



## The Staining Interactions of Aqueous Extract of Skin *Allium cepa* (Red Onion) on Some Selected Histological Tissues

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### Authors' contributions

This work was carried out in collaboration among all authors. Author MOM owner of the work and performed the design of the work. Authors AAH, MI, SYM carried out and sourced the materials for literatures review. Authors OOO and MKD did the overall cross the work to ensure that everything is in order. Authors AU and HK carried out the photomicrographs and interpretation. Authors RIT and UA managed the entire manuscript and dissection of the animals. Authors ATM and IM managed the overall review of the work. Authors NO, HMT and MS managed the references. Authors AAN and JMB carried out preparation of the stains used. Authors BAB, HA and FAD took care of the animal used and tissue processing. Authors ASA and AA carried out microtomy procedure. Authors SMS, AS and NAI managed the staining procedures and carried out screening of the slides. All authors read and approved the final manuscript.

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## ABSTRACT

**Introduction:** Histological stains are biological dyes which colour tissue in order to aid optical differentiation of tissue component. Dyes are coloured substance which impact colour for material such as textile, cosmetic, food, drugs, rubber plastics, hair, fur and tissues. There are two types of dyes namely; natural dyes and synthetic dyes. *Allium cepa* is an imperative, evergreen plant, which belongs to the family Amaryllidaceae commonly called bulb onion.

**Aim:** This research work aimed at evaluating the staining capability of onion extract as counter stain when haematoxylin was used as primary stain.

**Methods:** Tissue blocks sections of liver and kidney organs were made from the Wistar rat. Serial sections labelled A to M were made from each block and stained with Harris haematoxylin. Section A was counterstained with eosin, as control. Different preparations of extracts of the onion skin were used to counter stain sections of kidney and liver tissues. Group B to M were kidney and liver tissues stained as follows: B, E, H and K (5% aqueous and ethanolic extract with ferric chloride, 5% aqueous and ethanolic extract with potassium aluminium alum, and 5% aqueous and ethanolic extract without mordant, for 10 and 20 minutes). C, D, F, G, I, J, L and M (5% aqueous and ethanolic extract with ferric chloride, 5% aqueous and ethanolic extract with potassium aluminium alum and 5% aqueous and ethanolic extract without mordant for 10 and 20 minutes).

**Results:** The skin extract of *Allium cepa* stained the cytoplasm of cells and connective tissues in shades of reddish brown to yellowish brown. The study established the cytoplasmic counter-staining ability of the extract of *Allium cepa*.

**Conclusion:** It is therefore suggested that onion skin extract solution can be substituted for eosin due to its domestic availability, ease of preparation and above all, its good cytoplasm contrast with the nuclear stain.

*Keywords:* Staining; *Allium cepa*; tissues.

## 1. INTRODUCTION

Histological stains are substance or biological dyes which colour tissue in order to aid optical differentiation of tissue component [1]. Dyes are coloured substance which impact colour for material such as textile, cosmetic, food, drugs, rubber plastics, hair, fur and tissues. There are two types of dyes natural dyes and synthetic dyes [2,3]. Stains are prepared from dyes which have been manufactured to rigid specification or have been subjected to rigid quality assurance procedures to ensure that they are suitable for this specialized purpose [4]. Stains are also referred to as dyes which have affinity to a particular part of cell [5]. Dyes are chemical substance of chemical or synthetic origin, solution in a medium used to impart a desired colour to a non-food material like paper, leather, wood, textile and even cosmetics in a process known as dyeing. Tissues and their constituent cells are usually transparent and colourless when examined under the light microscope with little or no differentiation of the various structures [5]. Colouring, in other words, dyeing or staining of

the section of tissues makes it possible to see and study the physical features and relationships of the tissues and their constituent cells [5]. It so happens that different tissues and indeed, different components of the cell, show different affinities for most dyes or stains, it follows therefore that no single staining method will demonstrate all the tissue structures present [5]. Some dyes require the addition of mordants, oxidants, accelerators and adjustment of pH before they can stain tissues while others do not require these substances in other for them to stain tissues. That is, simple aqueous or alcoholic solutions of the dyes can be used as stains, generally called simple stain [1]. Mordants act as bridge between the dye and tissue, facilitator improve the quality of staining, while, accentuator and accelerator increase staining power of dyes to an optimal [5]. The colour of dyed fabrics depends on the nature of the chromophores as well as the substituent functional groups, the auxochromes, of the dye molecular species. Chromophores and auxochromes are considered the most important chemical constituent of dyes responsible for

textile coloration. Dye – yielding plants, unlike synthetic dyes, may contain more than one chemical constituent, each exhibiting a different color and properties, operating singly or in combination with the different groups, depending on their chemical structure and composition [6,5,7].

