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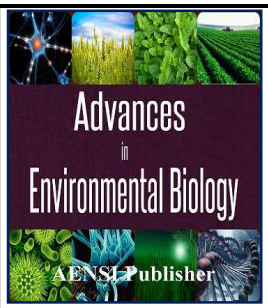

Staining *Leishmania* parasite by using different synthetic food coloring

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ABSTRACT

Background: The method of staining is the quickest and easiest methods used in the diagnosis, especially in the diagnosis of parasites, some of these stains are natural stain and others are chemical ,in recent years increased the use of synthetic food coloring because its more stable property, cheaper prize, high heat resistance, easy availability.**Objective:** The aim of this study is touse synthetic food coloring with different colors (green, red, blue, and yellow) for staining *leishmania* parasite and comparison it with giemsa stain.**Results:**three different concentrations were prepared (100%, 50% and 30%) from each food color and when measuring the pH turns out all the stains were acidic, the results showed that the high concentrations were better in staining parasite also green and red stains were the best, followed by blue and least efficient is yellowstain.**Conclusion:**the results referred to the possibility of using synthetic food coloring for staining *leishmania* parasite and can be using it in research and in laboratories because it its more stable, cheaper, high heat resistance, availability and easy preparation.

KEYWORDS:*leishmania*, giemsa stain, food colorings, natural stain.

INTRODUCTION

Parasitic infection dominate millions of people and cause fearful suffering and mortality especially in the population where arrival to health care is constrained [1]. Human visceral leishmaniasis (VL) is a sever disease geographically wide spread caused by *Leishmaniadonovani* in India, also by *Leishmaniainfantum* in north africa and south europe[2]. Parasite was transmitted by infected *Phlepotomus*spp and flies to humans]3]. Microscopically examination is the common method fordetecting visceral leishmaniasis[4]. In parasitological laboratory, the more delicate identification of parasite is depending on the detection of details of internal structures and morphological features after using synthetic or natural stains like eosin, hematoxylin, romanowsky stain, lugol's iodine, lactophenol cotton blue, giemsa stain, carmine….etc [5]. Giemsa stain was used to detect various parasites including blood pathogen like (*Babesia*spp, *L.donovani*, *Plasmodium* spp, *Trypanosomaspp* and *Microflaria* in thick and thin blood films, *L.donovani* in bone marrow and splenic aspirates, *Toxoplasma gondii* in imprints of brain, lung and other tissues, *Trypanosoma*in lymph node imprints and *Entamoebahistolytica* and *Giardia lambilia* in imprints of gastrointestinal biopsy [6].It differentiates nuclear and cytoplasmic morphology of parasites, WBCs, RBCs and platelets [7]. These traditional stains are expensive and imported substances, some of them may cause irritation, allergic reaction and burning sensation to skin, eye, nose and throat. The exposure of high concentration of iodine vapor can cause chest tightness, sever inflammation, airway spasm and fluid accumulation in the voice box and lungs [8]. Natural dyes extracted from plants, animals and minerals were used as essential staining material in the paint, texture and other industries [9]. Recently many researches were carried out to evaluate the effect of natural dyes to stain different parasites

[10]. Red beet (*Beta vulgaris L.*) extract had been used to stain various types of helminthic parasites[11], herbal dyes also utilized to stain Platyhelminthes[12], hibiscus (*Hibiscus rosa-sinensis L.*) and red beet extracts used to diagnosis ova of intestinal nematodes(*Trichuristrichura* and *Ascarislumbricoides*) [1].

Food colorings are any pigments, dyes or substances that added to food in small quantity during production to preserve flavor or enhance its taste and appearance [13] andin attracting the public for purchasing such products[14]and food colorings are among the important and prefer factors enhancing the goodness of food appearance and in attractive the public on purchasing such product[15],on a worldwide basis, the control of the use of food dyes is based on the Acceptable Daily Intake (ADI), which is based on the results of international research and the recommendations of the Codex Committee on Food Additives and Contaminants (CCFAC) [16].Depending on source, food colorings are either natural or synthetic. Natural colorants are extracted from plant materials, algae, insects, etc, while synthetic colorants are derived from chemical materials (petroleum product) and are used in food, medical devices, cosmetics and pharmaceuticals[17].

