

## COMPARATIVE STUDY OF IN-HOUSE AND MARKETING PREPARATION OF POLY-HERBAL CREAM USED FOR ANAL INFLAMMATION IN MICE

<sup>1</sup>\*Jija Lode, <sup>2</sup>Dindayal Darunde and <sup>3</sup>Sandip Umare

<sup>1</sup>University Department of Pharmaceutical Sciences, Nagpur.

<sup>2</sup>Government College of Pharmacy Amravati.

<sup>3</sup>Sharad Pawar College of Pharmacy Nagpur.

Article Received on  
11 July 2023,

Revised on 01 August 2023,  
Accepted on 22 August 2023

DOI: 10.20959/wjpr202315-29455

### \*Corresponding Author

Jija Lode

University Department of  
Pharmaceutical Sciences,  
Nagpur.

### ABSTRACT

The present study was to compare the in-house and marketed formulations. The cream was formulated using medicinal plants like Til oil (*Seasamum indicum*), Jastamadhu (*Glycerrhiza glabra*), Babool sal (*Acacia Arabica*), Lodhra sal (*Symplocos Racemosa*), Pipal sal (*Ficus Religiosa*), Wat sal (*Ficus bengalensis*), Udumber sal (*Ficus glomerata*), Haldi (*Curcuma/onga*), Madh (MeD, Khas (*Andropogam Muricatus*)). The formulation was studied to compare the efficacy of the in-house and marketed formulation using a suitable animal model. From the biochemical assessment, histopathological study and pharmacological evaluation of the tested formulation, it was found that the marketed formulation exhibited the strongest anti-inflammatory property which was quite comparable to the in-house formulation.

**KEYWORDS:** Polyherbal cream, Anal Inflammation, comparative study, croton oil, Curcuma.

### INTRODUCTION

Conventional pharmaceuticals have remained as the most extreme low-priced and easily accessible source of treatment inside the essential welfare care machine of terrible groups. The nearby community has a long record of conventional plant utilization for therapeutic purposes. In India, however, the most punctual references to the vegetation as cure appear in Rig-Veda, which is said to be composed between 1600-3500 BC. Afterward, the houses and mending use of therapeutic blooms have been examined in component and recorded

experimentally by utilizing noteworthy doctors (an inborn contraption of drugs) that are a crucial establishment of antiquated clinical mechanical know-how in India.<sup>[1]</sup> In the early 1900s, eighty% of all solutions had gotten from roots, barks and clears out and it is imagined that almost 25% of all tablets endorsed nowadays from the beginning of plant life. The plant kingdom, with 300,000 to 400,000 higher species (anticipated degrees reach from 215,000 up to 500,000, was persistently a key supply of later chemical substances for lively pharmaceutical substances and lead compounds. It is anticipated that most compelling five% to 15% of this earthly vegetation has been chemically and pharmacologically examined as systemic mold. Approximately 10,000 to 15,000 of the world's greenery have archived restorative makes utilize of and generally 150-two hundred were coordinates in western cure. Marine plant life like microalgae, microalgae (ocean growth) and blooming plants (together with mangroves) have been examined to a distant lesser volume and are broadly proposed in association with dietary, supplemental or ethno pharmacological employments. For over a long time, the US Countrywide cancer organized has assembled higher blossoms for screening, with the modern-day arrangement composed of ~ 30,000 species. At least importantly, a small percent of those have allegedly been screened for natural or phytochemical side interest until a decade back and gigantic numbers are carelessly being tried for his or her doable pharmacological cost these days. Basically, based on their research, the creators legitimize their presumption that the plant country still holds numerous species containing materials restorative taken a toll and for capacity pharmaceutical bundles, which haven't started to be discovered.<sup>[2]</sup> The torment is serious and taking after bowel development and it can last many minutes to a few hours. The throb is as a result of a fit of the anal sphincter (the inward circle of muscle within the butt-centric canal). Due to the throb, sufferers with this circumstance stress bowel movement and regularly endeavour to keep absent from it. This closes in essentially clogging with harder stools, which in flip increments hurt. Butt-centric gap may be a year or a little split interior the skin at the conclusion of the butt. (Returned passage). The circumstance is also known as gap. It is likely the conclusion result of weight constrained onto the divider of the butt-centric canal both by the section of troublesome stool or straining with a free stool.<sup>[3]</sup> The phrase "haemorrhoids" is inferred from the Greek "hama" = blood, and "rhoos" = streaming, and changed at the beginning, utilized by Hippocrates to supply clarification for them with the stream of blood from the veins of the butt. Ordinarily in society, it's alluded to as heaps. Heaps are a common affliction among grown-ups. More than half of guys and females mature 50 and more will have greater haemorrhoid signs at unspecified times within their future lifetime. Haemorrhoids are exceptional in kids. In any case, nowadays, various

