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Study the effect of tridax leaf extract on protein histo-chemistry of chick embryo blood vessels

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Abstract
Protein molecules are large and complex biomolecules molecules that play important roles as building blocks in the body. Every function of the body depends on the proteins. *Tridax* procumbens (Linn.) is used as a traditional herbal medicine in rural India for healing of wounds. (SRP Kethamakka *et. al.* 2014), [22] Wound healing is a complex biological process and consists of cellular remodeling; Tridax extract helps in fast wound healing and many cell types are involved and play a very important role in this process. (Manisha Mhaske *et. al*. 2016), [13] The medicinal property of Tridax *procumbence* has inspired the author for the protein histochemical study at vitelline blood vessels of 48 hrs and 96 hrs. chick embryo. (Sneha Mundada *et. al* 2010), [21]. This histochemical study reveals and compares protein content of the blood vessels in control and *Tridax* leaf extract treated samples. The author presented estimation of proteins and histochemical staining techniques of the proteins at tissue level. The author describes the Lowry method for quantitative estimation. (O.H. Lowry et.al.1951), [16] The histochemical protein staining performed by mercury bromophenol blue. The administration of *Tridax plant* leaf extract on developing blood vessels demonstrates the active effect on the blood vessels. The objective of this work is to find out and evaluate the healing effect of leaf extract histochemical and statistical analysis.

Keywords: chick embryo, protein, histochemistry, blood vessel

Introduction
Histochemistry has explored the knowledge of morphological structures and biochemical constituents of tissue, its insight into the function as well. Where it has localized an enzyme, hormone, or other entity of known biological activity to a cell type. Histochemistry has contributed insight into the cell's function (E. Campbelland M. A. Gibson 1971), [9]. The heterogeneity in the content of the biomolecules like proteins, carbohydrates and lipids can be detected very well by histochemistry. On the other hand, histochemistry has suggested a possible role for the constituent related to that of the structure. Proteins are vital macromolecules which perform very important roles in every aspect of life processes. These molecules are encoded in DNA from where transcribed and translated in the specific subcellular organelles and are translocated to their specific location. Some proteins play structural roles and are part of cytoskeleton and become integral part of cell membrane. Some proteins are secreted to the external environment and work as enzymes, and some act as hormones. Protein localization in cells and tissues encompasses the processes that establish and maintain proteins at specific levels. Therefore, analysis of histochemical localization of proteins and their quantification are important aspects of study in biology. P protein staining was performed with mercury bromophenol blue, and its estimation was done by Lowry's method. However, the author made efforts to apply these techniques to study the effect of Tridax leaf extract on the chick embryo blood vessels and collected data of essential protein biomolecule. Therefore, the histochemical effect of *Tridax* leaf extract is studied on the relative concentration and content of proteins. The data include histochemical and concentration details of proteins. This piece of research work was carried out under UGC minor research project, and the objective is to study medicinal property of herbal medicine. (Chandra Pratap Sing et.al.2016), [5].

Materials and Methods
This research work was carried out under UGC minor research project (File No: 47-698/13(UGC-WRO) 2015-2017. The freshly laid fertilized eggs (0 hrs. stage) of *Gallus domesticus* (White Leghorn Strain) were obtained from a poultry farm. The eggs were washed with distilled water and wiped with 70% ethanol and then incubated for 24 hrs. in BOD incubator at 37.50 C with a relative humidity of 70 - 80%. The identification of chick embryonic developmental stage was done. (Hamburger V, Hamilton HL.1951). The chick embryos were treated with 100 µl of distilled water as a carrier solvent control and some of chick embryo were treated with 100 µl of 1 ppm, concentration of Tridax leaf extract. (P. V. Gado et. al 2016), [17]. Treatment was given in Ovo through air sac route at 46 hrs. (HH stage 11-12) and incubated till 96 hrs. (4 days incubation) HH stage 24 and transferred to the incubator at 37.5^0 C. The treated and controlled eggs were manually rotated periodically avoiding yolk and albumin spillage through the incision made for treatment. Isolation of chick embryo vitelline blood vessels

