INTRODUCTION

Fixation is the process by which the tissues are preserved and protected from the process of putrefaction and autolysis. The objective of fixation is to retain cellular components in their respective compartments and to present cells with a distinct and detailed microscopical appearance.\textsuperscript{1,2}

Alexander M. Butlerov, a Russian chemist first discovered Formaldehyde in 1859, and later in 19th century Ferdinand Blum while working on Formaldehyde for disinfection accidentally found its tissue fixative ability. Since then, Formaldehyde is commonly used as 10% Neutral Buffered Formalin (NBF), which the most universally accepted fixative in routine diagnostic pathology. Formalin Fixed Paraffin Embedded Tissue (FFPET) stained with Hematoxylin and Eosin (H & E) has become the “gold standard” in histopathology having an advantage of being quick and cost effective.\textsuperscript{3,4}

Despite of its various advantages, attempts have been made to find other alternatives or to replace Formaldehyde. The major limitations of Formaldehyde as a fixative are as follows:

- During formalin fixation, certain antigenic sites are masked and consequently require antigen retrieval to unmask these antigens. Reagents used during this
procedure have varying pH and can only act at certain temperature which finally influence the immunostaining by producing false staining pattern.\textsuperscript{5}

- With the growing interest in understanding the genetic basis of disease, the ability to extract DNA from FFPET represents a valuable source of diagnostic material that can be used for genomic analysis and translational studies. Fixation by formaldehyde limits the quality & quantity of DNA, as nucleic acids are heavily modified by protein-nucleic acid and protein-protein cross linking. With an increase use of molecular testing in the present clinical scenario this is an another limitation of its use.\textsuperscript{6,7,8}

- In recent years, toxic nature of formaldehyde has been explored and is well documented by the number of International Agencies (European Union, International Agency for Research on Cancer, and World Health Organization). It has been proven as Group 1 carcinogen by International Agency for Research on Cancer. The Occupational Safety and Health Administration (OSHA) regulations declare it hazardous and have advocated its substitute. Repeated exposure or prolonged inhalation of Formaldehyde in occupational settings causes irritation of the mucous membrane (eyes, nose, mouth and upper respiratory tract). The available human-based evidence also indicates that Formaldehyde have a high carcinogenic potential, especially for nasal or nasopharyngeal tumors.\textsuperscript{9,10,11,12}
In order to overcome various limitations of formaldehyde, numerous substitutes are commercially available i.e. alcoholic formalin substitutes (Boon-fix, EthMeth, Kryo Fix, RCL 2, UMFix etc.) and non-alcoholic formalin substitutes (DMS, Glyo-Fixx, HistoCHOICE, Safe Fix II etc.) Various studies have been undertaken in surge of an ideal fixative but till date, there is not even a single fixative that fulfils all the criteria. According to Codex Alimentarius, “Honey is the natural sweet substance, produced by honeybees from the nectar of plants or from secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature.”

Honey is a natural product which constitutes about 200 substances, and is chiefly composed of sugar and water, of which sugar accounts for 95–99% of honey dry matter. The principle carbohydrate constituents of honey are fructose (32.56 to 38.2%) and glucose (28.54 to 31.3 %), others includes many amino acids, vitamins, several minerals, trace elements, enzymes, ascorbic acid, hydrogen peroxide etc. These constituent are responsible for the honey’s anti-oxidant, anti-microbial and anti-autolytic properties. It has the ability to penetrate the deeper tissue, and can also prevent autolysis and putrefaction. It has been widely used for its therapeutic effects i.e. anti-microbial,
anti-viral, anti-inflammatory, anti-oxidant effect, and also in wound healing, gastrointestinal tract & cardiovascular disease, diabetic patients, and certain neoplastic states.\textsuperscript{15}

Honey constitute high quantity of fructose, which at low pH break down to aldehydes and cross-links with the amino acids present in the tissue, which makes it a vital applicant to be used as a fixatives.\textsuperscript{18}

\begin{center}
\begin{tikzpicture}
  \node (A) {Fructose present in Honey};
  \node [below=of A] (B) {\textdownarrow Low pH};
  \node [below=of B] (C) {Breakdown to aldehydes};
  \node [below=of C] (D) {\textdownarrow};
  \node [below=of D] (E) {Aldehyde cross-link with tissue amino acids (Similar to the action of formaldehyde)};
  \node [below=of E] (F) {\textdownarrow};
  \node [below=of F] (G) {Tissue fixation};
\end{tikzpicture}
\end{center}

Being natural, eco-friendly and economical, the present research work has been undertaken to evaluate and explore the tissue fixation ability of Honey, and to determine its role in diagnostic pathology.