# *Lawsonia inermis* and para-phenylenediamine exerts an insidious effect on the epidermal, renal, and hepatic tissue with repeated application: a histological and morphometrical study

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## SUMMARY

Ground leaves of *Lawsonia inermis* (LI) made into a paste has been used from the Bronze Age as cosmetic application. Para-Phenylenediamine (PPD) is an organic compound that is added to LI to increase its aesthetic appearance and longevity. Addition of PPD increases the possibility of allergic contact dermatitis from LI tattoo combinations, and may be absorbed through the skin, affecting body organs. The current study was conducted to determine the effect of this mixture on the histology of skin, liver and kidney tissue. Thirty albino rats were grouped into five groups. The treatment protocol included a control group (I), application of LI paste alone for a period of two weeks (II), and four weeks (III), and a combination of LI and PPD for two weeks (IV), and four weeks (V). All rats in each group had an area on the lumbar region shaved and marked, and the paste was applied to this region. The rats were sacrificed after the experimental period and the tissues of interest removed for further histological and morphometric analysis.

Application of LI alone caused no observable histological changes, however, in combination with PPD, LI caused thinning of the epidermis, reduction in the quantity of collagen fibers in the dermis, distorted the arrangement of hepatocytes, caused infiltration of inflammatory cells into the liver tissue and distortion in the arrangement of renal tubular epithelial cells. The longer the exposure to LI and PPD, the more alteration to the normal histology of tissues.

**Key words:** Lawsonia inermis – Renal – Dye – Henna – Epidermis – Hepatic

## INTRODUCTION

*Lawsonia inermis* L. (LI) is a shrub from the family *Lythraceae,* also known with the following names: henna tree, Inai, hina, mignonette tree and Egyptian privet. It is used as a dye for coloring the epi-

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dermis of the hands, feet, hair, and fingernails, as well as fabrics—silk, wool, and leather (Ezzat et al., 2021). LI is one of the most important medicinal plants in use because of its medicinal and cosmetic properties. The plant has been present and used for several decades as drug, cosmetic and preservative in many cultures and tribes across the globe especially India and Africa (Ezzat et al., 2021; Aremu and Oridupa, 2022).

LI has been found to contain carbohydrates, phenolic, flavonoids, saponins, proteins, alkaloids, terpenoids, quinones, coumarins, xanthones, fat, resin and tannins (Aremu and Oridupa, 2022; Al-Snafi, 2019). Many phytochemicals including alkaloids, naphthoquinone derivatives, phenolics and flavonoids have been isolated from different components of LI. Pharmacological studies of the plant show that LI possesses antifungal (Rahmoun et al., 2013), antibacterial (Habbal et al., 2013), antiparasitic, molluscicidal, antioxidant, hepatoprotective, central nervous, analgesic, anti-inflammatory (Manuja et al., 2021), antipyretic, burn and wound healing, immunomodulatory, antiurolithiatic, antidiabetic, hypolipidemic, antiulcer, it is used as an antidiarrhoeal agent, diuretic, has been used as anticancer and has many other pharmacological effects (Aremu and Oridupa, 2022; Al-Snafi, 2019; Badoni et al., 2014). The antioxidant and anti-inflammatory properties of LI has been attributed to its phenolic and flavonoid contents (Rahmoun et al., 2013).

LI has been nominated in African alternate therapy and ayurvedic medicine as a purgative, abortifacient agent and astringent (Badoni et al., 2014). LI has also been reportedly used for some pharmacologic potentials, including its usage as pain reliever, anti-diabetic, hepatoprotective, immune booster, antioxidant, anticancer, anti-inflammatory and inhibitory action against several microbes (fungal, viral, trypanosome and plasmodium) (Al-Snafi, 2019; Manuja et al., 2021; Badoni et al., 2014; Khantamat et al., 2021). Biologically, it is also beneficial in the management of hair and scalp issues as well as in eye syndromes and hair loss (Ezzat et al., 2021).

The use of LI has been depicted in literary pieces and artwork indicating that LI has always played an important atomistic role in the everyday activities of many ancient cultures, supplying psychological and medicinal benefits, as well as being used for personal ornamentation and decoration (Habbal et al., 2013; Manuja et al., 2021). Although LI was historically applied to the hands and feet to protect against fungal pathogens and to hair to combat lice and dandruff, other traditional uses soon included the treatment of liver and digestive disorders, reduction of tissue loss in leprous individuals, and conditions like diabetic foot disorders and ulcers (Badoni et al., 2014). As indicated above, as many as 70 phenolic compounds were distilled and separated from various parts of the plant. Naphthaquinones, which include the dyeing component lawsone, have been connected to many of its pharmacological activities (Badoni et al., 2014; Khantamat et al., 2021).

The paste, decoction or infusion made from the leaves of LI has been used from the Bronze Age to dye skin, hairs and fingernails, especially at the times of festivals as a cosmetic application (Pradhan, 2012). In recent times, henna paste has been used for body art paintings and designs in many western countries. Henna application for cosmetic purposes is customary in the culture of Northern Nigeria, especially during celebratory occasions like weddings, naming ceremonies and religious festivals. Despite such widespread use in dyeing and body art painting, there has been many concerns about the use of the dye in combination with other substances, which makes the dye more pronounced with exposure to air. Para-phenylenediamine is one of such substances used in the manner described above.

