

Evaluation of the antioxidant activity effect of Henna (*Lawsonia inermis* Linn.) leaves and or vitamin C in rats

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Abstract: Aim: the purpose of this study was to evaluate the role of *Lawsonia inermis* leaves on the antioxidant enzyme activity in serum, kidney and liver cells. Methods: The present study was undertaken to determine whether Lawsonia leaves, a natural free radical scavenger, could ameliorate the oxidative stress in serum, kidney and liver in rats. Thirty Six Wister albino rats were divided into 6 groups. Group I: control group (no treatment), group II: administered vitamin C, as ascorbic acid (180 mg/kg BW), group III: administered Henna in low dose (200 mg/kg BW), group IV: administered Henna in high dose (1000 mg /kg BW), group V: administered Henna (200 mg/kg BW) + Vit. C (180 mg/kg BW) group VI: administered Henna (1000 mg/kg BW) + Vit. C (180 mg/kg body weight). The levels of CAT and GPX activity were measured. Also, hydrogen peroxidase, lipid peroxide in serum and tissue homogenates were evaluated. Results: There was an increased lipid peroxidase and hydrogen peroxidase in rats treated with high dose of henna compared to control group. However, there was a significant decrease in catalase activity with high dose of henna alone and henna with vitamin C. As regard to glutathione, all treatments increase its concentration especially with high dose of henna. The activity of GPX significantly increases with all treated animals especially with high dose of henna except in low dose of henna treated rats. Thus, these results conclude that high dose of henna enhanced the antioxidant defense against reactive oxygen species and can be a potential treatment to ameliorate the toxic effects associated with liver and kidney disease.

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1.Introduction

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. According to the World Health Organization, 2003 about 80 % of the population of developing countries being unable to afford pharmaceutical drugs rely on traditional medicines, mainly plant based, to sustain their primary health care needs. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs.

Lawsonia inermis Linn. commonly known as henna, is a finely ground brown or green powder originating from dried leaves of the plant *Lawsonia inermis* which is grown in dry tropical and subtropical zones, including North Africa, India, Sri Lanka, and the Middle East. Common names in English are Henna, Samphire, Cypress shrub; in Sanskrit are Mendhi, Mendika, Timir ; Arabic are Alhenna, Hinna French : Alcana d' orient Greek : Kypros Gujrat : Medi Hindi : Hena, Mhindi Marthi are Mendhi, Mendi; Tamil are Alvanam, Aivani and in Telugu are Goranta, Kormmi (Borade *et al.*, 2011).

Lawsonia inermis is a member of the family Lythraceae which consists of about 500 species, widely spread in tropical regions with relatively few

species in temperate regions (Musa and Gasmelseed, 2012). *Lawsonia inermis* is generally considered as a native of Africa and Asia. It is widely cultivated in tropical regions of the world in Sudan, Egypt, China, and India. Major producing countries are Sudan, Egypt and India (Borade *et al.*, 2011; Musa and Gasmelseed, 2012). *Lawsonia inermis* has been well investigated phytochemically by various researchers. The occurrence of β - sitosterol, lawsone, esculetin, fraxetin, isoplumbagin, scopoletin, betulin, betulinic acid, hennadiol, lupeol, lacoumarin, laxanthone, flavone glycosides, two pentacytic triterpenes glucoside, flavonoids, quinoids, naphthalene derivatives, gallic acid, coumarins, and xanthenes (Chaudhary *et al.*, 2010; Kamal and Jawaid, 2010; Borade *et al.*, 2011; Musa and Gasmelseed, 2012) in *Lawsonia* leaves has been reported. Earlier work establishes the use of henna as an alternative vegetable retanning agent (Musa and Gasmelseed, 2012).