The aim of this study is to know the ability of using synthetic food colorings to staining the parasite *L.donovani* and compared it with recommended stain (Giemsa stain).

MATERIALS AND METHODS

Food colorings:

Four differentsynthetic liquid food colorings (Red, Yellow, Blue, Green) were purchased from local market (Surya International, Ahmedabad. India). , three concentrations were prepared for each color (30%, 50% and 100%), by putting (30 and 50) ml in two bottles and complete the volume to 100 ml by adding distilled water then filtered the tow concentration and stock color with Whatman filter paper then collected in clean labeled bottles, also measured pH for all stains by using pH meter and stored at room temperature.

Leishmania strain:

Leishmaniadonovani strain was obtained from department of biology, Al-Mustansiriyah University. put one drop from*Ldonovani* culture on clean dry glass slides and making thin smearing, left to dry then fixed with put one drop from absolute methanol and left to dry till staining.

Staining methods:

Five slides prepare for each stain of different concentration. Slides put in different stains for 15 minutes and five slides colored with giemsa stain in order to compare it with the pigments used in this study. Then washed with distilled water and left to dry, then all slides were examined under light microscope.

RESULTS AND DISCUSSION

L.donovani parasite was respond to food colorings and stained well in comparison with giemsa stain as control stain depending for parasite staining**.** All food colorings (Red, Yellow, Blue, Green) with different concentrations gave a good result with compared with giemsa stain fig (1) shows *Leishmaina*promasitgote staining with giemsa stain nuclei ,cytoplasm and cell membrane . The results showed that the red figure (2) and green figure (3) stains were better than blue stain figure (4) in terms of the intensity of the staining of the parasite figure and the least visible and staining parasite is yellow stain figure (5). Also noted higher concentration of food colorings (100%) stained the parasite more darkly than the lower concentration and gave better staining result, there for it is recommended that for diagnostic purpose that these stains should be used in this concentration. This result was not agreeable with Okolie [18] ,when he used ethanol extracts of *Hibiscus sabdariffa* and *Azadirachtaindica* for staining of ova of intestinal parasites, the lower concentration gave better result than higher concentration, the variance between the staining abilities of higher and lower concentration may be explained by the fact that reduction in the concentration of the stain increases its permeability.

Natural colorants are unsteady and easily degraded during food processing while synthetic colorants show various preferences such as low pollution, high consistency to light, relatively lower production costs, high heat resistance, easy availability, etc. Therefore, in recent years synthetic food colors are used instead of natural colors in many food productions [19, 20].

This result may indicate that the natural stain can distribute through parasite's cell in different degree as Mohanad*et al.* [11] showed that when experimentally red beet (*Beta vulgaris L.*) extract had been used to stain different types of helminthes like *Lecithochiriumacutum*, *Taenia*spp.*Dipylidiumcaninum*and cysticercus cyst for *Taenia*spp. , *Toxascarisleonina*and *Toxocaracanis*and *Neoechinorhynchusiraqensis*, all these stained helminthes were tacked a good coloration with distinction their internal structure. The food colors have been used as natural colorant in food which could be healthier or at least not harmful. The most common artificial food dyes, the azo

dyes, contain aromatic azo compounds such as tartrazine and are widely used. Tartrazine is a synthetic lemon yellow azo dye primarily used as a food coloring [21] .