Until the middle of 19th century basically all dyes were natural product extracted from plant e.g haematoxylin from Mexican tree *Haematoxylon campechianum*. The dye derived from insect e.g carmine from body of a female insect *Dactilopius cacti*. Orcein dye was derived from *Cracus santivas* [1]. In Africa particularly in Nigeria there are number of natural dye plant which are capable of cultivation, just as they are cultivated in other countries around the globe. Recent studies have given useful result in of such abundant dye plant use as histological stains for same tissue component [8]. This is considering the huge amount of foreign exchange and process involved in obtaining suitable synthetic dye for histological purposes, and also to their hazard to human and animal health [8]. This has resulted in the withdrawal of some dyes as their hazard to human and animal become recognized [2]. With the wide concern favouring the use of eco - friendly and biodegradable materials, the use of natural dyes once again gain interest [9,10]. Therefore greater attention and effort is now channeled toward the use of natural occurring dye from plant, which is less expensive than costly synthetic [11,12]. This prompt an interest in this research work that explore the histological staining abilities of the dye extract of *Allium cepa* (red onion) on some selected histological tissue components in an attempt to faintly identified the ability of the solution as counter stain after haematoxylin as primary stain [13].

Onion belongs to the family amaryllidaceae, commonly called bulbs onion the genus *allium* is one of the largest monocotyledons general, It consist of many wild edible species and widely distributed in a temperate zones in the northern hemisphere [14,15]. The onion is a tunicate bulb, compressed or round, or oblong in figure, invested with a shining, thin, dry membrane, of a reddish or white color. It is less pungent to the taste than garlic, with some degree of sweetness, and a peculiar, well-known odor. Onion bulbs are of various shapes and sizes, usually globular, the layers being juicy [16,17]. A half hardy biennial herb, normally grown for its bulb as an annual and only carried forward into a

second year when seeds are required Bulb-truncate formed from thickened leaf bases (sheaths) outer layers are thin and fibrous leaves alternate and are produced from a flattened conical basal stem, they are cylindrical and leaf blades are hollow flowers are greenish white Seeds are smooth black, wrinkled when dry Stem- cone shaped from where leaves arise [18,19,7]. Onion belongs to the family amaryllidaceae, commonly called bulbs onion the genus *allium* is one of the largest monocotyledons general, it consist of many wild edible species and widely distributed in a temperate zones in the northern hemisphere, the place of origin is far reported to be in central Asia, and the Mediterranean regions are considered to be secondary countries of origin [14,7]. Onion has common names such as, red onion, brown onion, yellow onion, pink onion and White onion [20]. Onion is known as scallion or Green onion in USA, Cabolleta in Spain, Harapyaz in India, spring onion In Australia, batzal, yaroq in Israel, pa in Korea, Tazesoganin turkey, Escallion in Jamaica, Daunbowang in Malaysia, cabolinha in Brazil, Cebolla de verdeo in Argentina, Fruhlingszwiebel in Germany, chives In Caribbean, onion leaves in Bangladesh, shna [21,22].

## 2. MATERIALS AND METHODS

### 2.1 Study Location

Study was carried out in the Department of Histopathology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto, Nigeria.

### 2.2 Experimental Animal

Two (2) experimental Wistar rats were used for this research, which were purchased from animal farm and kept in a metal cage at the animal house of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto.

### 2.3 Animal Sacrifice

The Wistar rats were anaesthetised using chloroform vapour. The liver and kidney organs were carefully isolated and washed with normal saline and then transferred into 10% formalin fixative container.

### 2.4 Procurement of *Allium cepa* Plant

The dry red onion bulb with its skin was purchased at Kasuwar-Kara (Popularly known as

Kara Market) in Sokoto North Local Area of Sokoto and stored in cool and dried place before use.