assessments kingdom the frequency of haemorrhoids in children and in matured individuals. In joined together states, 3-sector of people have haemorrhoids in the long run of their lives, and roughly 1/2 of the people over age 50 need treatment, and at a smaller rate, around 4% are in look of logical treatment for the condition.<sup>[4]</sup> Haemorrhoid may be exceptionally common therapeutic feedback. Extrapolated predominance of haemorrhoid in India is and masses utilized for this estimation has been 105, 070,607. These measurements are calculated extrapolations of assorted predominance or frequency costs against the populace of a specific area.<sup>[5]</sup> A cream may be a topical instruction for the skin. Creams for utility to bodily fluid layers, including those of the rectum or vagina, are utilized. Salves can be considered pharmaceutical stock as indeed restorative salves are basically based on techniques created by drug stores and unmediated creams are massively utilized in a spread of skin circumstances (dermatoses). The utilize of the fingertip unit can be accommodating in directing how an entirety topical cream is required to cowl select regions. Creams are semisolid dose bureaucracy containing one or more medicate materials broken up or scattered in an appropriate base. This term has customarily been connected to semisolids that have a shockingly liquid consistency, defined as either water-in-oil or oil-in-water.<sup>[6]</sup>

## METHODS AND MATERIALS

The following plant materials was procured from Nagpur region Maharashtra, India and was authenticated by Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur.

1. Jesthamadhu
2. Babool Sal
3. Lajjavati parna,
4. Lodhar sal,
5. Pipal Sal,
6. Udumbara sal,
7. Wat sal
8. Haldi, Khas

## Preparation of in-house formulation

Take ingredients no 2-9 are weighted accurately and grinded into coarse power prepare extract. Extract mix with in Til oil. Prepare siddha oil, add melt cream base and stir it mix with honey, and add preservative again stir it and fill in suitable container.

**Table 1: Ingredients and its quantity for 100gm.**

Sr.no	Ingredients	Quantity.
1	<i>Til oil</i>	15ml
2	<i>Jesthamadhu</i>	3gm
3	<i>Babul sal</i>	3gm
4	<i>Lajjavati parna</i>	2gm
5	<i>Lodhar sal</i>	2gm
6	<i>Pipal sal</i>	2gm
7	<i>Wat sal</i>	2gm
8	<i>Udumbara sal</i>	2gm
9	<i>Haldi</i>	2gm
10	<i>Madh</i>	1ml
11	<i>Khas</i>	0.1ml
12	<i>Saiojak</i>	q.s.
13	<i>Preservative</i>	q.s.

**Marketed formulation**

The marketed formulation was procured from local drug market, Nagpur. Name of Company- Vaipani Herbal Pvt. Ltd.

Date of manufacturing- March-2015 Date of Expiration- Feb-2018 Quantity-20 gram.

**Pharmacological Evaluation**

Swiss Albino mice male mice weight 20-30 g were obtained from the animal house (Reg. No 92/1999/CPCSEA Date-28/04/1999) (Reg.No. IAEC/UDPS/2016/25.), Department of Pharmaceutical Sciences, R.T.M.N.U. Nagpur and were kept under standard lab condition. The animal were allowed to acclimatize to the environment for 7 days before the commencement of RTNU Nagpur University (date 09/08/ 2016). Grouping of animal was based upon individual studies and the formulation apply locally.