Para-Phenylenediamine (PPD) is an organic compound, which appears as a white to purple crystalline solidified matter that turns purple-to-black color when exposed to air. When mixed with an oxidizer (typically hydrogen peroxide) and applied, it penetrates shafts of hair and changes into an oxidized form whose molecules are too large to 'escape', thus creating long-lasting color very effectively. There is no better chemical for the job.

On the flip side, is is highly allergenic, and potentially dangerous to those who have developed a sensitivity to it. There are 'milder' chemicals similar to PPD (other phenylenediamines or tol-

uenediamines), which are a little less allergenic, but these are not quite as effective. It is also toxic by skin absorption, inhalation or ingestion, and used for production of aramid fiber, antioxidants, as a laboratory reagent, in photographic developing, and as a dye for hair and furs (National Center for Biotechnology Information, 2022). Very often, PPD is added to LI paste to make the color appear darker and to speed up the dyeing process (Kang and Lee, 2006).

The addition of PPD to LI, which is a common practice, may produce a more aesthetic appearance, but it may also increase the risk of allergic contact dermatitis from LI tattoo combinations, and a number of cases have been reported (Kang and Lee, 2006; Calogiuri et al., 2010; Uzuner et al., 2009; Turan et al., 2003). Given the increasing popularity with use of IL for cosmetic purposes among teenagers and older individuals, coupled with dearth of information of the effect of these substances on the tissues of interest, this study was undertaken to determine the effect of this mixture on the histology of the skin, as well as the liver and kidney tissue.

The ensuring information obtained will serve as information on the effect of the paste on these tissues over a period of time.

## MATERIALS AND METHODS

### Collection and identification of plant

The leaves of LI and PPD were obtained from the central (Monday) market, Maiduguri Borno State, Nigeria. The plant was identified and authenticated by a Taxonomist at the Department of Biological Sciences, Faculty of Sciences, University of Maiduguri, Borno state, Nigeria. The leaves were identified and authenticated by a taxonomist from the Department of Biological Science. The plant sample was deposited at the herbarium of the Department of Human Anatomy with a voucher number UM/HAH/2021/006.

### Preparation of plant extract

The powdered extract of the plant was obtained by shade-drying the leaves of LI, and then mechanically pulverizing the dried leaves to obtain the powdered product. This was then sieved by passing through a fine sifter to remove the larger particles. The fine powder was then weighed and collected into a dry container, and refrigerated prior to use.

### Experimental animals and animal husbandry

Thirty (30) male albino rats weighing between 91 and 160 g were purchased from the Department of Human Physiology, University of Maiduguri for the experimental study. They were 4-to-5 months old at the period of the experimental study. The animals were kept in the animal house for a period of one week to acclimatize.

Their accommodation was well ventilated, and room temperature was maintained according to laboratory guidelines (20-26$^{o}$C/68-78.8$^{o}$F); the cages were kept in hygienic conditions and under a natural light (13 hours) and dark (11 hours) schedule. The rats were fed with standard rat chow and water *ad libitum*.

### Experimental design

The thirty (30) male Wistar albino rats were assigned into five groups with six (6) animals each, using the block method to ensure an average weight of 135 g across each group.

Group I: Control group; nothing was applied to the skin of rats. Group II: 100 mg LI paste was applied once weekly for two weeks. Group III: 100 mg LI paste was applied once weekly for four weeks. Group IV: 100 mg LI paste + PPD was applied once weekly for two weeks. Group V: 100 mg LI paste + PPD was applied once weekly for four weeks.

### Experimental procedure

All the rats in each group had an area on the lumbar region of the back shaved to expose the skin in this region. This was done using a pair of scissors to remove the surface hair. The hair that was not removed was cleared using shaving cream (Veet, Reckitt, Canada). This was removed by cleaning the surface of the shaved region repeatedly with alcohol solution to remove the residue shaving cream and also to sterilize the skin over this region. The shaved skin was closely observed for a period of two days to examine for inflammation

or rashes which would indicate a reaction to the shaving cream. None of these were observed on any of the shaved skin. The exposed skin was then marked to obtain a region with an area of 2x4 $cm^2$. This area was marked for application of the powdered extract as determined by the experimental design. Topical application of the extract was repeated every 7 days for rats in groups II and IV for two weeks, and every 7 days for 4 weeks for rats in groups III and V.

**Animal sacrifice**

The animals were humanely sacrificed after two weeks and four weeks respectively, by administrating ketamine hydrochloride injection (0.1 mg/kg) as anesthesia. This was given as an intramuscular injection on the left thigh of the rats in all groups. The shaved skin over the marked region to which the extract was applied was carefully dissected out by separating the epidermis from the subcutaneous tissue. A median incision along the abdomen of the rats provided access to the liver and the kidney tissue, which were quickly dissected, fixed and histologically processed for further observation to determine the effect of the extract on these tissues.