## 2.5 Identification of *Allium cepa* Plant

Dried onion skin was identified in the Department of Pharmacognosy and Ethno pharmacy, Faculty of Pharmaceutical Sciences of Usmanu Danfodiyo University, Sokoto and was giving a voucher number PCG/ UDUS /Amry/ 0002. It was then kept in the Department of Histopathology School of Medical Laboratory Sciences Usmanu Danfodiyo University, Sokoto, Nigeria to prevent destruction of active ingredient by the sunlight.

## 2.6 Processing and Extraction of Onion Staining Solution

The dried onion skin was grounded into fine powder using a blender (Sonik Japan) the Aqueous and Ethanolic extraction were performed as follows:

### 2.6.1 Extraction using distilled water

To about 5 grams of the powder was dissolved in 100 ml of distilled water and heated on Bunsen burner to boil for 10 minutes. The solution was filtered with filter paper and the filtrate was poured in a plastic container giving 5% aqueous solution.

### 2.6.2 Extraction using distilled water and potassium alum as mordant

About 5 grams of the powder was dissolved in 100 ml of distilled water and heated on Bunsen burner to boil for 10 minutes. The solution was filtered with filter paper and the filtrate was saturated with Potassium Alum which turns greenish red the mordant solution was then poured in plastic container giving 5% aqueous alummordant solution.

### 2.6.3 Extraction using distilled water and ferric chloride as mordant

About 5 grams of the powder was dissolved in 100 ml of distilled water and heated on Bunsen burner to boil for 10 minutes. The solution was filtered with filter paper and the filtrate was saturated with ferric chloride which turns black the mordant solution was then poured in a plastic container giving 5% aqueous ironmordant solution.

### 2.6.4 Extraction using 70% ethanol

About 5 grams of the powder was dissolved in 100 ml of 70% ethanol the extract was allowed to stay for 24 hours at room temperature, the solution was filtered with filter paper and the filtrate was poured in a plastic container giving 5% ethanolic solution.

### 2.6.5 Extraction using 70% ethanol and potassium alum as mordant

About 5 grams of the powder was dissolved in 100 ml of 70% ethanol the extract was allowed to stay for 24 hours at room temperature, the solution was filtered with filter paper and the filtrate was saturated with Potassium Alum which turns greenish red the mordant solution was then poured in a plastic container giving 5% ethanolic alummordant solution.

### 2.6.6 Extraction using 70% ethanol and ferric chloride as mordant

About 5 grams of the powder was dissolved in 100 ml of 70% ethanol the extract was allowed to stay for 24 hours at room temperature, the solution was filtered with filter paper and the filtrate was saturated with ferric chloride which turns black the mordant solution was then poured in a plastic container giving 5% ethanolic ironmordant solution.

## 2.7 Tissue Slide Preparation

The kidney and liver tissues were processed using the standard paraffin wax processing schedule. Sections of 5  $\mu$ m thickness were cut from these blocks using rotary microtome, floated out in warm water bath, picked onto albumenised microscopic glass slide and allowed to fix on the hot plate.

## 2.8 Grouping and Staining Procedure for the Test

### 2.8.1 Grouping and concentration of *Allium cepa* extract as follows:

- A. eosin stain (control)
- B. 5% aqueous extract with ferric chloride
- C. 5% ethanolic extract with potassium alum
- D. 5% ethanolic extract
- E. 5% aqueous extract with ferric chloride
- F. 5% aqueous extract with ferric chloride
- G. 5% aqueous extract with potassium alum
- H. 5% aqueous extract

### 2.8.2 Staining procedure for test

Harris haematoxylin and 12 groups of *Allium cepa* extract were used to stain tissue sections following the method described below:

- Sections were dewaxed in three (3) categories of xylene namely; xylene I, II, and III respectively for 5 minutes each.
- The sections were hydrated in descending grades of alcohol, absolute, I, II, 95% and 75% for 2 minutes each and then to water.
- The sections were stained in Harris haematoxylin for 10 minutes.
- The sections were rinsed in water.
- The sections were differentiated in 1% acid alcohol for few seconds.
- The sections were rinsed in water.
- The sections were counterstained with different concentration of *Allium cepa* for 10 and 20 minutes.
- The sections were dehydrated in ascending grades of alcohol, 70%, 95% and 2 changes of absolute alcohol respectively.
- The sections were cleared in xylene.
- The sections were air dried for few minutes.
- The sections were then mounted with DPX.