**Aniaml Model<sup>[7]</sup>**

Haemorrhoids were induced to all the groups, except normal control group, by applying croton oil preparation (deionized water, pyridine, diethyl ether, and 6% croton oil in diethyl ether in the ratio of 1 : 4 : 5 : 10). Followed by an overnight fasting, sterile cotton swabs (4mm diameter) soaked in 100  $\mu$ L of croton oil preparation were inserted into the anus (recto anal portion, 20mm from anal opening) of all the study animals and kept for 10 seconds. A linear development of oedema was observed up to 7 to 8 hours after the croton oil application. Quantitative evaluation of croton oil-induced plasma exudation in the recto anal tissue of mice was determined by estimating the quantity of Evans Blue (EB) dye. EB dye (30mg/kg) was

injected through the tail veins of the animals, 30 min before the application of croton oil preparation to induce haemorrhoids. Twenty-four hours after the induction, animals of the respective groups were treated for 5 days. On the fifth day, 1 hour after the relevant treatment, blood samples were collected from retro orbital sinus for estimating the levels of TNF- $\alpha$  and IL-6. All animals were euthanized by exsanguination under deep isoflurane anaesthesia; their recto anal tissues (20mm in length) were isolated and weighed and the EB dye present in the tissue was extracted using 1mL of formamide. The absorbance of the sample was recorded using Synergy HT (multimode microplate reader, BioTek) at 620 nm and quantified using standard curve of EB dye.

**Table 2: Grouping of Animals.**

Group of animals	No of the Animals
Normal group	6
Induced group	6
Standard group	6
In-house group	6
Marketed 1 group	6

#### **Scoring severity of skin inflammation**

To score the severity of inflammation of the back skin, an objective scoring system was developed based on the clinical Psoriasis Area and Severity Index (PASI), except that for the mouse model the affected skin area is not taken into account in the overall score. Erythema, scaling, and thickening were scored independently on a scale from 0 to 4: 0- none; 1- slight; 2-moderate; 3- marked; 4- very marked.

#### **Biochemical analysis**

All the biochemical parameters were studied on the 15<sup>th</sup> operative day, the tissues from each were removed and each tissue was divided into three parts for the following study:

1. Estimation of protein and DNA.
2. Estimation of superoxide dismutase, and lipid peroxidation

#### **Procedures of biochemical estimations**

##### **Estimation of proteins and DNA**

##### **Preparation of homogenate for estimation of protein content**

For estimation of protein, tissue was first homogenized in 5% trichloro acetic acid and then centrifuged. The pellets were then washed with 10% trichloro acetic acid and then again suspended in 5% trichloro acetic acid. It was then kept for 15 min in a water bath maintained at 90°C. The contents were centrifuged and the precipitated proteins were suspended in 0.1 M

Tris-HCl, pH 7.4,<sup>[8]</sup>

### Estimation of protein content<sup>[8]</sup>

**Principle:** It is based on the principle that copper forms a complex with protein (tyrosine major and tryptophan to minor extent) and causes reduction of Folin ciocalteau reagent (phosphotungstic and phosphomolybdic acid) forming blue colour having absorptivity maximum at 600nm.

### Reagents

Alkaline copper reagent Copper sulphate: 20mg/L

Sodium potassium tartrate: 20mg/L Sodium carbonate: 20g/L

Sodium hydroxide: 40g/L

Folin Ciocalteau reagent (FCR): 2N

### Procedure

To 0.5ml of above homogenate, 6.0ml of alkaline copper reagent was added. This was then mixed properly and allowed to stand for 10 minutes. To this 0.5ml of Folin ciocalteau reagent was added with proper mixing and allowed to stand for 30 minutes. Finally, absorbance was taken at 600nm.

Standard curve was prepared by using standard bovin serum albumin in the concentration range of 10, 20, 40, 80 and 160 microgram/ml.

### Estimation of DNA content<sup>[9]</sup>

**Principle:** It is based on the principle that diphenylamine forms a complex with deoxyribose moiety of DNA having absorptivity maximum at 600nm.

### Reagents

Perchloric acid: 0.5M

Burton reagent (Diphenylamine: 15g/Sulphuric acid: 0.25M, Acetaldehyde: 0.05ml/L).

### Procedure

To the tissue sample, enough perchloric acid was added to cover the sample. It was then heated in a water bath for about 90 minutes at temperature between 70-80°C. This was then centrifuged at 300g to remove the cellular debris. To 1ml of the above supernatant, 2ml of Burton reagent was added, mixed properly and allowed to stand for 18 hours at 30°C. Finally,

absorbance was taken at 600nm. Standard curve was prepared following the same procedure by using standard DNA sample in the concentration range of 40, 80, 120, 160 and 200 microgram/ml.

