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# Research Paper

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## Anticarcinogenic Properties and Antioxidant Activity of Henna (*Lawsonia inermis*)

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The research was conducted to examines the anticarcinogenic properties of henna (*Lawsonia inermis*) extract (using chloroform as a solvent) by the microculture tetrazolium salt (MTT) assay on the human breast (dependent-hormone, MCF-7; non-dependent hormone, MDA-MB-231), colon (Caco-2), liver (HepG2) carcinoma cell lines and normal human liver cell lines (Chang Liver). The preliminary results showed that the henna extract displayed the cytotoxic effects against HepG2 and MCF-7 with IC<sub>50</sub>-value of 0.3 and 24.85 µg ml<sup>-1</sup>, respectively. However, no IC<sub>50</sub> values were obtained from Caco-2 and MDA-MB-231 cell lines at the concentration studied. This extract also did not show the IC<sub>50</sub> value against normal human liver cell lines, Chang Liver, indicated the selectivity of its cytotoxic properties. The antioxidative activities of this extract which could contribute to its cytotoxic properties were also studied. Antioxidant activity in henna was found to be the highest as compared to vitamin E or α-tocopherol. The strong cytotoxic properties of this extract could be due to its high antioxidant activities.

**Key words:** Henna, MTT assay, ferric thiocyanate, thiobarbituric acid, antioxidant

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## Introduction

Henna (*Lawsonia inermis* Linn) is a plant which grow wild in abandoned areas (Muhammad and Mustafa, 1994) and commonly known as 'Inai' in Sumatra or 'Pachar kuku' in Java. This plant is a worldwide known cosmetic agent used to stain hair, skin and nails (Hanna *et al.*, 1998). However, it is not only relevant to cosmetics. Alcoholic extracts of henna leaves showed mild antibacterial activity against *Micrococcus pyrogenes* var *Aureus* and *Eschericia coli* (Kirtikar and Basu, 1981). Henna was also reported to have tuberculostatic activity (Sharma, 1990). The leaves are used as a prophylactic against skin diseases. They are used externally in the form of paste or decoction against boils, burns, bruises and skin inflammations. A decoction is used as gurgle against sore throat (Rout *et al.*, 2001). The roots of this plant are useful in burning sensation, leprosy, strangury and premature greying of hair (Vaidyaratnam, 1995).

The major phytochemical constituent of henna, lawsone, was found to possess significant anti inflammatory, analgesic and antipyretic activities (Ali *et al.*, 1995). Recently, this compound has been reported to have the inhibitory growth effect against human colon carcinoma, HCT-15 cells (Kamei *et al.*, 1998). However, there is no study reported on the inhibitory effect of this plant against other carcinoma cell lines.

Therefore, this study was carried out to examine the anticarcinogenic properties of this plant towards several cancer cell lines including MCF-7 (human breast dependent-hormone carcinoma), MDA-MB-231 (human non-dependent hormone breast carcinoma), HepG2 (human liver carcinoma), Caco-2 (human colon carcinoma) and Chang Liver (human normal liver) cell lines using the MTT-based cytotoxicity assay. The antioxidative activities of this plant that may contribute to its cytotoxic properties were also studied.

## Materials and Methods

The research work was done at Department of Nutrition and Health Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia, from June 2000 to June 2001.

### Cytotoxicity study

**Plant material and extraction:** The leaves of *L. inermis* were collected at Faculty of Medicine and Health Sciences, UPM, Serdang, Selangor.

The dried leaves (100 g) of *L. inermis* were soaked in chloroform (CHCl<sub>3</sub>) at room temperature for a week. The extract was then filtered and evaporated at 40 °C under reduced pressure and subsequently air dried. Half of the dried residue was resuspended in absolute ethanol (C<sub>2</sub>H<sub>5</sub>OH) for cytotoxicity assay, whereas unsuspended dried residue was subjected to antioxidant activity study by following the method of Ali *et al.* (1996) with slightly modification.

