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Comparative study of Hematological variation in healthy and fungal infected Kalabans, *Bangana dero* (Hamilton, 1822)

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Introduction

Hematological studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fishery management disease investigations (Mulcahy, 1975; Sampath *et al*., 1993). However, fishes can adapt themselves according to the environmental conditions by changing their physiological activities up to optimum range but in extreme change in water quality i.e. dissolved oxygen level, free carbon dioxide level and hardness, pH, total dissolved solids and presence and virulence of pathogens can change the hematological parameters than normal values or control.

Qualitative and quantitative variations in haematological parameters including the red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), erythrocyte sediment rate(ESR) are significant findings for the determination of anemia, polycythemia, inflammation and infection while total white blood cell numbers (WBC) and differential leukocyte count viz, neutrophils, lymphocyte, eosinophils and monocyte are significant findings for the determination in pathological condition viz. leucocytosis and leucopenia (Eroh *et al*., 2003; Shah *et al*., 2009).

The analysis of blood indices has proven to be a valuable approach for analysing the health status of farmed animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani *et al*., 2001). Blood biochemistry parameters can be

also used to detect the health of fish (De Pedro *et al*., 2005). Exogenous factors, such as management (Svobodova *et al*., 2008), diseases (Chen *et al*., 2005) and stress (Cnaani *et al*., 2004), always induce major changes in blood composition. For example, significant fluctuations were detected in the concentrations of cortisol, glucose, cholesterol and other basic components in response to handling and hypoxic stress (Skjervold *et al*., 2001). The levels of cortisol and glucose are considered to be specific indicators of sympathetic activation during stress conditions **(Lermen *et al*., 2004**; Tekmedash *et al.*, 2014). *Abramis brama orientalis* were examined by (Hayatbakhsh *et al*., 2014) for evaluation changes of haematological parameters regarding to parasitic infection, physical damages, morphological modifications, growth retardation, hematological changes, immune response disturbance, endocrine disruption, and behavioral changes. Cortisol and glucose are two of the most common stress indicators. Cortisol may be useful only in acute stress experiments and monitored throughout time.

Bangana dero (Hamilton, 1822), a common fish of food value in the Indian Himalayan region was targeted for the study. Saprolegniosis is very common in this species at all life stages under coldwater condition and captive rearing. It was estimated that 12-20% loss has been occurred due to mycosis, directly as mortality and indirectly as growth retardation and stress condition to the fish. This species is commonly known as 'Kalabans' in India, 'Gardi' and 'Kathalegi' in Nepal, 'Kursa' in Bangladesh and widely distributed all along foot hill regions of Himalayan ranges. The body is ordinarily white and more linear having relatively small head and maximum size ~~is~~ has reported around 750 mm in total length. *B. dero* is a bottom feeding herbivorous (Masuda and Karki, 1980) and is a candidate species for aquaculture in mid altitudinal hills. *B. dero* (Ham.) is of immense economic value in the Uttarakhand state and is found almost throughout the year in the rivers Khoh, Nayar, Bhagirathi and Alaknanda of the Himalayan riverine ecosystems. Though, the faunistic studies and biology of this fish has been done by earlier researchers (Hora and Mukherjee, 1936; Lal and Chatterjee, 1962; Singh, 1964; Grover, 1971), hematological study is not yet to be reported. Attempt has been done to explore the hematological profile of wild and cultured stock of *B. dero* and its correlation with the very common saprolegniosis infection.

Materials and methods

40 Healthy and infected specimen of *B. dero* with the average weight of 470±12 g were collected during May-June, 2017 from the Directorate of Coldwater Fisheries Research (DCFR) farm in Bhimtal, Kosi and Ramganga river of Kumaon hills of Uttarakhand in India. Blood samples were taken from 10 healthy and 10 infected fish from each stock and hematological parameters of triplicate samples (3 times, 40 samples in each sampling) were measured.

Blood samples were collected from the caudal vain using sterilized disposable 2-ml syringes. Total erythrocyte count (TEC) and total leukocyte count (TLC) were determined in a human hemocytometer crystalline chamber using diluting fluids. Sahli's hemoglobinometer was used to estimate hemoglobin (Hb). Packed cell volume (PCV) was estimated using microhematocrit. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to (Reddy and Bashamohideen, 1989). Plasma cortisol was measured by RIA. Plasma glucose (mMol/L. serum) was assayed by analysis commercial kit through colorimetric method using an Auto-analyser Technician. Statistical analysis was done using one-way ANOVA at a 5% level of significance.