### **Estimation of lipid peroxidation (LPO) Reagents**

Sodium dodecyl sulphate (SDS) 20 % acetic acid solution (pH 3.5)

Thiobarbituric acid n-b utanol and pyridine (15:1, v/v)

### **Procedure**

To 0.2 mL of tissue homogenate, 0.1 mL of 8.1 % SDS, 0.75 mL of 20 % acetic acid solution (pH 3.5) and 0.75 mL of 0.8 % aqueous solution of TBA was added in stoppered tubes. The mixture was made up to 2 mL with distilled water, and then heated in an oil bath at 95°C for 60 min. After cooling with tap water, 0.5 mL of distilled water and 2.5 mL of mixture of n-butanol and Pyridine (15:1, v/v) were added and shaken vigorously. After centrifugation at 3000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured against blank containing 0.2 mL of distilled water in place of sample.<sup>[10]</sup>

### **Histopathological studies**

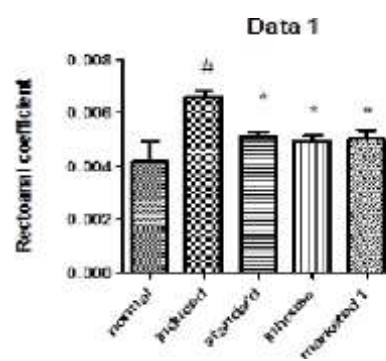
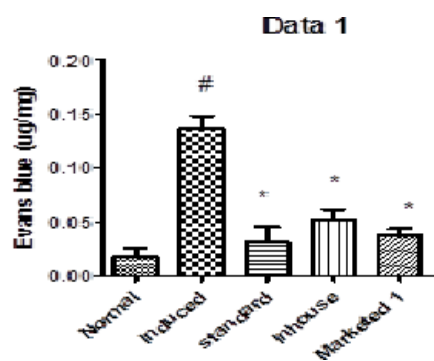
On the 5<sup>th</sup> operative day, the animals were anaesthetized, and tissue sampling was done. The tissues were immediately blotted, dried and fixed in formalin (10%), then dehydrated in acetone and embedded in paraffin wax for taking sections (4µm thickness) with the help of microtome. The transverse sections of the tissues were taken. The sections were then stained with haematoxylin-eosin and processed for photo microscopic examination using Nikon Trinocular Microscope.<sup>[7]</sup>

### **Statistical analysis**

The experimental results are expressed as mean  $\pm$  SEM, with six animals in each group followed by one-way analysis of variance (ANOVA). Newman-keuls test for multiple comparisons was applied for determining the statistical significance between different groups. Graph Pad Prism, version 5 software, was used for all statistical analysis. P values <0.05 were considered to be significant.



## RESULT AND DISCUSSION



**Figure 1: Estimation of Evans Blue. Figure 2: Estimation of Recto anal Coefficient.**

Values are mean  $\pm$  SEM of 6 parallel measurements.

Statistical significant test for comparison was done by ANOVA, followed (n=6). \*P< 0.05vs INDUCED, <sup>#</sup>P<0.05 vs. control