network were used for protein estimation The estimation of protein content of tissue sample of 0.170 mg was carried out by Lowry metho (O. H. Lowry et. al 1950), [16]. The doses of *Tridax* extract were given to developing embryo; blood vessels containing tissues were separated from embryo after treatment and fixed in Conroy's fixative. After fixation the tissues were transferred in absolute alcohol for dehydration, then cleaned in xylene & embedded in usual manner; the blocks were cut at 7 microns. Then de-paraffinized slides were transferred into the absolute alcohol. Then stained in mercury Bromo-phenol blue for 30 mins. to 2 hrs. Then transferred in 1% aqueous acetic acid for 5 mins. After washing in 1% aqueous acetic acid were cleaned in xylene and mounted in DPX. The dried slides containing sections were observed under microscope and photographs were taken to maintain record.

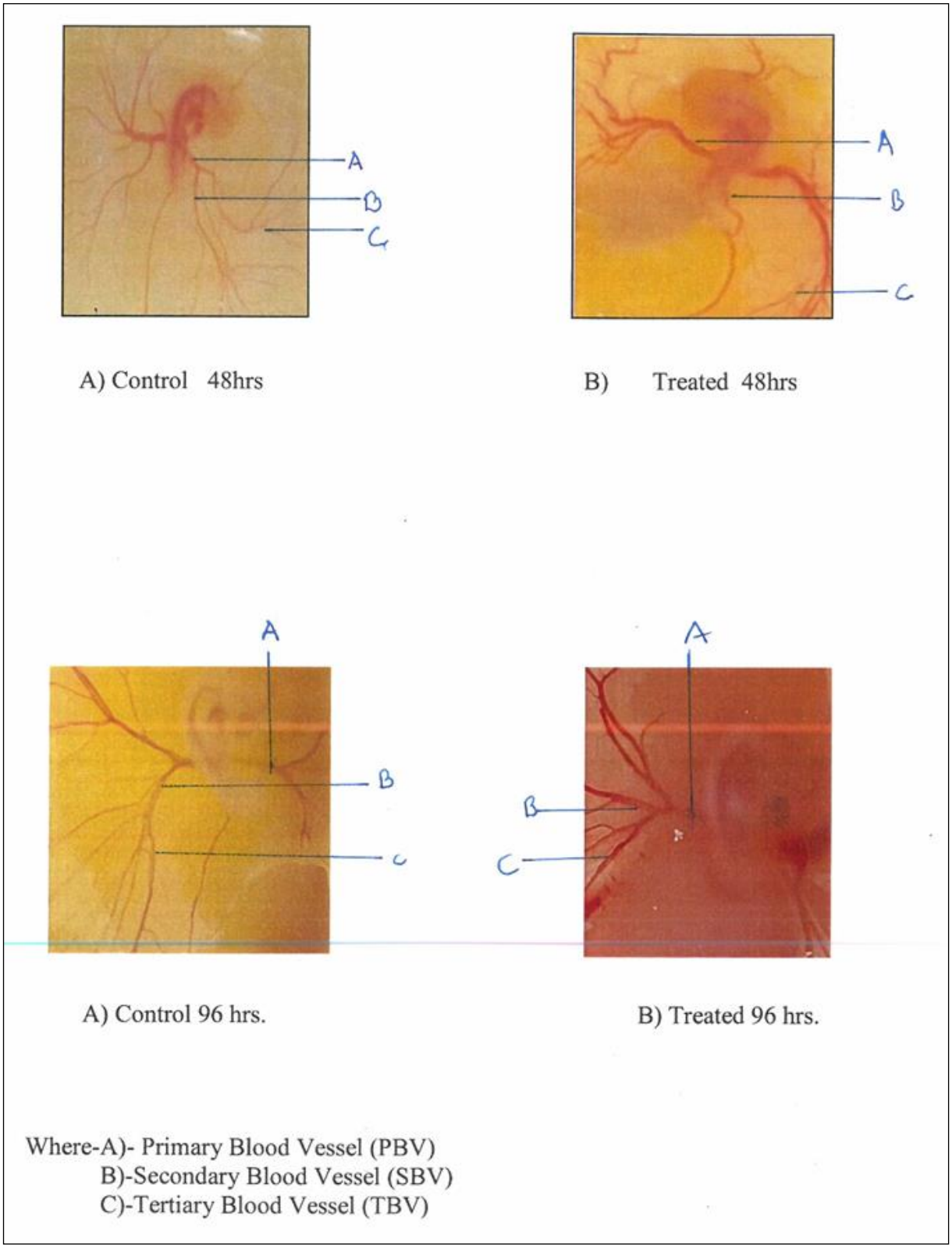

Plate 1: Whole mount of embryo

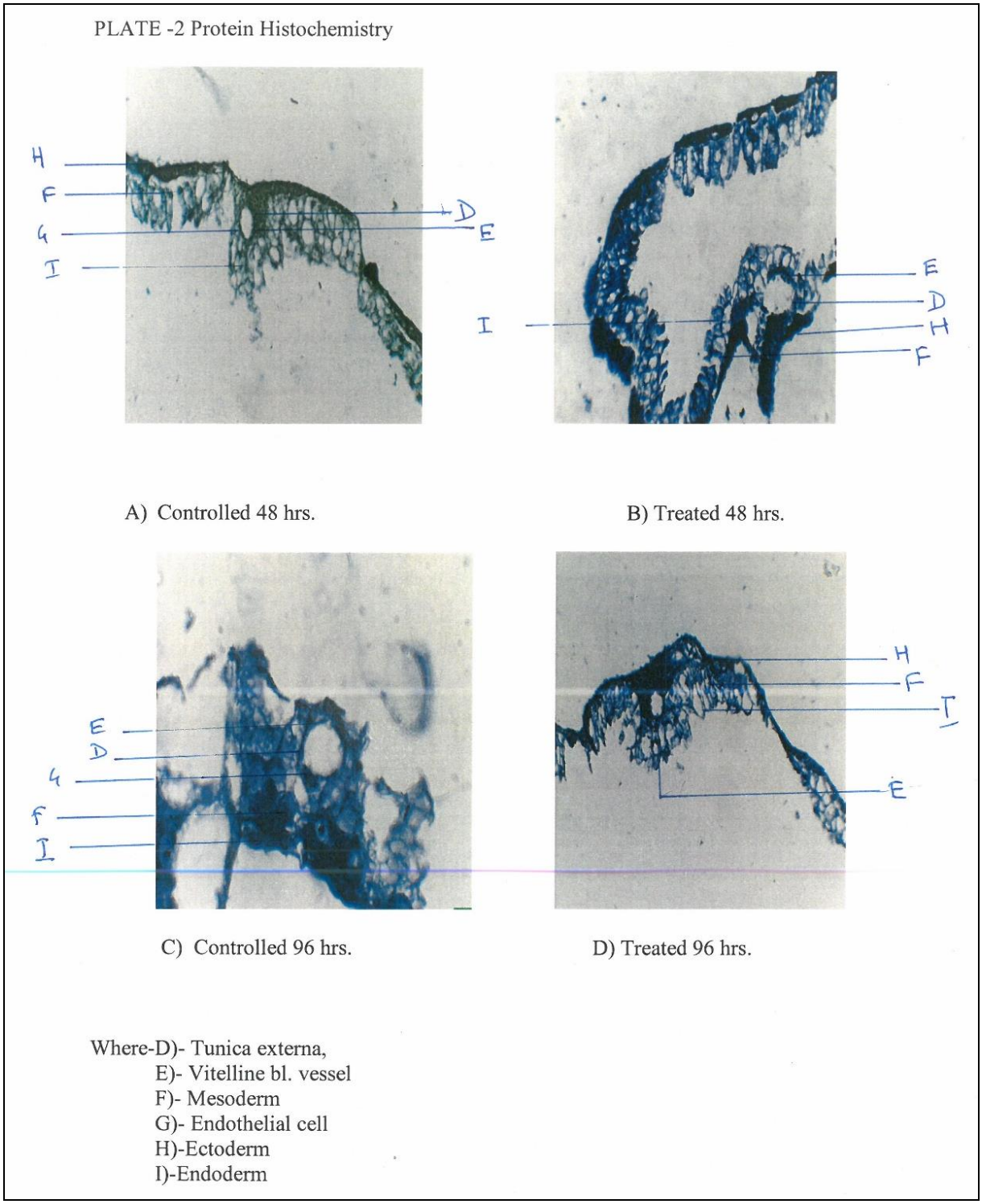

Plate 2: Protein Histochemistry

Table 1: Protein estimation

Sr. No.	Tissue	Incubation	Estimated protein content
1	Control	48hrs	0.060 mg/ 0.170 mg of tissue
2	Tridax extract	48hrs	0.063 mg/ 0.170 mg of tissue
3	Control	96hrs	0.072 mg/0.170 mg of tissue
4	Tridax extract	96hrs	0.078 mg / 0.170 mg of tissue