**Tissue preparation**

The skin, liver and kidney tissues were fixed for 24 hours to prevent autolysis and putrefaction. Thereafter, the tissues were trimmed and conveyed through a series of solvents as per schedule for dehydration, clearing and paraffin infiltration. The tissues underwent normal histological procedures, which included dehydration in ascending grades of alcohol (50, 70, 80, 95 and 100%), clearing using pure xylene, followed by impregnation in molten paraffin wax, and sectioning with a rotatory microtome. 5 µm sections were obtained and fixed on a clean albumenized surface of the slide of glass for Haematoxylin and Eosin staining.

**Morphological studies and histological observation**

The micrographs of the liver, kidney and skin tissues were observed under the microscope and areas of interest were measured using morphometric methods described by Attah et al., (2022). Images from the histological sections were produced using an Amscope light microscope (MB-JX-ISCOPE, Los Angeles, USA), to which a digital camera was attached (M500, X64, version 3.7). The images were photographed at using a magnification of X100 and X200. An ocular micrometer was previously standardized, and was also used to measure areas of interest in the histological slides for morphometric analyses also based on established methods described by Omar (2018; Zaki, (2015), and Attah et al., (2019). Morphometric analysis was performed using the computerized image analysis system, ImageJ 1.53a (Wayne Rasband, National Institutes of Health, USA).

The micrometer was used to calibrate the ImageJ application to establish the unit of measurement in micrometers (µm) instead of pixels. The micrometer used was the same objective and pixel resolution as that of the micrograph being examined.

The skin tissue was examined, and parts of the epidermis measured by using the free hand tool of imageJ application to determine the length of the various strata. Strata granulosum and basale were measured as a single unit, as they were not distinguishable in the micrograph. Stratum corneum and granulosa were measured as the distances form where the skin tissue originated to where each strata terminated. In the liver tissue, the width of the hepatocytes and sinusoidal spaces were measured for rats in each group. Each length was represented in µm.

Data from the above measurements were statistically analyzed using GraphPad Prism 8 software by using One-way Analysis of Variance (ANOVA) and expressed as mean ± SEM and percentage followed by Tukey Multiple Comparisons Test. $p<0.05$ was considered to be statistically significant.

**Ethical considerations**

The current research study was conducted in accordance with the University of Maiduguri Research and Ethical Committee guidelines, the ARRIVE guidelines (reporting of in vivo experiment), and the National Institutes of Health (NIH) guide for the CARE and use of laboratory animals (NIH Publications No. 8023, revised 1978). It was ap-

proved by the Ethical Committee of the Department of Human Anatomy, University of Maiduguri, on 21st March, 2021 with code number UM/HA/UGP 19.20-007.

## RESULTS

The layers of the epidermis were individually measured starting with stratum corneum. The strata were found to be thickest in group I, with a mean length of 16.5 µm, which was statistically thicker when compared to other groups. The group with the thinnest stratum corneum was group V, with a mean thickness of 2.5 µm (Fig. 1). The length of stratum granulosum in all groups was significantly thicker in rats in group III, which had a mean thickness of 3.8 µm compared to the other groups. The mean thinnest stratum granulosum was also found in the rats in group V (0.7 µm) (Fig. 2).

Stratum spinosum and basale were measured together in all groups, as these strata were not distinguishable in all groups. Rats in group III had the thickest of these combined layers (14.5 µm), and this value was statistically significant when compared to the other groups (Fig. 3). The thinnest strata basale and spinosum were found in rats in group V (2.7 µm).

The hepatic sinusoids were widest in livers of rats in group II and this value was statistically significant when compared to the width of sinusoids in group V (Fig. 4). The thickness of the hepatocytes in all group was not significantly significant, however, the hepatocytes of the rats in group II showed the greatest thickness (6.7 µm) whereas the thickness in group IV was 5.4 µm (Fig. 5).

The skin in the control group showed the typical features present in skin tissue with the basal layer resting on the dermis, composed of simple columnar epithelium with their numerous nuclei, which appeared basophilic with prominent nucleoli. Stratum spinosum was eosinophilic and the cells were not easy to distinguish. Their nuclei were lighter in color compared to the nuclei found in stratum basale. Stratum granulosum was thick and compact across the skin tissue and stratum corneum was observed as sheets of tissue that sloughed from the surface of the skin. The dermis of the rats in this group was composed of numerous robust collagen fibers within which blood vessels could be observed (Fig. 6A).

The cells in stratum basale were not as prominent as observed in group I, and stratum spinosum consisted of cells with round and clear nuclei, and some cells had one or more nucleoli. The cytoplasm of the keratinocytes in this layer were eosinophilic. Stratum granulosum was broader and more distributed when compared to the strata in group I. Stratum corneum was sparse and the dermis had less robust collagen fibers (Fig. 6B).