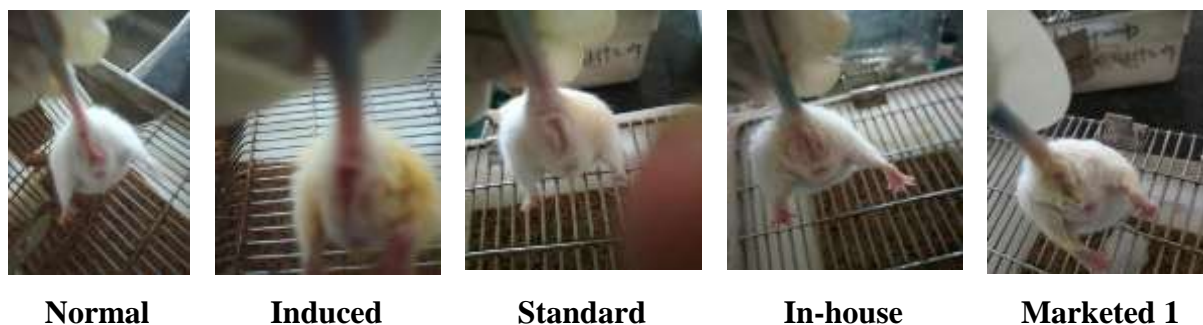
**Table No 3: Biochemical estimation.**

	Normal	Induced	Standard	In-house	Marketed 1
<b>Lipid peroxidation (LPO) (MDA nmol/unit mg of protein)</b>	1.914152 $\pm$ 0.461881	4.134798 $\pm$ 0.701478 <sup>#</sup>	1.683565 $\pm$ 0.653818 <sup>*</sup>	1.774445 $\pm$ 0.521328 <sup>*</sup>	1.781842 $\pm$ 0.51939 <sup>*</sup>
<b>DNA</b>	3.6228 $\pm$ 0.422572 <sup>*</sup>	0.35625 $\pm$ 0.058315 <sup>#</sup>	0.576633 $\pm$ 0.023147 <sup>*</sup>	0.540067 $\pm$ 0.090011 <sup>*</sup>	0.562533 $\pm$ 2.44949 <sup>*</sup>
<b>Protein</b>	1.010742 $\pm$ 0.124166 <sup>*</sup>	0.238472 $\pm$ 0.009518 <sup>#</sup>	0.61658 $\pm$ 2.44949 <sup>*</sup>	0.625733 $\pm$ 0.130098 <sup>*</sup>	0.661992 $\pm$ 2.44949 <sup>*</sup>

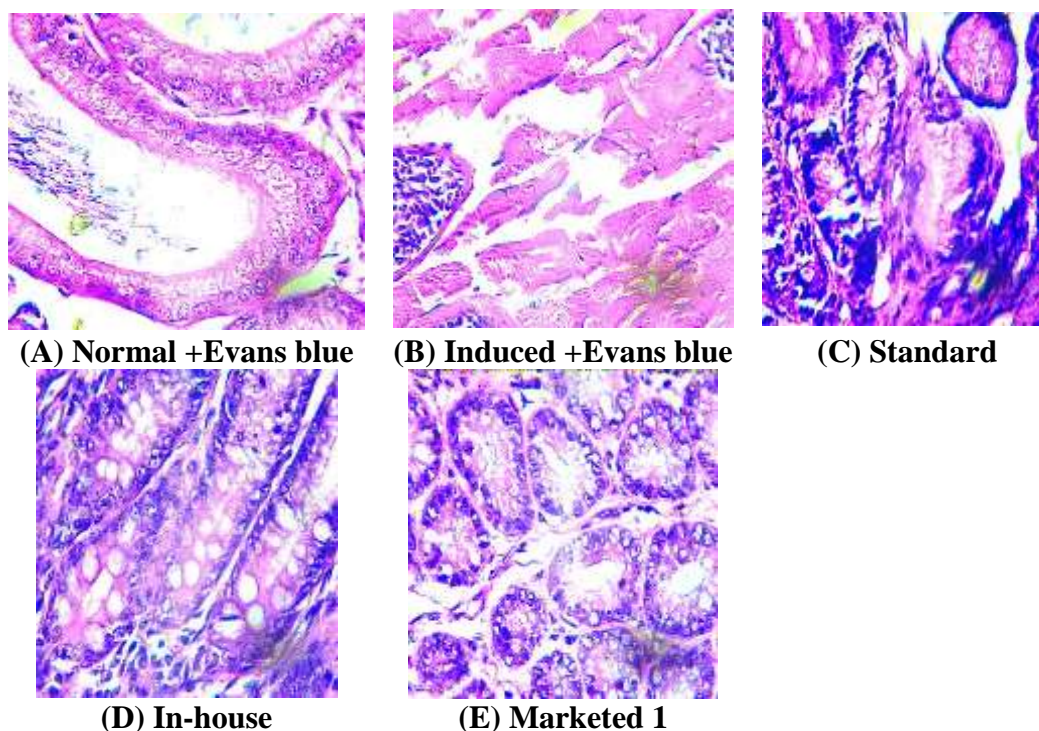
Values are mean  $\pm$  SEM of 6 parallel measurements.

Statistical significant test for comparison was done by ANOVA, followed (n=6). \*P< 0.05vs INDUCED, <sup>#</sup>P<0.05 vs. control.

### Externally Observation.





**Internally Observation****Histopathological observation**

(A) Section showing normal appearance and architecture in normal group.

(B) Marked to severe inflammation, congestion, haemorrhage, dilatation of blood vessels, degeneration, and necrosis can be observed in the section of untreated induced group.

(C) Section of rat treated with Pilex ointment as standard showing mild inflammation, marked congestion, degeneration, and necrosis.

(D) Showing minimal inflammation, congestion, haemorrhage, dilatation of blood vessels, degeneration, and necrosis.