Henna, is a traditional product with religious associations and has been widely used cosmetically and medicinally for over 9,000 years for traditional, folk and prophetic medicine in Africa, Asia, the Middle East and many other parts of the world. It was found to be analgesic, nootropic, antitrypanosomal, antidermatophytic, antimalarial, hepatoprotective, antioxidant, antidiabetic, immunomodulatory,

antibacterial, tuberculostatic, antifertility, antifungal, antiviral, in cancer treatment, in treating skin problems, headache, jaundice, anthelmintic, rheumatoid arthritis, ulcers, diarrhea, leprosy, fever, leucorrhoea, cardiac disease and enlargement of the spleen (Hemalatha et al., 2004, Endrini et al., 2007, Syamsudin and Winarno, 2008, Mikhaeil et al., 2004, Ali et al., 2001, Khan et al., 1991 and Ozaslan et al., 2008; Chaudhary et al., 2010; Kamal and Jawaid, 2010; Borade et al., 2011). In addition, henna leaves have been extensively used for centuries in the Middle East, the Far East and Northern Africa in cosmetics in dying hair, nails, hands and textile for its dyeing properties due to the strong binding of a lawsone to hair and skin, a naphthoquinone compound derived from henna (Chaudhary et al., 2010; Jiny et al., 2010; Kamal and Jawaid, 2010; Borade et al., 2011; Musa and Gasmelseed, 2012).

Based on the fact that extracts of *L. inermis* are still widely used to treat liver and renal diseases in traditional medicine, it is reasonable to assume that the possible mechanisms of the extract's therapeutic actions are due to the extract's ability to suppress oxidative processes and maintain endogenous antioxidant levels. However, there are no laboratory data on the extract's effects on endogenous antioxidants, such as glutathione. Therefore, we assessed the effects of increasing concentrations of an aqueous extract of *L. inermis* on markers of antioxidant status, in the liver and kidney and serum of male rats.

2. Materials and methods

Collection of plant material

The plant leaves were collected from Al-Madinah Al-Munawarah region. The plant was authenticated at College of Science and Arts, Qassim University (Voucher specimen number: MEDP 26). *Lawsonia inermis* leaves was washed, dried and crushed into fine powder with crushing machine. Then, an aqueous suspension from plant powder was prepared according to the required doses, simulating the traditional healers whom using it as suspended in water.

Experimental animals

Wister albino male rats (*Rattus norvegicus*) were supplied from the Animal House, Faculty of Pharmacology, King Saud University, Saudi Arabia. Body weight ranging between 100 - 150 g and age ranging from 2 - 3 months. Rats were kept in a hygienic plastic cages in a well ventilated room at 28±2 °C and light-dark cycle. Animals were kept in this room for one week before the experiment to adapt, fed on standard pellet diets (ARASCO Company, KSA). The experiment on rats was

conducted in accordance with the recommendations of King Saud University Guideline for Care and Use of Laboratory Animals.

Treatments

The drinking water for treated animals was mixed with the required doses of plant powders and offered *Ad libitum*. Rats were divided into six groups (36 individuals). Group I: control group (no treatment), group II: administered vitamin C, as ascorbic acid (180 mg/kg b. wt.), group III: administered Henna in low dose (200 mg/kg b.wt.), group IV: administered Henna in high dose (1000 mg/kg b.wt), group V: administered Henna (200 mg/kg b. wt) + Vit. C (180 mg/kg b. wt.), group VI: Administered Henna (1000 mg/kg b. wt) + Vit. C (180 mg/kg b. wt.). Rats were given Henna in proposed low and high doses while Vitamin C was given as reported by Abdelmoneium et al. (1997). The experiment lasted six consecutive weeks.

Antioxidant enzymes studies

Serum catalase (CAT) activity was measured by the UV colorimetric method of Aebi (1974) using H₂O₂ as substrate while serum glutathione peroxidase (GP_x) activity was measured by a modification of the colorimetric method of Flohe & Günzler (1984) using H₂O₂ as substrate and the reduced glutathione (GSH).

Oxidative stress analysis

Lipid peroxidation in the liver and kidney tissue homogenates was estimated by measuring thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content which is the end product of lipid peroxidation according to Buege and Aust (1978). In brief, 125 µl of homogenate supernatants were homogenized by sonication with 50 µl of Tris Buffer Solution (TBS), 125 µl of TCA-BHT in order to precipitate proteins and centrifuged (1000 g, 10 min, 4°C). 200 µl of obtained supernatant were mixed with 40 µl of HCl (0.6 M) and 160 µl of TBA dissolved in Tris and the mixture was heated at 80°C for 10 min. The absorbance of the resultant supernatant was read at 530 nm. The amount of TBARS was calculated by using an extinction coefficient of 15610'5 mM⁻¹ cm⁻¹.

Hydrogen peroxide was determined by the spectrophotometric method of the spot test Machly & Chance (1954) and which was modified by Feigl (1958).