**Culturing of cells:** HepG2, Caco-2, MDA-MB-231, MCF-7 and Chang Liver cell lines were obtained from American Type Culture Collection (ATCC, USA). The medium for HepG2 and Chang Liver were Minimum Essential Medium with Earle's salt (Gibco, USA). While growth of Caco-2, MDA-MB-231 and MCF-7 were using Dulbecco's Modified Eagle medium (Gibco, USA). The cells were cultured in their own medium supplemented with 10% of fetal calf serum, 100 IU ml<sup>-1</sup> penicillin and 100 µg ml<sup>-1</sup> of streptomycin (Gibco, USA) using 25 cm<sup>2</sup> flasks (Nunc, Denmark), in a CO<sub>2</sub> incubator (Sanyo, Japan) at 37 °C.

**MTT assay (Roche diagnostic, USA):** The viability of cells was determined with trypan blue. Exponentially growing cells were harvested, counted by using hemocytometer, and diluted with medium, yielding a concentration of 1x10<sup>5</sup> cells ml<sup>-1</sup>. From this cell suspension, 100 µl were pipetted into 96-well microtiter plates (Nunc, Denmark) and this well were incubated for 24 hours in 5 %

CO<sub>2</sub> incubator (Sanyo, Japan) at 37 °C. The diluted range of extract was added to each well with the final concentration of the test extracts being 0.03, 0.1, 0.3, 3, 10 and 30 µg ml<sup>-1</sup>. After adding the extract samples, new medium were added to make up the final volume of 200 µl each well. The plate was incubated in 5 % CO<sub>2</sub> incubator (Sanyo, Japan) at 37 °C for 96 hours. Then, 20 µl of MTT reagent (Roche, USA) was added into each well. This plate was incubated again for 4 hours in CO<sub>2</sub> incubator (Sanyo, Japan) at 37 °C. After incubation, 200 µl solubilization solution (Roche, USA) was added into each well. The cell was then left overnight at 37 °C, 5 % CO<sub>2</sub> incubator. Finally, the absorbance was read with the ELISA reader (LX-800).

$$\% \text{ Cytotoxicity} = \frac{\text{OD sample (mean)}}{\text{OD control (mean)}} \times 100\%$$

OD = Optical density

### Antioxidant activity

**Ferric thiocyanate (FTC) method:** The method of ferric thiocyanate was followed from Kikuzaki and Nakatani (1993), which was slightly modified from Mitsuda *et al.* (1967) and Osawa and Namiki (1981). FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. The peroxide reacts with ferrous chloride (FeCl<sub>2</sub>) to form a reddish ferric chloride (FeCl<sub>3</sub>) pigment. In this method, the concentration of peroxide decreases as the antioxidant activity increases. A mixture of 4 mg of sample was placed in 4 ml of absolute ethanol (Merck), 4.1 ml of 2.52 % linoleic acid (Sigma) in absolute ethanol, 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial (ø = 38 mm, h = 75 mm) with a screw cap and then placed in an oven at 40 °C in the dark. To 0.1 ml of this solution, 9.7 ml of 75 % ethanol and 0.1 ml 30 % ammonium thiocyanate (Sigma) was added. Exactly 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5 % hydrochloric acid (HCl) to the reaction mixture, the absorbance was measured at 500 nm every 24 hours until the absorbance of the control reached maximum. The control and standard were subjected to the same procedures as the sample, except that for the control, only the solvent was added, and for the standard, 4 mg sample was replaced with 4 mg of vitamin E.

**Thiobarbituric acid (TBA) method:** The method of Ottolenghi (1959) was used to determine the TBA values of the samples. The formation of malonaldehyde is the basis for the well-known TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100 °C), malonaldehyde binds TBA to form a red complex that can be measured at 532 nm. The increase amount of the red pigment formed correlates with the oxidative rancidity of the lipid. Two 2 ml of 20 % trichloroacetic acid (CCl<sub>3</sub>COOH) and 2 ml TBA aqueous solution were added to 1 ml of sample solution prepared as in the FTC procedure, and incubated in a similar manner. The mixture was then placed in a boiling water bath for 10 min. After cooling, it was centrifuged at 3,000 rpm for 20 min and the absorbance of the supernatant was measured at 532 nm. Antioxidant activity was determined based on the absorbance on the final day.