### 3. RESULTS

Complete extraction of red onion skin extract gave a dark reddish colour. Addition of mordants changed the colour of the extract to greenish red and black respectively. With ferric chloride as mordant the extract turned black, while the mordanted extract with potassium aluminium alum turned greenish red. Various solutions prepared from the Ethanolic and Alcoholic extract stained sections of Liver and Kidney shades of reddish brown to yellowish brown in 10 and 20 minutes at room temperature, also the warm solutions stained sections for 10 and 20 minutes. Generally the cytoplasm of the tissue sections was stained along with the nucleus with a little optical differentiation, but the staining ability of 5% ethanolic extract was found to be remarkably effective for the connective tissues with ferric chloride mordanted solution thus, showing the best staining ability [7].

The stained slides were examined to determine the staining capability of aqueous and ethanolic extract of mordanted and non-mordanted solutions on the histology of the kidney and liver sections. The cytoplasm of kidney sections stained in shades of pinkish and the nucleus stained in shades of purplish (Fig. 1a) control. Cytoplasm of group B stained in shades of yellowish brown and the nucleus stained in shades of pale yellow when compared with the control (Fig. 1B). Cytoplasm of group C stained in shades of reddish brown and the nucleus stained in shades of pale reddish brown when compared with the control (Fig. 1c). Cytoplasm of group D stained in shades of reddish brown and the nucleus stained in shades of pale reddish brown with little optical differentiation when compared with the control (Fig. 1d). Meanwhile, the cytoplasm of group E was stained in shades of yellowish brown and the nucleus stained in shades of pale yellow brown when compared with the control (Fig. 1E). Cytoplasm of group F stained in shades of reddish brown and the nucleus was stained in shades of pale brown when compared with control (Fig. 1F). Cytoplasm of group G stained in shades of reddish brown and the nucleus was stained in shades of pale brown when compared with control (Fig. 1G). Cytoplasm of group H stained in shades of yellowish brown and the nucleus was stained in shades of pale yellow when compared with control (Fig. 1H). Cytoplasm of group I stained in shades of yellowish brown and the nucleus was stained in shades of pale yellow when compared with control (Fig. 1I). The cytoplasm of group J stained in shades of reddish brown and the nucleus was stained in shades of pale brown when compared with control (Fig. 1J). Cytoplasm of group K stained in shades of yellowish brown and the nucleus was stained in shades of pale yellow when compared with control (Fig. 1K). Cytoplasm of group L stained in shades of reddish brown and the nucleus was stained in shades of pale reddish when compared with control (Fig. 1L). Cytoplasm of group M stained in shades of reddish brown and the nucleus was stained in shades of pale reddish brown when compared with control (Fig. 1M).

The cytoplasm of the liver sections stained in shades of pinkish and the nucleus stained in shades of purplish (Fig. 2A) control. Cytoplasm of group B stained in shades of yellowish brown and the nucleus was stained in shades of pale yellow when compared with the control (Fig. 2B). Cytoplasm of group C stained in shades of reddish brown and the nucleus was stained in

shades of pale reddish brown when compared with the control (Fig. 2C). Cytoplasm of group D stained in shades of reddish brown and the nucleus was stained in shades of pale reddish brown with little optical differentiation when compared with the control (Fig. 2D). Cytoplasm of group E stained in shades of yellowish brown and the nucleus was stained in shades of pale yellow brown when compared with the control (Fig. 2E). Cytoplasm of group F stained in shades of reddish brown and the nucleus was stained in shades of pale brown when compared with control (Fig. 2F). Cytoplasm of group G stained in shades of reddish brown and the nucleus was stained in shades of pale brown when compared with control (Fig. 2G). Cytoplasm of group H stained in shades of yellowish brown and the nucleus was stained in shades of pale yellow when compared with control (Fig. 2H). Cytoplasm of group I stained in shades of yellowish brown and the nucleus was stained in shades of pale yellow when compared with control (Fig. 1I). Cytoplasm of group J stained in shades of reddish brown and the nucleus was stained in shades of pale brown when compared with control (Fig. 2J). Cytoplasm of group K stained in shades of yellowish brown and the nucleus was stained in shades of pale

yellow when compared with control (Fig. 2K). Cytoplasm of group L stained in shades of reddish brown and the nucleus was stained in shades of pale reddish when compared with control (Fig. 2L).