Results and Discussion

Microscopic observations of embryo whole mount of HH 24 (4.0 Days) show that the interatrial septum has fused with the dorsal and ventral endocardial cushions as they fuse to divide the atrioventricular canal. As the interatrial septum fuses to the cushions, it obliterates the interatrial communication, foramen primum, but at the secondary interatrial communication in the form of multiple perforations develops in the middle portion of the

interatrial septum allowing continued shunting of blood from the right to left atria, thus bypassing the non-functional pulmonary system. The growth of the heart and its expansion of the ventricular bend and the primitive atria have led to the loss of the original tubular character of the heart. The late phase of cardiac looping has begun (HH 24–34), which corresponds to the phase of cardiac septation. (Waldo KL. *et. al*. 2001), [24]

In the *Tridax* extract treated embryo showing that *Vasoturm* (vessels of the vessels) and their capillaries are not uniform, i.e., question mark shaped embryo with some broadness at cephalic region, vitelline veins right and left have a normal shape to that of the control. It also shows some curves in venation on the right and left side of embryo. In controlled, the Primary Blood Vessel (PBV) are normally developed with proper dilated manner and tapering further. The Secondary Blood Vessel (SBV) and Tertiary Blood Vessel (TBV) also showed normal growth pattern like PBV. (Deshpande *et. al* 2018), [6].

In treated, the Primary Blood Vessel (PBV) are normally developed with proper dilated manner and tapering further. The Secondary Blood Vessel (SBV) and Tertiary Blood Vessel (TBV) also show some irregular shape. (Manisha Mhaske 2016), [13]. In the controlled tissues of protein histochemistry tunica externa of vitelline blood vessel is thin fibrous connective tissue layer, the middle layer of tunica media is also thickened with some smooth muscle layer to that of the tunica externa, the inner layer is of longitudinally oriented cell lining of the lumen thickened. Overall thickness with normal lumen is observed. In the treated tissues of protein histochemistry tunica externa, ectoderm of vitelline blood vessel is thick fibrous connective tissue layer, the middle layer of tunica media, mesoderm, is also more thickened with some smooth muscle layer to that of the tunica externa, the inner layer endoderm, is of longitudinally oriented flattened cell of endothelial cells lining of the lumen thickened. Overall thickness in blood vessel section with narrow lumen is observed.