(E) Showing minimal inflammation, congestion, haemorrhage, dilatation of blood vessels, degeneration, and necrosis showing the near normal architecture.

Histopathological evaluations (presence of inflammatory cells) were performed over the inflammatory reactions involved in the development of inflammation due to croton oil

application. The results of present studies showed severe extravasation of EB dye and increased levels of pro-inflammatory enzyme, in experimental animals. The pathological changes were supported by histological changes of the recto anal portion exhibiting severe vasodilatation and inflammatory cells infiltration. In the present study, croton oil has been used as inducer to induce experiment. Croton oil causes inflammation due to the release of soluble factors involving inflammatory lipid metabolites (prostaglandins, leukotrienes and lipoxins), kinins (bradykinins and chemokines), nitric oxide, and cytokines (TNF- $\alpha$  and IL-6) with hypertrophy of the mucosal cells and haemorrhagic spots. Interestingly, 5 days of treatment with inhouse, marketed 1, marketed 2, marketed 3, and standard cream Pilex, have significantly ameliorated the inflammatory hallmarks of croton oil-induced haemorrhoids in mice compared to induced mice. From the literature survey it has been reported that the plants used in formulation such as *Sesamum indicum*, *Glycyrrhiza glabra*, *Acacia Arabica*, *Mimosa pudica*, *Symplocos racemosa*, *Ficus religiosa*, *Ficus bengalensis*, *Ficus glomerata*, *Curcuma longa*, *Andropogon muricatus* have shown potent anti-inflammatory properties and antioxidant. above mention plants have shown the presence of major phytochemical marker like Curcumin, Loturine, and colloturine, Rutin, Friedelin, Teraxosterol, Lupol, B-amyrin, (Julies s. Jurenka 2009, Krishnaveni and Mirunalini, 2010, Mandal and Shete, et al. 2010) they have already reported potent anti-inflammatory activity. Thus the observed fast recovery from anal inflammation may be attributed presence of this phytoconstituents which belong to category flavonoid, polyphenols, tannins, thus from the overall observation we may assumed that marketed 1 and marketed 2 shown maximum protect from croton oil induced haemorrhoid as compare to other formulations. Thus from overall study, we have successfully evaluated physiochemical, phytochemical, chromatographical, pharmacological, potential of different formulation used in treatment of anal inflammation (pile, proctitis, fissure). Thus study may be helpful in presenting standard monograph which may help consumer as well as interested researcher for studying the quality, efficacy and safety of such formulation.

### Conflicts of Interest

The authors have stated that there is no Conflicts of Interest.

### REFERENCES

1. Saleh Hosseinzadeh, Azizollah Jafarikukhdan, Ahmadreza Hosseini Raham Armand. (2015) "Th Application of Medicinal Plant in traditional and modern medicine".
2. Jourgen Krause and Gailene Tobin 21 century.

3. St. Georges (2013) “Anal fissures” department of colorectal surgery.
4. Bharat Gami, “Hemorrhoids-A common ailment among adult, causes & treatment” Academic science International journal of pharmacy and pharmaceutical sciences, 2011; 3(5).
5. Satej Banne, shraddha Nayak, Yogesh Girigaon, Nalinikanta Parida, Mahanta Saleh Hosseinzadeh, Azizollah Jafarikukhdan, Ahmadsreza Hosseini Raham Armand. (2015) “The Application of Medicinal Plant in traditional and modern medicine”.
6. Tanesh Sahu, Tarun Patel, Sagar Shahu, Bina Gidwani “ skin cream as topical drug delivery system” Journal of Pharmaceutical and Biological Sciences, 2016.
7. Mohammed Azeemuddin, Gollapalle Lakshminarayanashastry Viswan, Mohamed Rafiq, Agadi Hire Math Thippeswamy, Mirza Rizwan Baig, Kethaganahalli Jayaramaiah Kavya, Pralhad Sadashiv Patki, and Ramakrishnan Shyam (2014) research article An Improved Experimental Model of Hemorrhoids in Rats: Evaluation of Antihemorrhoidal Activity of an Herbal Formulation.
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.T., Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 1951; 193: 265–276.
9. Burton, K., A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochemical Journal, 1956; 62: 315– 323.
10. Niehaus WG, Samuelson B Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. Eur J Biochem, 1968; 6: 126-130.