Cytoplasm of group M stained in shades of reddish brown and the nucleus was stained in shades of pale reddish brown when compared with control (Fig. 2M). The cytoplasm of liver sections stained in shades of pinkish and the nucleus was stained in shades of purplish (Fig. 2a) control. Cytoplasm of group G and H stained in shades of reddish brown and the nucleus was stained in shades of pale reddish brown when compared with the control (Fig. 2H and 2I). Cytoplasm of group I and J stained in shades of yellowish brown and the nucleus was stained in shades of pale yellow when compared with the control (Fig. 2J and 2K). Cytoplasm of group K and L stained in shades of reddish brown and the nucleus was stained in shades of pale reddish brown with little optical differentiation when compared with the control (Fig. 2L and 2M).

Photomicrograph of the kidney sections stained with H&E and *Allium cepa* extract.

**Table 1. Identification of Hydrogen iron concentration of various solution of the extract using pH meter**

Mixture of the Extract	pH-value
Aqueous solution of the onion extract	3.11
Aqueous extract solution with potassium alum	1.51
Aqueous extract solution with ferric chloride	1.26
Alcoholic (ethanol) solution in 70% Alcohol	3.66
70% Alcoholic extract solution with potassium alum	1.94
70% Alcoholic extract solution with ferric chloride	0.01

**Table 2. Showing Solution with Mordant and Final colour**

S/N	Aqueous Solution and Mordant	Final colour
1	Aqueous solution Iron for 10minutes	Brown
2	Aqueous solution Iron for 20minutes	Brown
3	Aqueous solution Alum for 10minutes	Brown
4	Aqueous solution Alum for 20minutes	Brown
5	Aqueous solution Without mordant for 10 minutes	Brown
6	Aqueous solution Without mordant for 20 minutes	Brown
7	Ethanollic solution Iron for 10minutes	Yellowish
8	Ethanollic solution Iron for 20minutes	Yellowish
9	Ethanollic solution Alum for 10minutes	Yellowish
10	Ethanollic solution Alum for 20minutes	Yellowish
11	Ethanollic solution Without mordant for 10minutes	Yellowish
12	Ethanollic solution Without mordant for 20 minutes	Yellowish

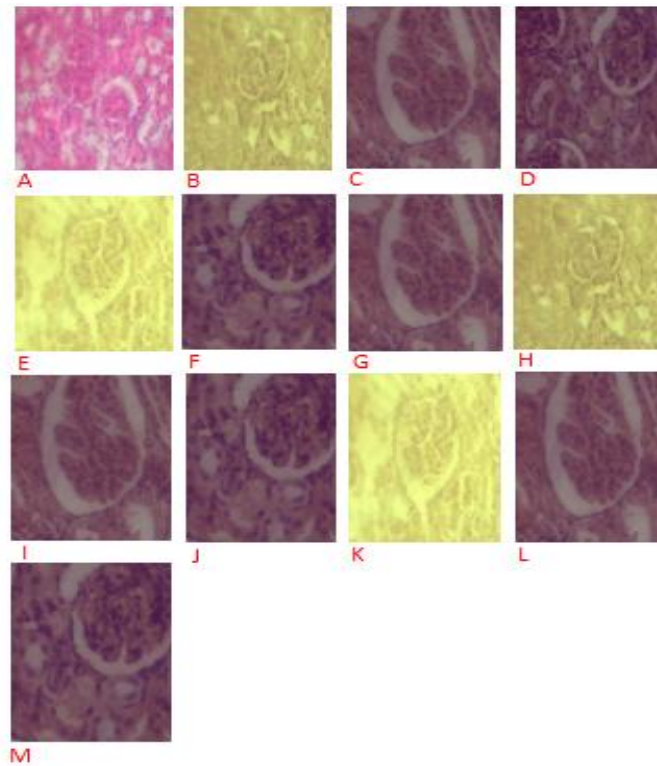
#### 4. DISCUSSION

During staining, dye molecules in stain appear as a certain colour and attached to a specific site or cellular structure. Combination of stains may be necessary to affect certain tissue demonstrated. This goes to show that a dye must ionize in solution to produce coloured cations and anions which are capable of uniting with tissue components to form coloured compounds [23]. Complete extraction of red onion skin extract gave a dark reddish colour. Addition of mordants changed the colour of the extract to greenish red and black This finding was contrast to the work reported by [20] using ferric chloride as mordant the extract turned black, while the mordanted extract with potassium aluminium alum turned greenish red.