The estimated protein content by Lowry method in controlled tissue in 48 hrs. incubated embryonic tissue is 0.060 mg and in 96 hrs. tissue it is 0.063mg. In treated tissue in 48 hrs. incubated embryonic tissue is 0.072mg and in 96 hrs. tissue it is 0.078mg. Therefore, it may be concluded that the protein content is slightly elevated because of Tridax leaf extract. (Waterborg J.H. 1994), [23].

Conclusion

The developing embryo showed normal growth with control and the Tridax leaf extract treated blood vessels, the angiogenesis is well developed. Histochemical structure is normal with normal intensity of blue color that stained the protein content. The *Tridax* extract treated embryo showed some more protein content in histological section and more blue color indicating that some more protein content is detected, which may be the response to *Tridax* extract. Due to reduction of the lumen of the vessels sealing of wound takes place very fast at a microscopic level.

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Etihical Approval

As per the university standard University Grants Commission (WRO) Pune, approved and gave financial assistance for this research project. (File No: 47-698/13(UGC-WRO)

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Competing Interests

Authors have declared that no competing interest exists.

Authors' Contributions

This work was carried out at Department of Zoology Fergusson College (Autonomous) Pune under UGC minor research project. The author read and approved the final manuscript

References

1. Bhagwat DA, Killedar SG, Adnaik RS. "Antidiabetic activity of leaf extract of Tridax procumbens". Intnl. Journal Green Pharma,2008:2:126-128.
2. Bob G, Sanders. Kimberly Kline "Expression of serum proteins in the developing chick embryo"ELSEVIER Comparative Biochemistry and Physiology Part B: Comparative Biochemistry,1977:58(1):97-101. https://doi.org/10.1016/0305-0491(77)90133-X
3. Bouman HG, Broekhuizen LA, Baasten AM, Gittenberger-de Groot AC, Wenink AC. Stereological study of stage 34 chicken hearts with looping disturbances after retinoic acid treatment: disturbed growth of myocardium and atrioventricular cushion tissue.J. *Anat Rec*.,1972:48(2):242-250. doi: 10.1002/(SICI)1097-0185(199706)248:2<242::AID-AR11>3.0.CO;2-P.