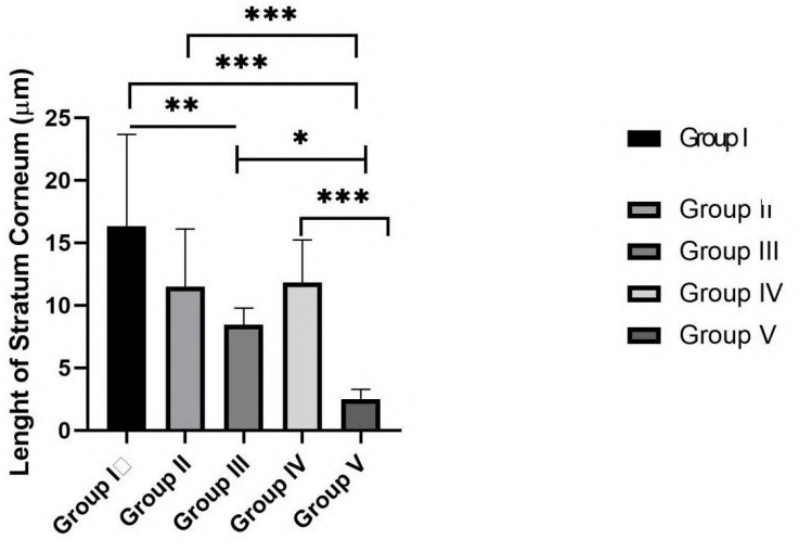


**Fig. 1.-** Length of stratum corneum in all groups (* - p<0.05, **p<0.01, ***-p<0.001, µm - micrometers, n=6, N=30).

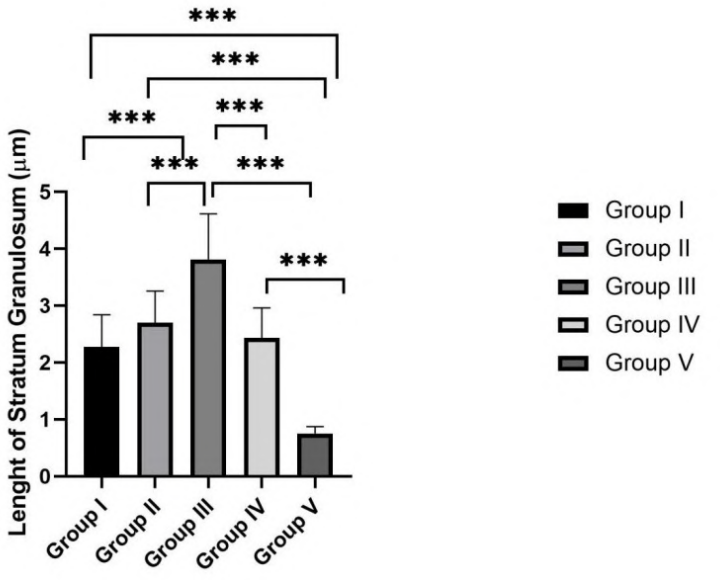


**Fig. 2.-** Length of stratum granulosum in all groups (* - p<0.05, **p<0.01, ***-p<0.001, µm - micrometers, n=6, N=30).