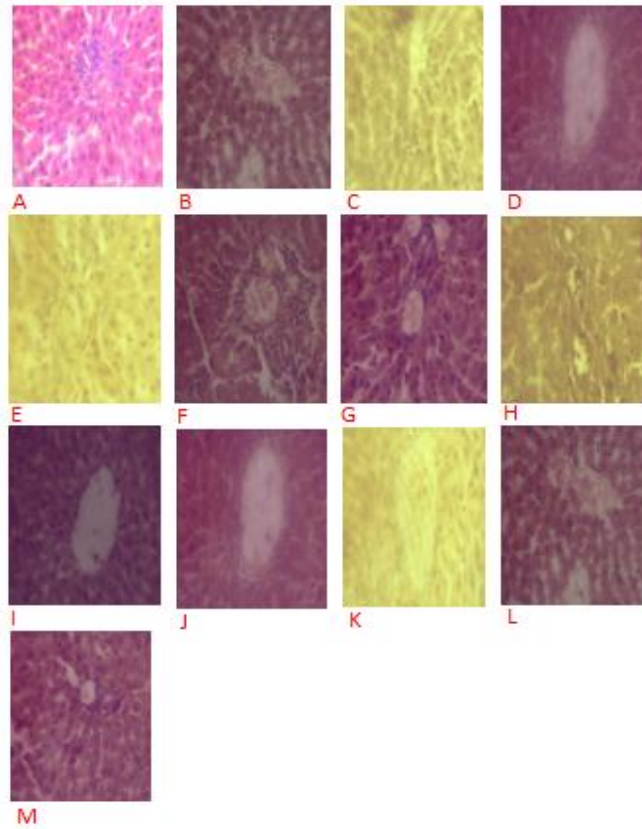
Various solutions prepared from the Ethanolic and Alcoholic extract stained sections of Liver and Kidney shades of reddish brown to yellowish brown in 10 and 20 minutes at room temperature, warm solutions also stained sections in 10 and 20 minutes. Generally the cytoplasm of the tissue sections was stained

along with the nucleus with a little optical differentiation, but the staining ability of 5% ethanolic extract was found to be remarkably impacted on the connective tissues with ferric chloride mordanted solution showing the best staining ability the findings were in line with the reported by [20].

Cytoplasm of group B stained in shades of yellowish brown and the nucleus stained in shades of pale yellow when compared with the control (Fig. 1b). Cytoplasm of group C stained in shades of reddish brown and the nucleus stained in shades of pale reddish brown when compared with the control (Fig. 1c). Cytoplasm of group D stained in shades of reddish brown and the nucleus stained in shades of pale reddish brown with little optical differentiation when compared with the control (Fig. 1d). This findings were in agreement with findings reported by [3,12]. Cytoplasm of group E stained in shades of yellowish brown and the nucleus stained in shades of pale yellow brown when compared with the control (Fig. 1e). Cytoplasm of group F stained in shades of reddish brown and the nucleus stained in shades of pale brown when



**Fig. 1. Cytoplasm of Group B to M stained in shades of reddish brown to yellowish brown. And Group C has the closest morphological resemblance to the control group (Group A)**  
*(Please increase the sizes of the micrograph)*



**Fig. 2. Cytoplasm of Group B to M stained in shades of reddish brown to yellowish brown. And Group J has the closest morphological resemblance to the control group (Group A)**  
*(Please increase the sizes of the micrograph)*

compared with control (Fig. 1f). Cytoplasm of group G stained in shades of reddish brown and the nucleus stained in shades of pale brown when compared with control (Fig. 1g). Cytoplasm of group H stained in shades of yellowish brown and the nucleus stained in shades of pale yellow when compared with control (Fig. 1h). Cytoplasm of group I stained in shades of yellowish brown and the nucleus stained in shades of pale yellow when compared with control (Fig. 1i). Cytoplasm of group J stained in shades of reddish brown and the nucleus stained in shades of pale brown when compared with control (Fig. 1j). Cytoplasm of group K stained in shades of yellowish brown and the nucleus stained in shades of pale yellow when compared with control (Fig. 1k). Cytoplasm of group L stained in shades of reddish brown and the nucleus stained in shades of pale reddish when compared with control (Fig. 1l). Cytoplasm of group M stained in shades of reddish brown and the nucleus stained in shades of pale reddish brown when compared with control (Fig. 1m).