Statistical analysis

Data were analyzed using GLM procedure of SAS (2000) to determine the effect of treatments on traits studied. The fixed model was as follows:

$Y_{ij} = \mu + T_i + e_{ij}$ where:

Y_{ij} = an observation on the j th individual,

μ = the overall mean,

T_i = the fixed effect of the i th treatment.

ϵ_{ij} = random error assumed to be independent normally distributed with mean = 0 and variance σ^2 .

3. Results

As illustrated in table(1), the supplementation of vitamin C alone didn't affect ($p > 0.05$) the activity of lipid peroxidase. Henna alone at its high dose (1000 mg/kg/day) resulted in the highest ($p > 0.01$) activity (44.6 mIU) of lipid peroxidase, however the addition of vitamin C didn't show enhancements of this activity. All treatments except vitamin C alone significantly ($p > 0.01$) increased the lipid peroxidase activity as compared with control.

As for hydrogen peroxidase, there found significant ($p < 0.01$) differences in all treated rats compared to control rats. However, there were no statistical differences among all treatment tested on the hydrogen peroxidase activity. Also, Henna at the high dose resulted in the highest activity (1.49 mIU) as compared to control (0.25 mIU), which means that this treatment appreciably increased the enzyme activity by about 6 folds its normal level in control rats.

All treatments, except low dose of henna, exhibited enhancement ($p > 0.01$) of catalase activity. The highest catalase activity was obtained with the high dose of henna which represented 3.1 folds more than control-adding vitamin C to henna didn't improve its ability for eliciting catalase activity (Table 1)

Glutathione concentration in kidney tissues were increased ($p > 0.01$) in all treated rats. This increase was highest in high dose of henna (1.15 mIU) which comprised 1.6 times more than control (0.72mIU). There were no significant ($p > 0.05$) differences between low dose of henna, vitamin C or vitamin C plus henna at either dose.

All treatments, except the low dose of henna significantly ($p < 0.01$) increased the activity of Glutathione peroxidase. There were no significant differences ($p > 0.05$) among high dose of henna, vitamin C, low dose of henna plus vitamin C and high dose of henna plus vitamin C. The highest increase in the activity of glutathione peroxidase was found in rats given the high dose of henna (135.35). This increase in glutathione peroxidase activity comprised 1-2 folds of that found in control rats.

Table (2) presents data of the effect of henna / vitamin C on the rat's liver enzyme activities. Lipid peroxidase activity was found to be the highest at the high dose of henna (80.70 mIU) as compared with control (41.5 mIU). However, henna at the low dose didn't exhibit significant ($p > 0.05$) increase in lipid peroxidase over that found in control rat livers. Vitamin C alone or in combination with low dose of

henna approximately exhibited similar response on the enzyme activity. Combining vitamin C with the high dose of henna wasn't efficient more than giving high dose of henna alone (77.9 vs 80.7mIU).

The activity of liver hydrogen peroxidase considerably increased ($p < 0.01$) in all treated rat's livers as compared with controls. Also, the highest increase in the activity of this enzyme was shown at the high dose of henna (1.59 mIU) as compared with control (0.31 mIU). This means that about 5 times of the activity found in livers of control animals. Henna at the low dose resulted in the less increase of enzyme activity. Combining vitamin C with henna at either level didn't show a better enhancement over giving henna alone.

Catalase activity was found to be at its highest activity when rats were given the high dose of henna (2.95 mIU). This significant increase of liver catalase amounts for 3.12 folds that found in control rat's liver (0.95 mIU).

However, the low dose of henna haven't show significant ($p > 0.05$) enhancement of the enzyme activity. Administration of vitamin C (1.62 mIU) alone or in combination with low (1.66 mIU) or high (2.09 mIU) dose of henna didn't improve the response of the enzyme activity over giving the low dose of henna alone (1.53 mIU).

Glutathione concentration in rat's livers was found to be the highest at the high dose of henna (2.38). Administration of low dose of henna alone resulted in a significant increase ($p < 0.01$) in the glutathione as compared with that obtained with vitamin C alone or its combination with the low dose of henna. However, the combination of vitamin C to the high dose of henna didn't show an enhancement over giving the high dose of henna alone.