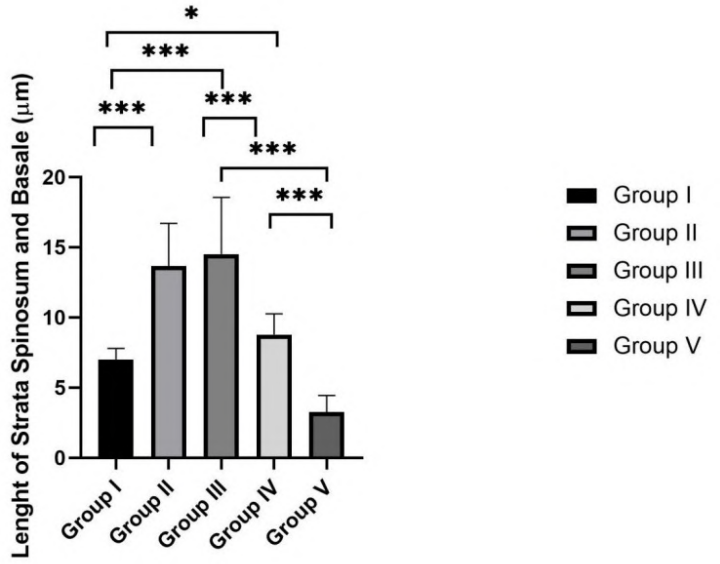


**Fig. 3.-** Length of strata spinosum and basale (* - p<0.05, **p<0.01, ***-p<0.001, µm - micrometers, n=6, N=30).

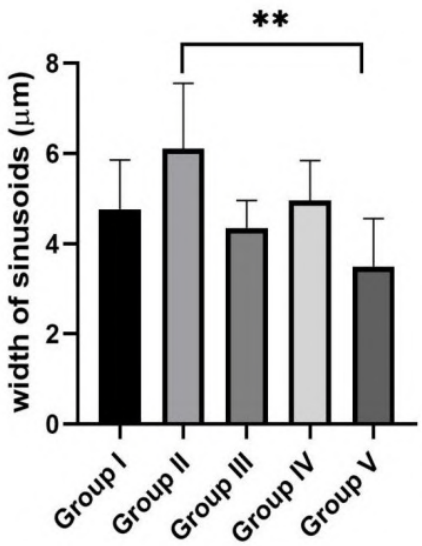


**Fig. 4.-** Width of sinusoids in all groups (* - p<0.05, **p<0.01, ***-p<0.001, µm - micrometers, n=6, N=30).

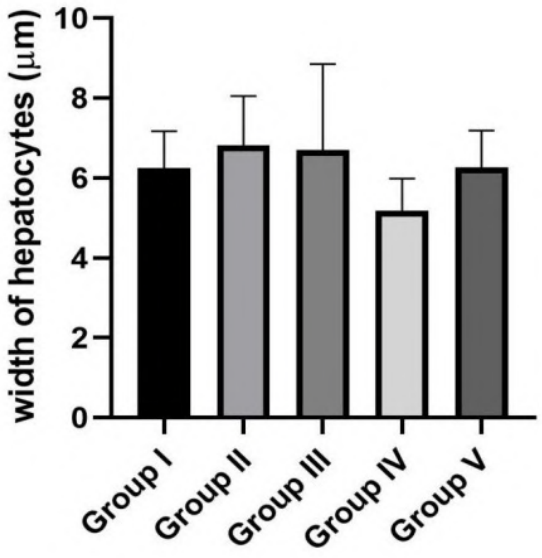


**Fig. 5.-** Width of hepatocytes in all groups (* - p<0.05, **p<0.01, ***-p<0.001, µm - micrometers, n=6, N=30).

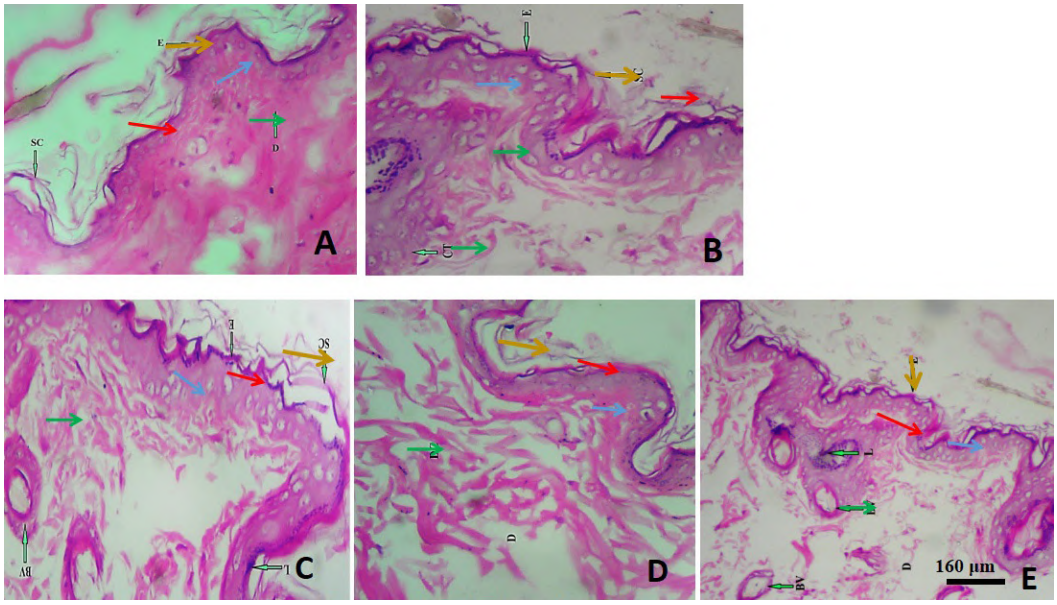


**Fig. 6.- A-E**: skin of the rats in the experimental groups (I-V) showing the epidermal layers and dermis: Stratum corneum SC (yellow arrow) which is mostly eroded in groups IV and V. Stratum granulosum (red arrow) is unaltered in all groups and stratum spinosum (blue arrow) was thinnest in group V. The dermis D (green arrow) in all experimental groups had blood vessels and collagen fibers. BV - blood vessel. H & E staining. Magnification = x200; Scale bar A-E = 160 µm.

The skin of the rats in group III showed similar appearance with rats in group II. However, stratum basale appeared sparser, stratum granulosum was more compact and continuous, and stratum corneum was relatively thicker. The blood vessels were more abundant in the dermis, and collagen fibers were sparsely arranged (Fig. 6C). Figure 6D represents the skin in rats in group IV, showing very thin strata spinosa and basale with very few keratinocytes present in these layers. Stratum granulosum was continuous over the surface of the skin, and stratum corneum was equally sparse. The dermis showed collagen fiber bundles which were loosely arranged.

The micrograph representing the skin of rats in group V showed a thinner epithelium with little defined stratum basale. Stratum spinosum was easier to distinguish and the keratinocyte nuclei