All used food colorings in this study were acidic substances pH (4.4 – 3.7) because citric acid found within their content. Avwioro [22] reported that the pH degree of the stains determine their ability to staining the tissue structure. Basic structures are stained with acidic stain and the acidic structures are stained with basic stain. In addition to that, the acidity play an active role in inhibiting bacteria and other microorganism's growth, this was agreeable with Emarah*et al*. [23] when used vinegar as a natural dye in staining of some tapeworm specimens and other parasite's eggs ,or for staining different microorganisms such as fungi[24] vinegar kept their shape and their internal structure also provide a safe alternatives to carcinogenic chemicals used in usual staining techniques, because it usually used in human nutrition[25]. After choosing a suitable water as a solvent for dyes because of the lower price the abundance of conducted testing the stability of these pigments to change the degree of heat as pigments showed good stability for use as evidence. Furthermore the synthetic food coloring are cheaper, faster and easier compared with chemical stains.

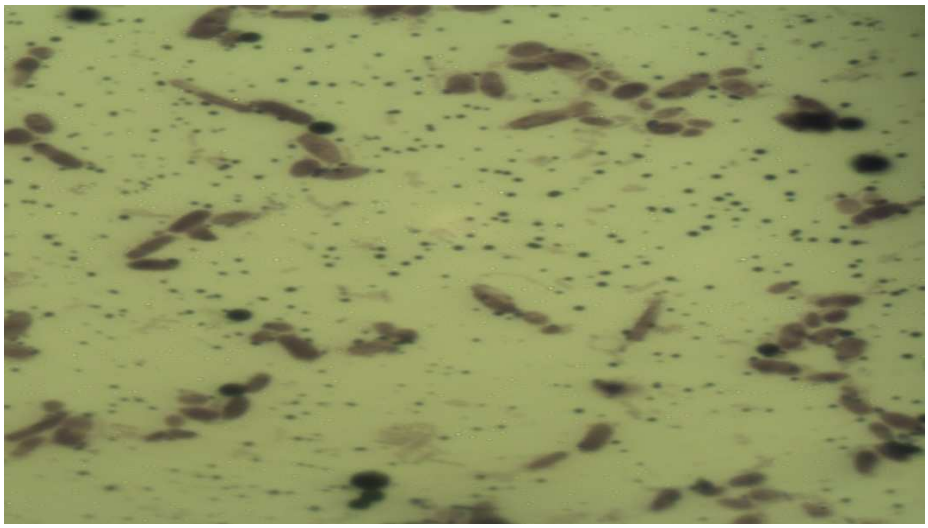

Fig. 1: Stained *L.donovani*promastigote by giemsastain **(100 X).**

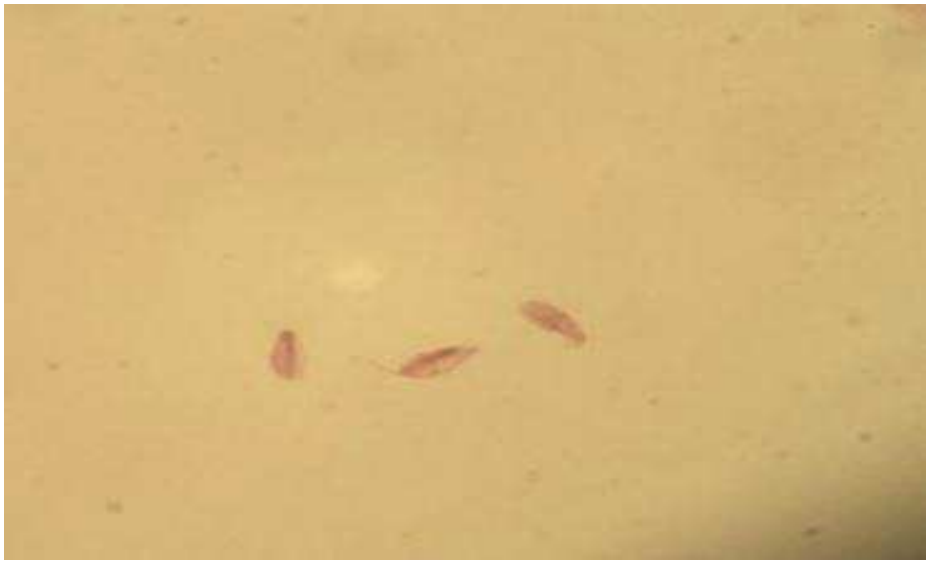

Fig. 2: Stained *L. donovani*promastigotes by red stain (100%) **(100X).**

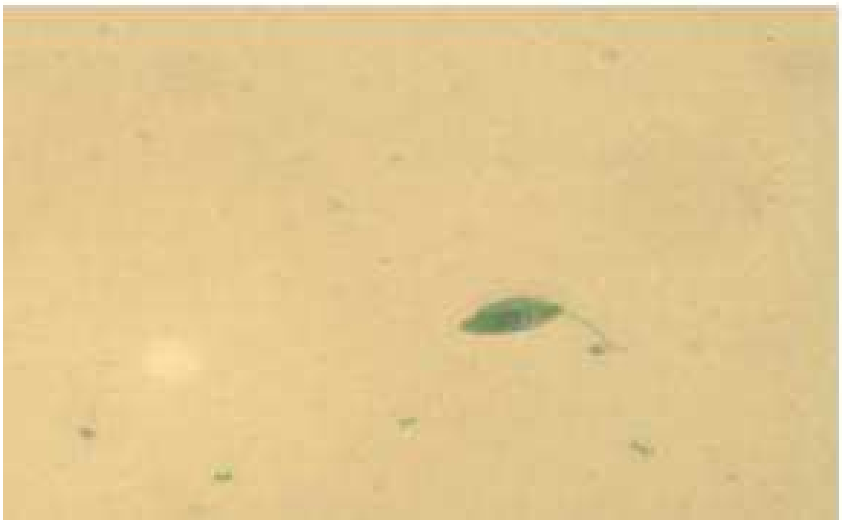

Fig. 3: Stained*L. donovani*promastigotebygreen stain(100%) **(100 X).**

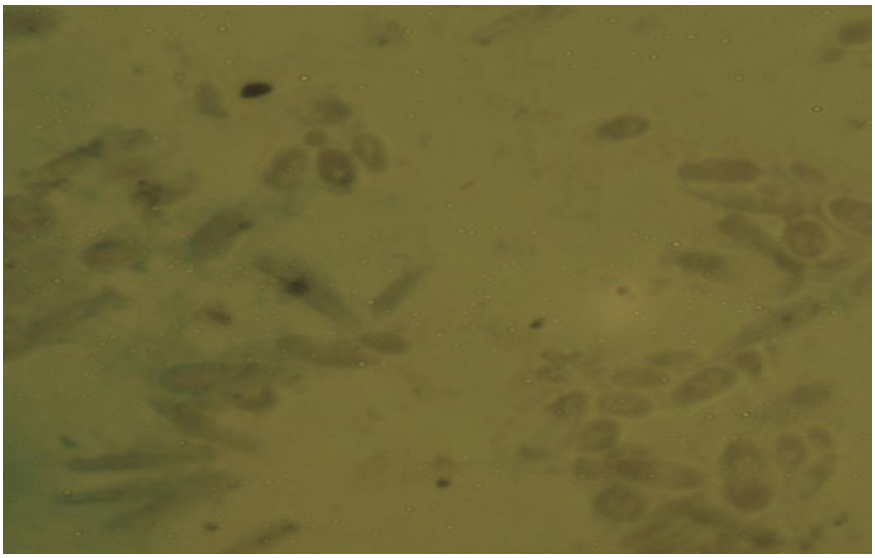

Fig. 4: Stained*L. donovani*promastigote by blue stain(100%) **(100 X).**

Fig. 5: Stained*L. donovani* promastigote by yellow stain(100%) **(100 X).**

Conclusion:

In this present study, we can conclude that synthetic food coloring agood stains for staining leishmania parasite and can be using instead of gimesa stain in research and in laboratories ,also it consider more stable, cheaper, high heat resistance and easy preparation**.**

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