**Statistical analysis:** All determinations were carried out in sixplicates and the independent sample t-test was analyzed by using Statistical Package for Social Sciences Software (SPSS).

## Results

**Cytotoxicity:** The chloroform extract of henna displayed the strongest cytotoxic effect on human liver carcinoma cell line (HepG2) followed by human dependent-hormone breast carcinoma cell line (MCF-7) with IC<sub>50</sub> value of 0.3 and 24.8 µg ml<sup>-1</sup>,

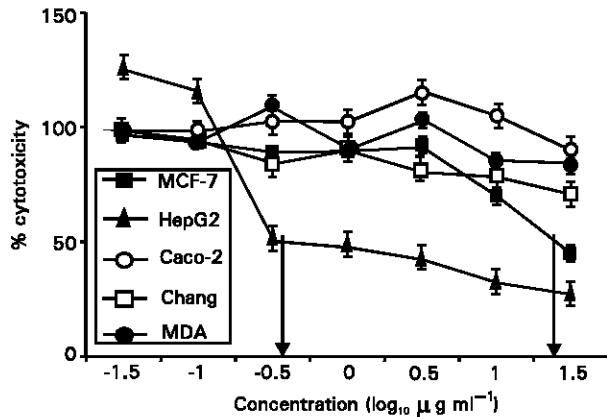


Fig. 1: The cytotoxic effect of chloroform extract of henna on human liver carcinoma cell lines (HepG2), human dependent-hormone breast carcinoma cell lines (MCF-7), and human colon carcinoma cell lines (Caco-2).

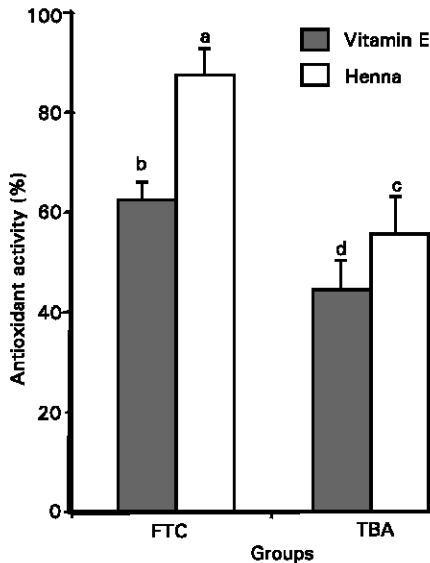


Fig. 2: The total antioxidant activity of chloroform extract of henna by using FTC and TBA method. Values are means  $\pm$  SEM (vertical lines). Bars with different letters are significantly different at  $p < 0.05$ . FTC: Ferric thiocyanate, TBA: Thiobarbituric acid

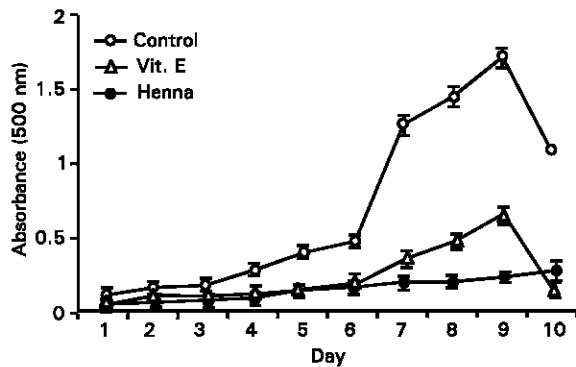


Fig. 3: Absorbance value of samples at 0.02 % concentration using FTC method

respectively. However, no  $IC_{50}$  values were obtained from Caco-2 and MDA-MB-231 at the concentration tested (Fig. 1).