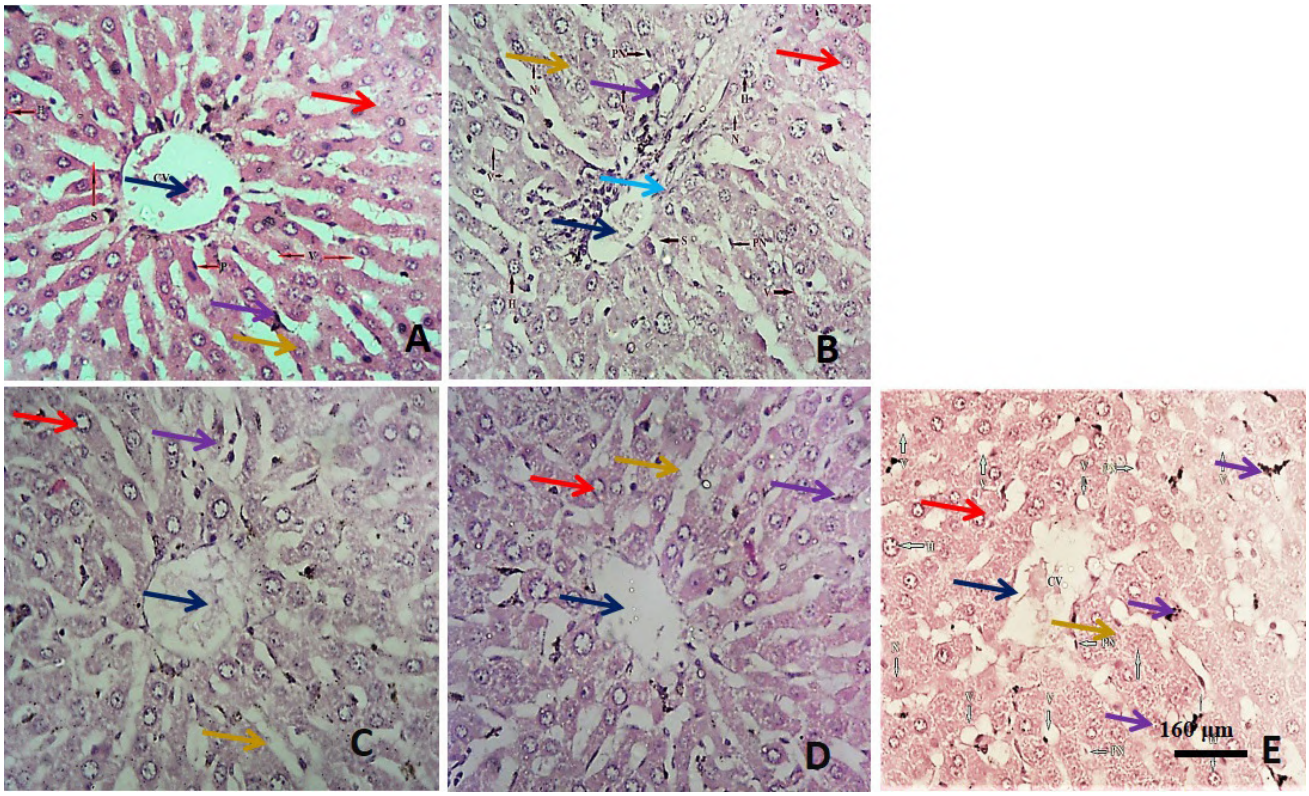


**Fig. 7.- A-E**: micrographs of the liver in groups I-V showing the central vein (navy arrow) surrounded by flat endothelial cells, the hepatocytes (red arrow) form columnar cords that radiate towards the central vein. This arrangement is more disorganized in group V. Sinusoids (yellow arrow) are found between the columnar cords. Kupffer cells (purple arrow) were more abundant in group IV and an aggregation of lymphocytes were found in the space surrounding the central vein in group II. H & E staining. Magnification = x200; Scale bar A-E = 160 µm.

were more clearly defined by their round and clear nuclei. Stratum granulosum was continuous and stratum corneum was barely observed. Numerous blood vessels were however found in the dermis, and very few collagen fibers were loosely arranged in the dermis (Fig. 6E).

Figures 7A-E show the liver tissue in all groups. Figure 7A showed the liver tissue in group I. The hepatocytes radiated towards the central vein separated by sinusoids. The cells had eosinophilic cytoplasm and prominent dark-staining, centrally located nuclei with 2-4 nucleoli (Fig. 7A). The liver tissue of rats in group II showed an aggregation of lymphoid/inflammatory cells around the central vein and pericentral regions, and several Kupffer cells located in the sinusoidal spaces. Lymphocytes are distinguished from the hepatocytes using H & E stains by observing their deep staining, prominent nucleus and a relatively small amount of agranular and poorly stained basophilic cytoplasm. These cells are easily differentiated from the histological appearance of hepatocytes, which are polygonal cells with abundant granular eosinophilic cytoplasm, centrally placed round/ovoid nuclei, and prominent nucleoli. The hepatocytes are also arranged in plates that are a single cell thick.

The hepatocytes were wider when compared to that in the rats in group I (Fig. 7B). The liver of rats in groups III and IV had identical histological structures with radiating hepatocytes and several ameboid-shaped Kupffer cells, which were easily identified by their ovoid, indented and/lobulated nuclei, and inflammatory cells found in the sinusoidal spaces attached to the sinusoidal endothelial cells (Figs. 7C and D). Figure 6E shows the liver tissue in group V. The hepatocytes appeared disorganized and the cytoplasm was granular in appearance, and the sinusoids appeared narrow and closely spaced compared to the other groups as was also observed in Fig. 4. The width of the sinusoid in groups II and VII appeared slightly wider when compared to the rats in groups III and V, who were exposed to the extract for a longer period of time.

