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Preliminary Study to Determine the Effects of Chemical solutions (Mahalabiah& Serratia) Added to the Henna on Biochemical and Hematological Parameters in white rabbits

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Abstract

The objective of the study was to determine the Influence of some chemical additives to Henna and most actively traded on some liver and kidney functions and complete blood count in white rabbits.15 rabbit divided into three groups, each group containing five rabbits. The first group which used as a control was a topical application of henna on the dorsal region of the rabbits after removing hair. The second group had been used henna with Mahlbaih. Henna was used with Serratia in third group. Rabbits were slaughter of after 24 hours to get the blood to require analyzes.

The results of this study showed a significant increase in AST, ALT and increase in the concentration of Urea and Creatinine in the two groups, which used Mahalbiah and Serratia. There is no variability in CBC count between three groups.

Key Words: Henna, Liver, Kidney, Blood, Rabbits, Chemical additives.

Introduction:

Henna or hina (*Lawsonia inermis*, family Lythraceae) is a flowering plant or shrub native to tropical and subtropical regions of Africa, and Southern Asia. Preferred Scientific Name: Lawsonia Inermis. Other Scientific Names: Lawsonia alba Lam. Trade Name: Henna. Most Popular Common Names:Arabic; Henna, Henne, Hine, Hina, Hene, Heni. Pakistan, India & Bangladesh; Mendhi, Mehndi, Mehendi [1, 2, 3, 4].

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Scientific Classification of henna plant [5, 6, 7]:

- Subkingdom: Tracheobionta
- Superdivision: Spermatophyta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Rosidae
- Order: Myrtales
- Family: Lythraceae
- Genus: Lawsonia L
- Species: Lawsonia Inermis L

Henna is commercially cultivated in Morocco, Libya, Sudan, India, Pakistan, Yemen, and other countries [8-11], It contains a burgundy dye

molecule, lawsone (2-hydroxy-1,4-naphthoquinone). This dye molecule has the ability to bond with proteins, and consequently has been widely used in body art to dye skin, hair and fingernails, and to dye silk, leather, and wool. Henna body art is made by applying henna paste to the skin. Henna paste is prepared by drying the henna leaves and grinding them to powder, and then this powder is mixed with oil or water to form the paste. When this henna paste is applied to the skin the dye (lawsone) migrates from the paste to the outermost layer of the skin; more lawsone will migrate if the paste is left on the skin for a longer time, thus creating a red-brown stain [12-15].

In the Arab world and Indian subcontinent henna was used for skin decoration and hair dying during social celebrations, and during marriage ceremonies. People celebrate by adorning the bride, and sometimes the groom with henna [16].

Lawsonia inermis Linn. (Lythraceae) is a very useful medicinal plant in all parts of the world [12, 13, 17]. The leaf powder of henna sap was used for staining hair, nails and beard. The leaves of Lawsonia inermis are used to treating poliomyelitis and measles among the Yoruba tribe of South Western Nigeria. The seeds of henna have been reported to possess deodorant action and are used in most cases of gynecological disorders such as menorrhagia, vaginal discharge and leucorrhoea [1, 4]. The leaves of Lawsonia inermis with those of Hibiscus rosa-sinensis, Eclipta prostrata and seeds of Abrus precatorius when they are taken in equal quantities and ground into paste which is soaked in sesame oil for 5 days is used as hair oil by the tribes of Andra Pradesh, India (18). In Turkey, henna which is an extract of Lawsonia sp. is used as hair dye and nail dye in many cultures as decorative dye centuries. Henna is widely used in the cosmetic industry as dyeing agent also in India [17]. Reports show that methanolic root extracts of Lawsonia is used in Nigeria for cosmetic purposes, as antimalarial as well as for abortifacient purposes. The powdered roasted seed is mixed with gingerly oil to make a paste which is used for the treatment of ring worm [18]. Decoction of the leaves is used for aseptic cleaning of wounds and healing [15, 16]. L. inermis is also used by some individuals as ‘blood tonic’, thus implying its multifaceted use [17].

Women of all ages use henna for skin decoration, and it is considered an essential part of the wedding ceremonies and other social celebrations. Despite the wide spread use of natural henna, reports of allergic contact dermatitis to natural henna and influences of some solutions added to henna are very rare in the literature [17].

Due to the frequent use of henna plant on the skin and hair among women in the general Arab societies, Libyan community in particular, frequent addition of chemicals unknown origin and unknown composition of henna paste to make the inscriptions dark-colored, long-term and that the most famous solutions Serratia and Mahalbiah and the lack of research or previous studies to assess health impact of these substances added to henna. This study was conducted to determine the effect of these additives to the henna on the functions of the liver, kidneys, and complete blood count in the white rabbits.