**Antioxidant Activity:** The total antioxidant activity of chloroform extract of henna by using FTC and TBA methods showed that there is a significant ( $p < 0.05$ ) differences between the total antioxidant activity of henna compared with  $\alpha$ -tocopherol (Vit. E) (Fig. 2). The chloroform extract of henna had the highest activity (87.6 %), followed by  $\alpha$ -tocopherol (62.5%) by using FTC method. Based on the TBA method, henna also had the significantly higher antioxidant activity (55.7 %) compared to  $\alpha$ -tocopherol (44.4%). The comparison of both methods showed that henna had the significantly ( $p < 0.05$ ) higher total antioxidant activity in both methods.

The individual activity of samples by the FTC method showed that henna had the least increase in absorbance values, followed by  $\alpha$ -tocopherol from day 1 to day 5, but the levels increased on day 6, reached maximum levels on day 9 and finally dropped on day 10 due to the malonaldehyde content (Fig. 3). The low absorbance value indicated the high level of total antioxidant activity.

### Discussion

The use of chemotherapeutic drugs in cancer therapy involves the risk of life threatening host toxicity. Henna (*Lawsonia inermis* Linn) has medicinal properties and this study revealed its potential cytotoxic and antioxidant activities. In this experiment, the extract was prepared by using chloroform. Although not all possible cytotoxic compounds are extracted by this method, thus it is expected to extract most of the cytotoxic compounds from the plants (Smit *et al.*, 1995).

The anticarcinogenic properties of this extract was determined by using MTT-based cytotoxicity assay and the selectivity effect of this extract towards normal liver cell lines, Chang Liver, was also determined by using MTT assay. This assay is based on the reduction of a soluble tetrazolium salt, by mitochondrial dehydrogenase activity of viable tumor cells, into an insoluble colored formazan product, which can be measured spectrophotometrically after dissolution. Under the experimental conditions of this study, the enzyme activity and the number of formazan formed were proportional to the number of cells. Reduction in the number of cells by a particular agent (cytotoxicity) can generally be explained by cell killing and/or inhibition of cell proliferation. The  $IC_{50}$  value (the drug concentration causing 50% inhibition of the tumor cells) was used as a parameter for cytotoxicity (Smit *et al.*, 1995).

According to Wall *et al.* (1987) any plant extracts which have the  $IC_{50}$  value below  $20 \mu g ml^{-1}$  can be accepted as a potent cytotoxic extract. The  $IC_{50}$  value of henna extract toward human liver cancer cell line, HepG2, which was much lower than  $20 \mu g ml^{-1}$  ( $0.3 \mu g ml^{-1}$ ) indicated a potential anticarcinogenic properties of this plant (Fig. 1). These results showed similarities between *in vitro* and *in vivo* experiments in terms of anticarcinogenic effects. In the previous animal study, henna supplementation showed the reduction of the severity of hepatocarcinogenesis (Rosnah *et al.*, 1998). It has shown good correlation between the cytotoxic effect of this plant and its antioxidant activity. Antioxidants are known to alleviate oxidative stress which is generally perceived as one of the major causes for the accumulation of mutations in the genome. Antioxidants are believed to provide protection against cancer (Ames, 1983). Peroxide is gradually decomposed to lower molecular compounds during the oxidation process and these compounds here measured by FTC and TBA methods (Fig. 2). The amount of peroxide at the primary stage of linoleic acid peroxidation were measured by FTC method, whereas TBA method measures at the secondary stage (Kikuzaki and Nakatani, 1993). From the FTC result, henna showed the least increase in absorbance values, followed by  $\alpha$ -tocopherol from day 1 to 5 day, but the levels increased on day 6, reached

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maximum levels on day 9 and finally dropped on day 10 (Fig. 3). This reduction is due to the accumulation of malonaldehyde compounds from linoleic acid oxidation, which is not stable. Further oxidation causes malonaldehyde to be converted to secondary products such as alcohols and acids that cannot be detected (Spanier *et al.*, 1992).

As a conclusion, antioxidant activity in henna chloroform extract was found to be highest, followed by vitamin E or  $\alpha$  tocopherol. The strong cytotoxic properties of this extract could be due to its high antioxidant activities. A more detailed investigation focusing on the active compounds is of interest for their molecular nature as well as their mechanisms of action.

**Acknowledgment**

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