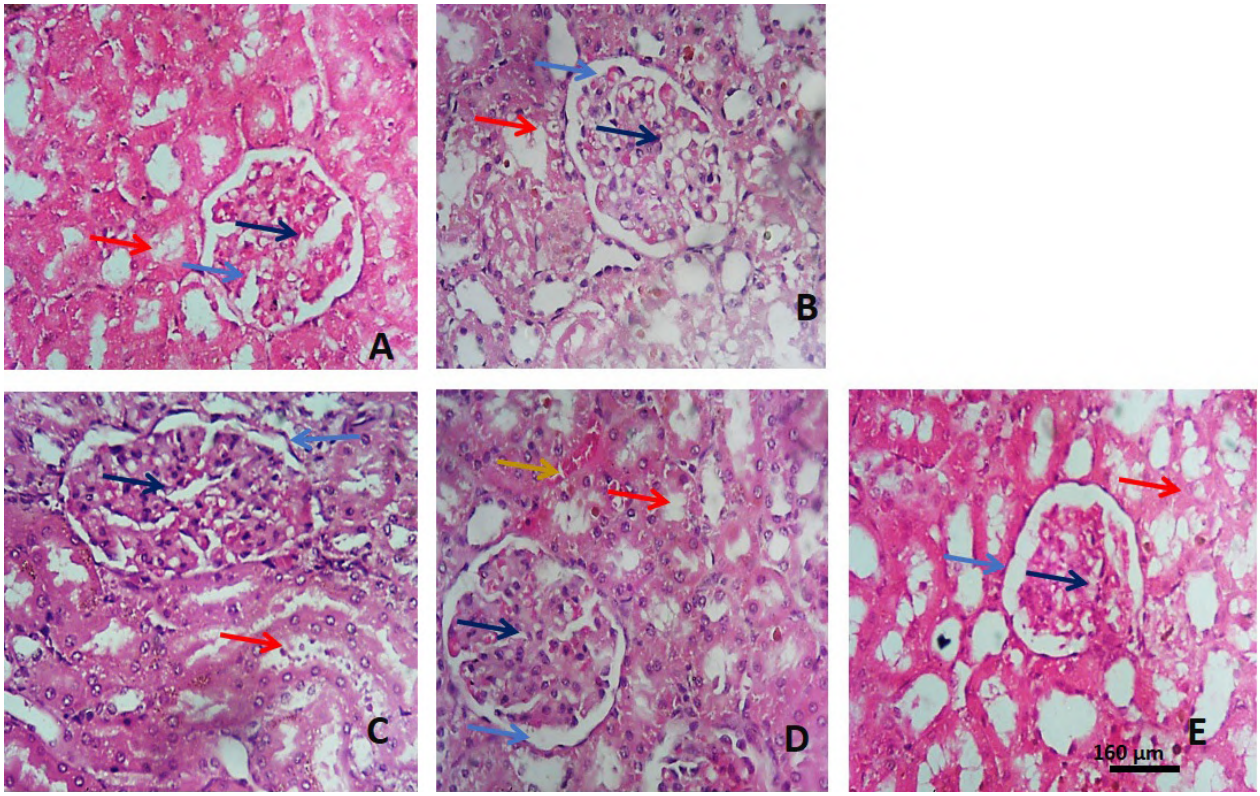


**Fig. 8.- A-E**: kidney tissue in groups I-V. The glomerulus in all groups were surrounded by Bowman's space and membrane (blue arrow). The renal tubules (red arrow) were dilated and distended in group V when compared to the other groups. There was a little interstitial bleeding (mustard arrow) in the renal parenchyma in group VI. H & E staining. Magnification = x200; Scale bar A-E = 160 µm.

Figures 8 A-E show the kidney tissue in all groups. Figure 8A shows the normal renal architecture showing the glomerulus surrounded by Bowman's capsule and space. The renal tubules contained simple cuboidal cells, and these had clear luminal spaces (Fig. 8A). The kidney tissue in groups II was similar to that found in the control group and the renal tissue showed similar histological arrangement (Fig. 8B). The kidney tissue in group III has dilated renal tubules (Fig. 8C), and in group IV there was some interstitial bleeding found in the renal parenchyma (Fig. 8D). The renal histology in group V showed slightly dilated renal tubules, which showed slight distortion in the arrangement of tubular lining cells (Fig. 8E).

## DISCUSSION

Henna (LI) has been used aesthetically and as a medicament for more than 9,000 years, as documented by archaeological and anthropological evidence. These records indicate that henna traditions originated from early times in the Mediterranean, Nubia, Libya, Tunisia, Arabia, Assyria, Mesopotamia, Persia, and India (Othman et al., 2020).

A paste made of LI leaves is usually used for coloring the skin, hair and fingernails for cosmetic purposes. Henna body art is presently popular as adornment for weddings and other celebrations in India, South Asia, the Middle East and Africa (Aremu and Oridupa, 2022; Othman et al., 2020). The traditional use of LI as a natural dye seems not to exert toxic effects and appears biosecure (Khantamat et al., 2021). However, many times, the dye is mixed with PPD before use, and even though allergy to natural henna is not usual, the addition of PPD to the natural henna increases the risk of allergic contact dermatitis (Othman et al., 2020; Al-Suwaidi and Ahmed, 2010).