With regard to glutathione peroxidase activity in liver tissues, all treatments except the low dose of henna increased ($p < 0.01$) the enzyme activity over control. Also, the high dose of henna gave the highest response (156.4 mIU) which amounts for 23% more activity than that found in control livers. There were no significant differences ($p > 0.05$) between the high dose of henna, vitamin C, low dose of henna plus vitamin C or high dose of henna plus vitamin C on the enzyme activity.

Table (3) exhibits data of the antioxidant enzymes in rat serum as influenced by henna and vitamin C. All treatments except vitamin C significantly ($p < 0.01$) increased lipid peroxidase activity in rat's serum. The high dose of henna resulted in the highest increase (26.24 mIU) in the enzyme activity. This elevation amounts to 2.6 times increase than that found in control rat serum. Vitamin C alone didn't change ($p > 0.05$) the enzyme activity over the control. However, henna alone at either level

or in combination with vitamin C enhanced the enzyme activity by a fold ranging between 1.7 and 2.6 times that found in control serum.

Hydrogen peroxidase activity in rat serum was found to be the highest (0.528 mIU) at the high dose of henna and the lowest (0.103 mIU) in control serum. There were no significant differences ($p > 0.05$) between low dose of henna, vitamin C, vitamin C combined with low or high dose of henna on the enzyme activity. Although, all treatments resulted in higher ($p < 0.01$) enzyme activity than control with enhancement percentages ranging between 300-500%, more than control. Also, combining vitamin C with henna didn't surpass the effect of giving animals henna alone.

Serum content of catalase was found to be the highest when the high dose of henna was given (0.982 mIU). All treatments, except the low dose of henna revealed significant ($p < 0.01$) increases in the enzyme activity over the control. Combining vitamin C with the high dose of henna caused a significant ($p < 0.01$) decrease in its enhancement capability on the catalase activity. Moreover, there were no significant differences ($p > 0.05$) on the enzyme activity between the low dose of henna, vitamin C, low dose of henna plus vitamin C or high dose of henna plus vitamin C.

Glutathione concentration in rat serum was found to be the highest ($p < 0.01$) in the high henna – treated rats (0.499) and the lowest in control rats (0.312). All treatments resulted in significant ($p < 0.01$) increases of glutathione over the control in rat serum. The fold of increase ranged between 1.2 -1.6 times of control. Similar to the results obtained with previous enzymes, there were no significant ($p > 0.05$) effects due to combining vitamin C with henna on the glutathione concentration in rat serum.

Glutathione peroxidase activity in rat serum (Table 3) was found to reach the highest value at the high dose of henna (75.19 mIU) and the lowest value at control (61.91 mIU). Although, all treatments, except the low henna, revealed significant differences than control ($p < 0.01$) the differences between the none control treatments were not statistically ($p > 0.05$) significant. The fold of increase over the control levels ranges between 1.06 and 1.21.

Also, no significant difference was found when vitamin C was combined to henna at either level. As for kidney function (Table 4), levels of lipid peroxidase, catalase, glutathione and glutathione peroxidase can be used as biomarkers for the integrity and functionality of renal tissues.

Table 1: Effect of henna/vitamin C on the antioxidant enzymes activities of rat's kidney (mean \pm SEM)*

Treatment (mg/kg/d)	Lipid peroxidase mIU	Hydrogen peroxidase mIU	Catalase mIU	Glutathione mIU	Glutathione peroxidase mIU
Control	17.124 \pm 1.52 ^a	0.246 \pm 0.04 ^a	0.850 \pm 0.08 ^a	0.719 \pm 0.04 ^a	111.444 \pm 3.57 ^a
Henna (200 mg)	30.251 \pm 4.40 ^{bc}	0.900 \pm 0.40 ^b	1.376 \pm 0.26 ^{ab}	0.927 \pm 0.05 ^b	117.291 \pm 5.90 ^{ab}
Henna (1000 mg)	44.599 \pm 5.11 ^d	1.485 \pm 0.13 ^b	2.651 \pm 0.31 ^c	1.148 \pm 0.07 ^c	135.345 \pm 9.19 ^c
Vitamin C (180 mg)	23.644 \pm 1.12 ^{ab}	0.967 \pm 0.04 ^b	1.454 \pm 0.08 ^b	0.878 \pm 0.01 ^b	132.402 \pm 2.57 ^{bc}
Henna (200 mg) +Vitamin C (180 mg)	28.724 \pm 1.64 ^{bc}	0.932 \pm 0.05 ^b	1.490 \pm 0.13 ^b	0.925 \pm 0.02 ^b	131.727 \pm 2.16 ^{bc}
Henna (1000 mg) +Vitamin C (180 mg)	36.431 \pm 1.02 ^{cd}	1.299 \pm 0.05 ^b	1.877 \pm 0.10 ^b	1.007 \pm 0.01 ^b	133.441 \pm 2. ^c

* Means in the same column with different superscripts are significantly different ($P < 0.01$).