4. Carolina de Souza Guerra, Yamba Carla Lara Pereira, João Paulo Mardegan Issa, Kelly Galisteu Luiz, Elaine A, Del Bel Guimarães *et al*. "Histological, Histochemical, and Protein Changes after Induced Malocclusion by Occlusion Alteration of Wistar Rats" Bio Med Research International, 2014. | Article ID 563463 | https://doi.org/10.1155/2014/563463
5. Chandra Pratap Singh, Pawan Kumar Mishra, Surya Prakash Gupta. "Design and Formulation of Tridax procumbens based Polyherbal Cream for Wound Healing Potential" Scholars Research Library Der Pharmacia Lettre,2016:8(12):15-21. ISSN 0975-5071 USA CODEN: DPLEB4
6. Deshpande Kale, Bhonde Datar. "Establishment of an in Ovo chick embryo yolk sac membrane (YSM) assay for pilot screening of potential angiogenic and anti-angiogenic agents". J.Cell Biology International,2018:42(11):1474-1483.
   DOI: 10.1002/cbin.11051
7. D Ribatti, A Vacca, L Roncali, Dammacco F. The chick embryo chorioallantoic membrane as a model for in *vivo* research on angiogenesis. Int. J. Dev. Biol.,1996:40:1189-1197.
8. Postma DS, Logue S, Pecorak JB. Prazma J. "Histochemistry of glycogen in the inner ear" Springer, The Histochemical Journal,1978:10:53-61. https://doi.org/10.1007/BF01003414
9. Campbell E, Gibson MA. A histological and histochemical study of the development of the pineal gland in the chick, *Gallus domesticus* Canadian Journal of Zoology, 1970. https://doi.org/10.1139/z70-225
10. Eram Fauzia, Tarun Kumar Barbhuyan, Amit Kumar Shrivastava, Manish Kumar, Paarth Garg, Mohsin Ali Khan *et al*. "Chick Embryo: A Preclinical Model for Understanding Ischemia-Reperfusion Mechanism" Front. Pharmacol., 2018, 1-12. https://doi.org/10.3389/fphar.2018.01034
11. Hamburger V, Hamilton HL. "A series of normal stages in the development of the chick embryo". Pub Med Dev Dyn.,1951, 1992:195(4):231-72. doi: 10.1002/aja.1001950404.
12. Kishor Dhanji Pendharkar. "Study of *Tridax* leaf extract on chick embryo vitelline blood vessels" Uttar Pradesh Journal of Zoology,2021:42(22):47-51.
13. Manisha Mhaske, Ghanshyam Gonjari. "Antiangiogenic Effect of Tridax procumbens (L.) Leaf extract by chick chorio-allantoic membrane (CAM) Assay; International Journal of Innovative Research in Science, Engineering and Technology,2016:5:1140-1149.
14. Manner J, Perez-Pomares JM, Macias D, Munoz-Chapuli R. The origin, formation and developmental significance of the epicardium: a review. J.*Cells Tissues Organs.*,2001:169(2):89-103. doi: 10.1159/000047867
15. Morse DE, Rogers CS, McCann PS. "Atrial septation in the chick and rat": a review. *J Submicrosc Cytol*.,1984:16(2):259-72.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. "Protein measurement with the Folin phenol reagent" J Biol Chem.,1951:193(1):265-275. PMID: 14907713
17. Gado PV, Salokhe SG, Deshpande SG. "Biochemical changes induced by Bio neem (0.03%) formulation in chick embryogenesis (Gallus domesticus)" International Journal of Environmental & Agriculture Research,2016:2(10):151-161.
18. Quiring DP. The development of the sino-atrial region of the chick heart. J Morphol.,1933:55:81-118. https://doi.org/10.1002/jmor.1050550106
19. Ramesh Petchi R, Vijaya C, Parasuraman S. "Anti-arthritic activity of ethanolic extract of Tridax procumbens (Linn.) in Sprague Dawley rats." Pub Med: Pharmacognosy Res.,2013:5(2):113-117. Apr-Jun; 5(2): 113–117. doi: 10.4103/0974-8490.110541
20. Salahdeen HM, Yemitan OK, Alada AR. "Effect of aqueous leaf extract of Tridax procumbens on blood pressure and heart rate in rats". African Journal of Biomedical Research,2004:7:27-29:7.
21. Sneha Mundada, Ruchi Shivhare. "Pharmacology of Tridax procumbens a weed: Review" Int. J. of Pharm Tech Research,2010:2(2):1391-1394.
22. Kethamakka Meena SRP, Deogade S. "Jayanti Veda (Tridax procumbens) Unnoticed Medicinal plant by Ayurveda Journal of Indian System of Medicine,2014:1:6-20.
23. Waterborg JH, Matthews HR. Methods The Lowry method for protein quantitation. Mol Biol.,1994:32:1-4. doi: 10.1385/0-89603-268-X:1.
24. Waldo KL, Kumiski DH, Wallis KT, Stadt HA, Hutson MR, Platt DH et al. "Conotruncal myocardium arises from a secondary heart field". Development,2001:128(16):3179-3188. doi: 10.1242/dev.128.16.3179.
25. Yanli Liu Jinghui, Zhou Bello, odinga Musa, Hayat Khawar Xin Yang Yuli Cao Xiaojun Yang. "Developmental changes in hepatic lipid metabolism of chicks during the embryonic periods and the first week of post hatch" ELSEVIER Poultry Science,2020:99(3):1655-1662. https://doi.org/10.1016/j.psj.2019.11.004
26. Yuji Yokouchi. "Establishment of a chick embryo model for analysing liver development and a search for candidate genes Development, Growth, differentiation", The Japanese Society of Developmental Biology,2005:47(6):357-366. https://doi.org/10.1111/j.1440-169X.2005.00812.x