Hair removal in the current study was achieved by chemical methods by using creams also called depilatories, which are cosmetic preparations used to remove hair from the skin. They work by breaking down the sulphur bonds in the hair's keratin that are sensitive to strong alkaline and

deoxidation agents. The active ingredient for deoxidizing the sulphur bonds is a salt of thioglycolic acid (Tsai et al., 2021). Chemical depilation generally does not destroy the dermal papilla, and hair is able to grow back afterwards, because relatively small amounts of chemicals are used (Pohl et al, 2013). In the current study, the depilatory cream did not appear to affect the dermis and epidermis, as the cream was applied to the surface of the skin in all groups and the control group showed no adverse effect and the histological structures appeared intact.

**Effect of LI paste on the histology of the skin**

The use of LI paste in the current experimental study presented very little changes on the skin tissue when used alone. The strata of the skin showed similar appearance with the control group when only henna was used, and this is in agreement with many studies previously conducted (Aremu and Oridupa, 2022; Al-Snafi, 2019; Othman et al., 2020; Al-Suwaidi and Ahmed, 2010). However, the skin tissue that was treated with LI and PT showed a change in the skin architecture, as the thickness of the epidermal layer and quantity of collagen in the dermis was reduced in group V. In the current experimental study, no signs of allergy were observed with the use of LI and PPD over a period of time. Other researchers have reported allergic reactions on the skin following the use of LI and PPD (Kang and Lee, 2006; Calogiuri et al., 2010; Uzuner et al., 2009; Turan et al., 2003).

**The effect of LI on the histology of the liver**

There is adequate literature available to support the fact that substances applied to the epidermis can be easily absorbed through blood vessels in the dermis, which consequently affects other organs in the body, including the liver and kidney tissue (Feldmann and Maibachk, 1970; Franz, 1978). The liver tissue in the group treated with LI and PPD showed disorganization of the hepatic tissue. This result is similar to studies carried out by Eissa et al. (2021), and Lee et al. (2015), where it is discovered that PPD induced allergic activity and increased the probability of tumors in the kidney, liver, thyroid gland and urinary bladder, although its effect on dermal papilla cells remained to be clarified. PPD was also found to induce several cytotoxic effects through modification of miRNA expression levels (Lee et al., 2015).

**The effect of LI on the histology of the kidney**

The kidney tissue showed slight distortion of the arrangement of renal tubular cuboidal epithelium in the group that was exposed to LI and PPD for a period of 4 weeks. This agrees with studies carried out by Eissa et al. (2021), Lee et al. (2015), and Devecioğlu et al. (2001), which found that kidney parts of the PPD-poisoned victims had regions exhibiting extreme tubular necrosis. In addition, there were cellular ghosts that had lost cellular information and sloughed into the luminal cavity, which was not observed in the same study. In addition, Ibrahim et al. (2006) demonstrated a distortion of the lobular structure of the kidney, accompanied with the thickening of the Bowman capsule of the kidney.

In the current study, the period of exposure to LI and PPD was proportional to the alteration of tissue, as most changes to skin, hepatic and renal tissue were observed in rats in group V. This also agrees with research carried out by Eissa et al. (2021), who determined that, with time, more absorption through the skin occurs, and PPD appeared in the urine samples of subjects exposed to PPD and LI, and the quantities found were directly proportional to the period of use and dermal exposure, which led to noticeable effects in the morphology and number of blood cells and extended to renal and liver functions.

## CONCLUSION

In the current study, LI and PPD were applied topically to the skin over a period of time to determine its effect on the epidermal, dermal, renal, and hepatic tissues. LI was first applied alone before a mixture of LI and PPD were applied. At the end of the experimental period, the authors determined that application of LI alone did not cause a lot of changes to the histological architecture of the organs listed. However, in combination with PPD, LI caused a thinning of the epidermis, and reduction in the quantity of collagen fibers in the dermis. It also caused distortion in the arrangement of hepatocytes and caused infiltration

of inflammatory cells in the liver tissue, as well as distortion in the arrangement of renal tubular epithelial cells. More study is recommended to test the effect of the combination of LI and PPD on these tissues over a longer period of time and on other tissues. Also, serum markers of liver function and urinalysis should be tested to observe the effect of the extract on the function of the kidney and liver. Other staining techniques aside from H and E should be performed to confirm an increase in collagen fibre and immune cell infiltration.

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## AUTHOR'S CONTRIBUTIONS

Conceptualization: SEJ, MAA, SHG and MOOA, Acquisition and design: SEJ, MAA, and SHG, Methodology: SEJ, MAA, SHG and MOOA, Administrative support/Supervision: SHG and MOOA Provision of study materials: SEJ, MAA, and SHG Data collection and assembly: SEJ, MAA, SHG and MOOA Data analysis and interpretation: SEJ, MAA, SHG and MOOA Funding Acquisition: SEJ, MAA, SHG and MOOA Writing: Review and Editing: SEJ, MAA, SHG and MOOA Final approval of manuscript: SEJ, MAA, SHG and MOOA.

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