Table 2: Effect of henna/vitamin C on the antioxidant enzymes activities of rat's liver (mean \pm SEM)

Treatment** (mg/kg/day)	Lipid peroxidase mIU	Hydrogen peroxidase mIU	Catalase mIU	Glutathione mIU	Glutathione peroxidase mIU
Control	41.468 \pm 0.764 ^a	0.309 \pm 0.014 ^a	0.945 \pm 0.092 ^a	1.453 \pm 0.049 ^d	126.82 \pm 3.971 ^a
Henna (200 mg)	51.923 \pm 6.99 ^{ab}	0.961 \pm 0.427 ^b	1.529 \pm 0.296 ^{ab}	1.909 \pm 0.093 ^{bc}	138.101 \pm 6.313 ^{ab}
Henna (1000 mg)	80.666 \pm 4.740 ^c	1.585 \pm 0.143 ^c	2.946 \pm 0.347 ^c	2.383 \pm 0.244 ^a	156.383 \pm 10.212 ^c
Vitamin C (180 mg)	62.030 \pm 2.079 ^b	1.033 \pm 0.048 ^{bc}	1.616 \pm 0.096 ^b	1.597 \pm 0.092 ^{cd}	153.113 \pm 2.858 ^{bc}
Henna (200 mg) +Vitamin C (180 mg)	62.525 \pm 2.962 ^b	0.996 \pm 0.062 ^{bc}	1.656 \pm 0.150 ^b	1.790 \pm 0.083 ^{bd}	152.363 \pm 2.408 ^{bc}
Henna (1000 mg) +Vitamin C (180)	77.932 \pm 0.828 ^c	1.387 \pm 0.057 ^{bc}	2.085 \pm 0.111 ^b	2.049 \pm 0.051 ^{ab}	154.268 \pm 2.829 ^{bc}

** Means in the same column with different superscripts are significantly different ($P < 0.01$).

Table 3: Effect of henna/vitamin C on the antioxidant enzymes activities of rat's serum (mean \pm SEM)

Treatment** (mg/kg/day)	Lipid peroxidase mIU	Hydrogen peroxidase mIU	Catalase mIU	Glutathione mIU	Glutathione peroxidase mIU
Control	10.073 \pm 0.899 ^a	0.103 \pm 0.004 ^a	0.315 \pm 0.030 ^a	0.312 \pm 0.019 ^a	61.913 \pm 1.985 ^a
Henna (200 mg)	17.795 \pm 2.589 ^{bc}	0.320 \pm 0.142 ^b	0.509 \pm 0.098 ^{ab}	0.403 \pm 0.025 ^b	66.050 \pm 3.866 ^{ab}
Henna (1000 mg)	26.235 \pm 3.006 ^d	0.528 \pm 0.047 ^c	0.982 \pm 0.115 ^c	0.499 \pm 0.030 ^c	75.191 \pm 5.106 ^b
Vitamin C (180 mg)	13.908 \pm 0.659 ^{ab}	0.344 \pm 0.016 ^{bc}	0.538 \pm 0.032 ^b	0.384 \pm 0.008 ^b	73.556 \pm 1.429 ^b
Henna (200 mg) +Vitamin C (180 mg)	16.896 \pm 0.859 ^{bc}	0.332 \pm 0.020 ^{bc}	0.552 \pm 0.050 ^b	0.402 \pm 0.010 ^b	73.181 \pm 1.204 ^b
Henna (1000 mg) +Vitamin C (180 mg)	21.430 \pm 0.604 ^{cd}	0.462 \pm 0.019 ^{bc}	0.695 \pm 0.037 ^b	0.438 \pm 0.007 ^b	74.134 \pm 1.414 ^b