Materials and Methods:

In this study, the using of the following materials:

Henna:

It was purchased from supermarkets in brack city; known as (Hanna taj) which is a powder, green in color Light placed in a paper bag weighs approximately 100 grams.

Mahalbiah and Serratia:

They are unknown composition solutions were added to the henna in order to increase the darker color and worn on the body for a long time, was purchased from a supermarkets in brack city. These solutions are oily liquid known as Mahalbiah a yellow color, Serratia with orange color, both placed in glass vials each containing 20 ml of the solutions.

Experimental animals:

Only female were used during our present investigation. The animals (n=15) were procured from stock animal facility of Department of Zoology, Sebha University, we divided it randomly into three groups (Control, Group 1, Group2) , five rabbits per group. The animals were acclimatized to the laboratory condition prior to treatment and given food and water.

Group I (Control):

It included five rabbits average weight 1125 ± 170.78 grams was topical application of henna on the dorsal region of the rabbits after hair removal procedure of which were covered treatment area using a transparent nylon for 24 hours and was used henna with water only .

Group 2 (Mahalbaih):

Included five rabbits average weight 820 ± 164.32 grams, it was topical application of henna on the dorsal region of the rabbits after hair removal procedure of which were covered treatment area using a transparent nylon for 24 hours. Henna was used in this group is henna mixed with water and added Mahlbaih, which was divided by the treatment of rabbits as follows:

- Three rabbits were treated using 20 ml of Mahlbaih per 100 grams of henna.
- One rabbit was treated using 40 ml of Mahlbaih per 100 grams of henna.
- One rabbit was treated using 60 ml of Mahlbaih per 100 grams of henna.

Group 3 (Serratia):

It included five rabbits average weight 1420 ± 311.45 grams, was topical application of henna on the dorsal region of the rabbits after hair removal procedure of which were covered treatment area using a transparent nylon for 24 hours. Henna was mixed with water and added Serratia, which was divided treatment of rabbits which are as follows:

- Three rabbits were treated using 20 ml of Serratia per 100 grams of henna.
- One rabbit was treated using 40 ml of Serratia per 100 grams of henna.
- One rabbit was treated using 60 ml of Serratia per 100 grams of henna.

Methods:

The collection and separation of samples:

After topical application of Henna action for 24 hours on the dorsal region removed them hair for rabbits have been slaughtered and assemble blood sampled and divided into whole blood collection tubes containing blocker clot EDTA in order to work full picture of the blood, and the blood collection tubes do not contain any anti coagulant to get the blood, which was separated using a centrifuge speeds of 3000 r / min for 5 minutes and then the separation of the serum in a dry and clean tubes to be used to measure AST , ALT, Urea and Creatinine.

Statistical analysis:

All data was presented as means ±SD.

Statistical analysis was performed using T-test. P-value < 0.05 was taken into consideration for determining significance.

All statistical procedures were computed using SPSS 10.0 software.

Results:

As shown in Table (1) the mean of red blood cells (RBC) in the first group (control) was 4.82 ± 0.125x10^{12} /L, in the second group

(Mahalbiah) 5.35 ± 0.50 $x10^{12}$ /L, in the third group (Serratia) 4.79 ± 0.211 $x10^{12}$ /L .No significant difference between control and Mahalbiah ,Serratia groups. Mean of white blood cells (WBC) in the first group was 10.70 ± 1.65$x10^{9}$ /L. in the second group 5.80 ± 1.85 $x10^{12}$ /L; in the third group 7.37 ± 4.83 $x10^{9}$ /L. There was no significant difference between the first group and third group (p-value >0.05), while the observed difference statistically significant among the first group and second group (p-value< 0.05).
The results showed also that the mean of platelets (PLT) in the first group was 400 ± 111 $x10^{9}$ /L, in the second group 337 ± 92.50 $x10^{9}$ /L, in third group 339 ±271 $x10^{9}$ /L, there are no significant differences between the first group and the second and third groups.
Mean of hemoglobin (Hb) in the first group was 10.93 ± 1.02 g/dl ,in the second group11.67 ± 1.05 g/dl, in third group 10.60 ± 0.102 g/dl there are no significant differences between the control group and the two groups. The results showed that the mean of size of red blood cells (MCV) in the first group was 69.17 ± 3.86 ft, in the second group 65.43 ± 3.89 ft, in Third group 67.03 ± 2.10ft . There was no differences significantly between the control group and second, third groups.
The results of this study showed that the mean of ALT in the serum of rabbits of first group (Control) was 16.5±11.7 U/L and the second group (Mahalbiah) was 59.7±12.3 U/L and the third group (Serratia) 64.0±14.2 (table2). A significant difference was found between the first group and the second, third group (p-value < 0.05).
The results of this study also showed that the average AST activity in the serum of rabbits of first group was 24.67±6.66 U/L, and the second group was 44.0±6.56 U/L, and the third group was 75.0±13.7 U/L (table 2). A significant difference was found between the first group and the second and third groups (p-value <0.05).
The results of this study showed that the average concentration of Creatinine in the serum of rabbits of first group (Control) was 0.80 ± 0.55 mg /dl and the second group (Mahalbiah) was 2.16 ± 0.37 mg /dl and the third group (Serratia) 2.50 ± 0.20 mg /dl (table2). A significant difference was found between the first group and the second and the third group (p-value < 0.05).
The results of this study also showed that the average urea concentration in the serum of rabbits of first group was 46.3 ± 11.2 mg /dl and the second group was 79 ± 10.5 mg /dl and the third group 87.7 ± 14.2 mg /dl (table 3). A significant difference was found between the first group and the second and third groups (p-value <0.05).