The cytoplasm of liver sections stained in shades of pinkish and the nucleus stained in shades of purplish (Fig. 2a) control. Cytoplasm of group B stained in shades of yellowish brown and the nucleus stained in shades of pale yellow when compared with the control (Fig. 2b). Cytoplasm of group C stained in shades of reddish brown and the nucleus stained in shades of pale reddish brown when compared with the control (Fig. 2c). Cytoplasm of group D stained in shades of reddish brown and the nucleus stained in shades of pale reddish brown with little optical differentiation when compared with the control (Fig. 2d). Cytoplasm of group E stained in shades of yellowish brown and the nucleus stained in shades of pale yellow brown when compared with the control (Fig. 2e). Cytoplasm of group F stained in shades of reddish brown and the nucleus stained in shades of pale brown when compared with control (Fig. 2f). Cytoplasm of group G stained in shades of reddish brown and the nucleus stained in shades of pale brown when compared with control (Fig. 2g). Cytoplasm



of group H stained in shades of yellowish brown and the nucleus stained in shades of pale yellow when compared with control (Fig. 2h). Cytoplasm of group I stained in shades of yellowish brown and the nucleus stained in shades of pale yellow when compared with control (Fig. 2i). Cytoplasm of group J stained in shades of reddish brown and the nucleus stained in shades of pale brown when compared with control (Fig. 2j). Cytoplasm of group K stained in shades of yellowish brown and the nucleus stained in shades of pale yellow when compared with control (Fig. 2k). Cytoplasm of group L stained in shades of reddish brown and the nucleus stained in shades of pale reddish when compared with control (Fig. 2).

Cytoplasm of group M stained in shades of reddish brown and the nucleus stained in shades of pale reddish brown when compared with control (Fig. 2m). The cytoplasm of liver sections stained in shades of pinkish and the nucleus stained in shades of purplish (Fig. 2a) control. Cytoplasm of group G and H stained in shades of reddish brown and the nucleus stained in shades of pale reddish brown when compared with the control (Fig. 2h and 2i). Cytoplasm of group I and J stained in shades of yellowish brown and the nucleus stained in shades of pale yellow when compared with the control (Fig. 2j and 2k). Cytoplasm of group K and L stained in shades of reddish brown and the nucleus stained in shades of pale reddish brown with little optical differentiation when compared with the control (Fig. 2L and 2M).

Agrimedia et al. [21] did appreciable work on the onion skin extract and reported that ethanolic extract of *Allium cepa* stained cytoplasm of cells and connective tissues in shades of reddish brown to yellowish brown. Routine eosin stain of the control was used to compare the cytoplasm staining of other groups (B to M). Eosin dye stained the cytoplasm pink in all the tissues section, and the blue colour of the nuclei was well preserved. Extract of group B to M stained the cytoplasm of all the tissue sections reddish brown to yellowish brown along with the nucleus with little optical differentiation. Both nuclei and cytoplasm therefore appeared beamishly reddish brown to yellowish brown. This may be due to the addition of ferric chloride and potassium aluminium alum. Extract of group B to M stained the cytoplasm excellently well, and preserved the tissue constituents distinctly, though the colour impacted on cytoplasm varies slightly from the control, group A. This variation can be attributed to differences in the extraction solvent,

concentration, mordant used, and ph. While the staining characteristic of the extract have been established in this study, the staining ability of 5% extract in 70% ethanol was found to be remarkably impacted on connective tissues with ferric chloride as mordant showing the best staining ability and may be substituted for eosin in diagnostic histopathology staining.

## 5. CONCLUSION

The histological characteristics of the tissues were well demonstrated details similar to H&E stained sections. The cytoplasm of the tissue appears distinct and well stained along with the nucleus. The use of red onion skin extract as a counter stain when haematoxylin was used as primary stain showed that the dye extract was a good counter stain as it stained connective tissues and cytoplasm well. It may therefore be concluded that *Allium cepa* (red onion) skin extract is a promising histological stain that can serve as a useful stain for histological diagnosis.

## ETHICAL APPROVAL

Animals ethics committee approval has been taken to carry out the research.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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