** Means in the same row with different superscripts are significantly differ ($P < 0.01$).

Table 4: Ratio of kidney relative to serum antioxidant enzymes activities in rats given henna /vitamin C

Treatment	L.P*	H.P	Cat	Glut	G.P
Control	1.7	2.39	2.7	2.3	1.8
Henna (200 mg)	1.7	2.81	2.7	2.3	1.8
Henna (1000 mg)	1.7	2.76	2.7	2.3	1.8
Vitamin C (180 mg)	1.7	2.81	2.7	2.3	1.8
Henna (200 mg + Vit. C (180 mg)	1.7	2.81	2.7	2.3	1.8
Henna (1000 mg) + Vit. C (180 mg)	1.7	2.81	2.7	2.3	1.8

*L.P= Lipid peroxidase, H.P = Hydrogen peroxidase, Cat = Catalase, Glut = Glutathione, G.P = Glutathione peroxidase.

Table 5: Ratio of liver relative to serum antioxidant enzymes activities in rats given henna /vitamin C

Treatment	L.P	H.P	Cat	Glut	G.P
Control	4.12	3.0	3.0	4.66	2.05
Henna (200 mg)	2.92	3.0	3.0	4.74	2.09
Henna (1000 mg)	3.07	3.0	3.0	4.78	2.08
Vitamin C (180 mg)	4.46	3.0	3.0	4.16	2.08
Henna (200 mg) +Vitamin C (180 mg)	3.70	3.0	3.0	4.45	2.08
Henna (1000 mg) +Vitamin C (180 mg)	3.64	3.0	3.0	4.68	2.08

In this experiment, there were no changes in the kidney / serum ratios of the glutathione and the enzymes, catalase, lipid peroxidase and glutathione peroxidase. This finding confirms that the kidney release system of such metabolites might be within the tolerant average which must not impact any kind of stress on the renal function. Hydrogen peroxidase was the only enzyme showing sensitivity to the treatments as its activity increased in all treatments by about 18% than control.

Evidently, treatment didn't cause great changes in the ratio of Enzymes released of liver relative to those found in serum. Catalase, hydrogen peroxidase and glutathione peroxidase were the most predictor enzymes for the synchronous relation between liver and serum bio-levels of these metabolites.

Surprisingly, henna at either level or combined with vitamin C resulted in a decrease in the ratio of lipid peroxidase activity released from liver tissues relative to that found in blood serum.

4. Discussion:-

Phytochemical investigations of *L. inermis* have shown predominantly the presence of phenolic

compounds (coumarins, flavonoids, naphthalene and gallic acid derivatives) which could be glycosylated (Ben Hsouna *et al.*, 2011). Other compounds, such as triterpenoids, steroids and aliphatic hydrocarbons have been also isolated from this plant (Siddiqui *et al.*, 2003). Several studies have been reported on the beneficial effects of these phenolics, because of some interesting new findings regarding their biological activities.

Free radicals are well known to induce cellular and tissue pathogenesis leading to oxidative processes (Halliwell *et al.*, 1992).

Free radicals and other reactive oxygen species (ROS), are known to take part in lipid peroxidation, which causes food deterioration, and are reported to be a causative agent of various diseases (Nichenamela *et al.*, 2006).

Antioxidants act as radical-scavengers and inhibit lipid peroxidation and other free radical mediated processes. Actually, there is a growing interest in the substitution of synthetic antioxidants used in food preservation, which involve toxic side effects, with natural ones. Polyphenols with their redox properties can play an important role in

adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Galato *et al.*, 2003). The differential scavenging activities of the extracts against radicals may be referred to the different mechanisms of the radical antioxidant reactions in these assays.

The diversity in radical scavenging shown in these assays may be due to factors like stereo selectivity of the radicals or the differential solubility of the extracts (Yu *et al.*, 2002). Therefore, phenolic compounds seems to be the responsible of the antioxidant activity by its 'chain-breaking' property that can counteract peroxy and alkoxy radical generated during lipid peroxidation to prevent continual hydrogen abstraction and thus inhibiting chain propagation step.