Table 1: Mean± SD of RBC, WBC, PLT, HG, MCV in three groups

		MCV(fl)	**HB (g/dl)**	**PLT ($x10^{9}$ /L)**	**WBC ($x10^{9}$ /L)**	**RBC($x10^{12}$ /L)**
Control	Mean±SD	69.17±3.86	10.93±1.02	400±111	10.70±1.65	4.82±0.125
Mahalbiah	Mean±SD	65.43±3.89	11.67±1.05	337±92.5	45.80±1.85	5.35±0.50
	p-value	0.3	0.4	0.5	0.04	0.2
Serratia	Mean±SD	67.03±2.10	10.60±2.10	339±271	7.37±4.83	4.79±0.21
	p-value	0.4	0.6	0.7	0.3	0.8

Table 2: Mean ± SD of ALT, AST in three groups

		AST (U/L)	ALT (U/L)
Control	Mean ±SD	24.67±6.66	16.50±11.70
Mahalbiah	Mean ±SD	44.0±6.56	59.70±12.30
	P- value	0.03	0.02
Serratia	Mean ±SD	75.0±13.70	64.0±14.20
	p-value	0.02	0.02

Table 3: Mean ± SD of Creatinine and Urea in three groups

		Creatinine (mg/dl)	Urea (mg/dl)
Control	Mean ±SD	0.80±0.55	46.30±11.20
Mahalbiah	Mean ±SD	2.16±0.37	79.0±10.50
	P- value	0.03	0.03
Serratia	Mean ±SD	2.50±0.20	87.70±14.20
	p-value	0.02	0.03

Discussion

Many chemicals are added to the henna to increase the color dye from henna, Among the most famous substances that are added to the henna in the Arab societies in general and the Libyan community in particular is Serratia and Mahalbaih ,a current commercial solutions in unknown markets source and unknown chemical composition . Despite the widespread use of these materials significantly and frequently among women, but it has never conducted research or studies on the impact of the use of such substances on human health [10, 12].

This study was conducted to determine the impact of these materials on the functions of the liver and kidneys and complete blood count of rabbits white with a dose of 20 ml of mahalbiah and serratia per 100 gram of henna.

In this study the henna is ready sold in known as the henna taj markets women employed on a permanent basis and frequently, the results showed that there is a rise in the average concentration of liver enzymes AST, ALT, as well as a rise in the average of both urea and creatinine concentration in both the added Mahalbiah Group and Group added Serratia compared to the group that used her only henna.

It was expected to find a higher concentration of liver enzymes, Urea, Creatinin in both group (Serratia and Mahalbiah) because it is not a natural material made with chemical properties [2, 18, 19].

But the results showed that there is also a rise in the average concentration of liver enzymes, Urea, Creatinin in the results of rabbits that have been used with henna only possibly due to the henna used henna business may not be pure or contain additional materials undeclared to the consumer because it is supposed to henna Sofa naturally is one of the medicinal plants used in many treatments so we analyzed liver enzymes, Urea, Creatinin rabbits did not put her henna at all were the results within the normal range to them, and this can be confirmed in other research in the future using natural henna leaves are ground and processed in the lab [2, 3, 5].

When we changed the added dose of henna in each of Serratia and Mahalbiah to 60 ml per 100 gram of henna it led to animal death in both groups after about two hours of setting the mixture, it was noticed the deterioration of the case of animals once the

Blend Mode them and put them in its own fund, where respiration rate began at least gradually until she died about two hours later and may have suffered a severe case of poisoning. As well as the dose of 40 ml per 100 gram of henna have also led to the death of animals, but after a period of somewhat longer than the dose gram of henna 60 ml per100 [3, 5, 11, 12, 19].

As for the results of CBC in the three groups there are no significant differences in the average of red blood cells (RBC) and platelet (PLT) and (Hb) and the average size of red blood cells (MCV) with the exception of a significant difference in (WBC) between the first group (control) and seconed group (Mahalbiah).

More research and studies to assess the health impact of these substances that adding to the henna and promote health awareness among women in Libyan society about this harmful unknown chemical material is our recommendations.

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