0.01^{a}	71.77±0.17^{c}
Standard	130.73±0.02^{c}	145.17±0.01^{c}	94.45±0.28^{c}
Cream base	69.38±0.01	17.51±0.01	8.55±0.21
Extract	101.86±0.01^{b}	124.71±0.01^{c}	64.54±0.25^{c}

Each value represents Mean±SEM, n = 6, $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$ compared to positive control group. One way ANOVA followed by Dunnett's test

Table 2: Effect of different formulations on blood LPO in UV-B induced psoriatic rat

Groups	Inhibition of LPO (%)
Positive control	-
Test 1	20.99±0.01
Test 2	61.48±0.01^{b}
Test 3	34.56±0.01^{a}
Standard	63.18±0.01^{b}
Cream base	4.38±0.01
Extract	28.76±0.01

Each value represents Mean±SEM, n = 6, $^{a}p<0.05$; $^{b}p<0.001$ compared to positive control group. One way ANOVA followed by Dunnett's test

Antioxidants act as free radical scavengers that destroy single oxygen molecules (free radicals) in the body, thereby protecting against oxidative damage of cells. SOD, CAT and GSH are the well known enzymes present in plasma which act as antioxidants by transforming reactive oxygen species and reactive nitrogen species into the stable compounds and involved in a scavenging of the excessive free radicals. The restoration of blood SOD, CAT and GSH levels by the treatment with test creams are indicating that the inbuilt protective mechanism is being restored.

CONCLUSION

O/W creams of *Cassia tora* leaves methanolic extract minimized the UV-B induced oxidative stress in rats and restored all the biochemical parameters towards normal level. Hence, it is concluded that *Cassia tora* has significant antioxidant activity.

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