The free radicals react with proteins and lipids resulting in damage to the molecules. They remove hydrogen atoms from unsaturated lipids, thus initiating lipid peroxidation. In the present study, the capability of *L. inermis* to protect the oxidative stress was investigated. The treatment with *L. inermis* extract improved the functional status of the liver. A previous study (zumrudal *et al.*, 2008) reported that aqueous extract of *L. inermis* did not show any prominent inhibition of lipid peroxidation (i.e. no decrease in the level of MDA in the liver cells of rats).

The results of the present study, however showed difference in results and showed a significant dosage -dependent increase in the capacity to inhibit lipid peroxidation in the rat liver, the reason might be attributed to the presence of toxic substances like Para-phenylene diamine (PPD), nickel, cobalt, and so forth in commercially available *L. inermis* powder (Kang and Lee, 2006) which was used for the previous study (Zumrudal *et al.*, 2008), and hence it might not have inhibited lipid peroxidation.

The results of Cun jar *et al.*, (2011) reported that phenolic content of *L. inermis* extract showed significant positive correlation with free radical scavenging and cytoprotective potential against chromium-induced toxicity.

Reactive oxygen species (ROS), such as superoxide anions and H₂O₂, are produced throughout cells during normal aerobic metabolism. The intracellular concentration of ROS is a consequence of both their production and their removal by various antioxidants. A major component of the antioxidant system in mammalian cells consists of three enzymes, namely, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These enzymes work in concert to detoxify superoxide anions and H₂O₂ in cells. The present results indicated that pre-treatment of *L. inermis* extract caused increases in the activities of antioxidant enzymes. The antioxidant

enzyme system plays an important role in the defence of the cells against oxidative damage. The high dose of henna (1000 mg/kg/day) expressed the best results to enhance the synthesis (in liver) and secretion (in serum) of the antioxidant enzymes. Our findings are in agreement with previous findings which reported that phenolic acids are important antioxidant components in *Acacia confuse*, among which gallic acid had the highest antioxidant activities (Tung *et al.*, 2009).

Hepatoprotective activity has been reported in the aqueous extract of henna against carbon tetrachloride (CCl₄) induced liver toxicity, significantly reduced the elevated serum marker enzyme levels, serum bilirubin, liver lipid peroxidase but increased serum protein, liver glutathione peroxidase in rats. These actions were achieved by counteracting the curare of liver weight, hepatic lipid peroxidation and serum alkaline phosphatase induced by carbon tetrachloride (Rajesh and Lath, 2004). As shown in table (5), the relative secretion of the antioxidants in treated rats paralleled that found in control. This finding confirms that the levels of henna/vitamin C designated to the animals in the current study fall within the physiological allowed dose. The only enzyme exhibiting significant synthesis decrease of liver tissues was lipid peroxidase.

Additionally, the changes of the activities of renal antioxidant enzymes were investigated to evaluate the renal levels of oxidative stress. The level of MDA, which was the decomposed product of low-density lipoproteins and an index for lipid peroxidation, was also studied. Daily treatment of *L. inermis* for six weeks significantly increased the activities of the antioxidant enzymes especially at the high dose. Henna surpassed vitamin C in its protective effect. Moreover, combining henna with vitamin C couldn't enhance its activity. The present findings are in agreement with other studies reporting that flavonoids and polyphenols were able to significantly enhance the activities of GPx, SOD and CAT (Badary *et al.*, 2005; Tirkey *et al.*, 2005; Morales *et al.*, 2006).

Chaudhary *et al.* (2010) mentioned that *L. inermis* have also been used in traditional medicine which contains carbohydrates, proteins, flavonoids, tannins and phenolic compounds. This plant is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products. One of the unique finding of the present study is that the administration of henna up to 1000 mg/kg B.W for rats didn't cause any kind of damage or stress to the metabolic system (i.e. kidney). The tolerance of both kidneys and liver to

the treatment would support the point that *L. inermis* is one of the best antioxidant herb having no adverse effects on body health.

The synchrony between liver and kidney functions in one side and their biomarkers released in blood circulation in the other side would support the safe antioxidative effects of henna.

Conclusion

In conclusion, it can be inferred that *L. inermis* extract have high antioxidant activity which may inhibit the oxidative toxicity that may lead to cell death. The plant can serve as a prospective source of natural phenolics and other metabolites which could prove to be precursors for designing effective drugs against liver and kidney toxicity. The activity may be attributed to the individual or combined action of phytoconstituents present in *L. inermis*.

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