Result and discussion

Blood samples collected form 10 healthy and 10 infected fish from each wild and cultured stock were analysed in triplicates. The result was summarized in the Table 1. The fungal infection was characterized as saproligniosis with the observation of elongated tapering zoosporangia formed at the tips of somatic hyphae which were appearing darker and more granular having pear shaped primary zoospores. The data reveal that Hb and TEC were significantly lower in infected farmed and wild fish while TLC was significantly higher in infected stock in wild as well as in cultured stock. Similarly, there was a significant difference between infected and non-infected *B. dero* in terms of cortisol, glucose which was measured as stress indicator.

Table 1: Changes in hematological parameters (means±SD) of *Bangana dero* due to *Saprolegnia* sp infection (n = 20).

Parameters	S 1	S 2	S 3	S 4
Hemoglobin (Hb; %g)	5.65 ±0.22	4.12 ±0.27*	5.24 ±0.08	4.23 ±0.11*
Total erythrocyte count (TEC; 106/mm^3)	1.56 ±0.07	1.32 ±0.05*	1.44 ±0.04	1.20±0.073*
Total leukocyte count (TLC; 103/mm^3)	16.3 ±0.4	18.4 ±0.1*	16.36 ±0.56	19.2±0.360*
Packed cell volume (PCV; %)	30.63 ±0.35	24.35 ±0.39	30.65 ±0.80	22.34±0.417
Mean corpuscular volume (MCV; μm^3)	196.59 ±7.86	183.72 ±7.12	212.98 ±7.92	185.62±12.00
Mean corpuscular hemoglobin (MCH; pg)	36.28 ±2.10	31.07 ±2.32	36.44 ±0.81	35.2±3.014
Mean corpuscular hemoglobin concentration (MCHC; %)	18.44 ±0.54	16.9 ±0.88	17.12 ±0.62	18.93±0.640

* Significantly different from the healthy $p<0.05$

*S 1- Wild healthy, S 2- Wild infected, S 3- Farmed healthy, S 4- Farmed infected

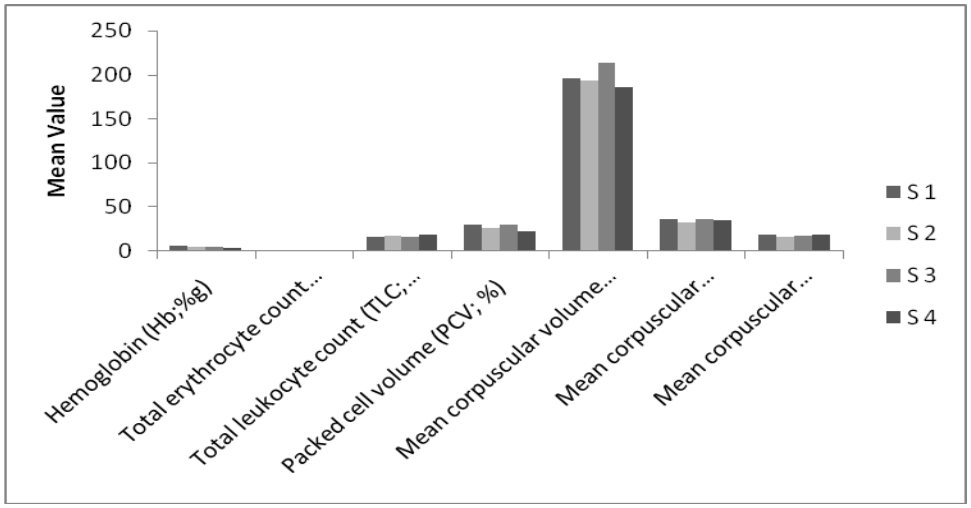

Figure 1: Changes in hematological parameters of *Bangana dero* due to *Saprolegnia* sp infection.
***S 1- Wild healthy, S 2- Wild infected, S 3- Farmed healthy, S 4- Farmed infected**

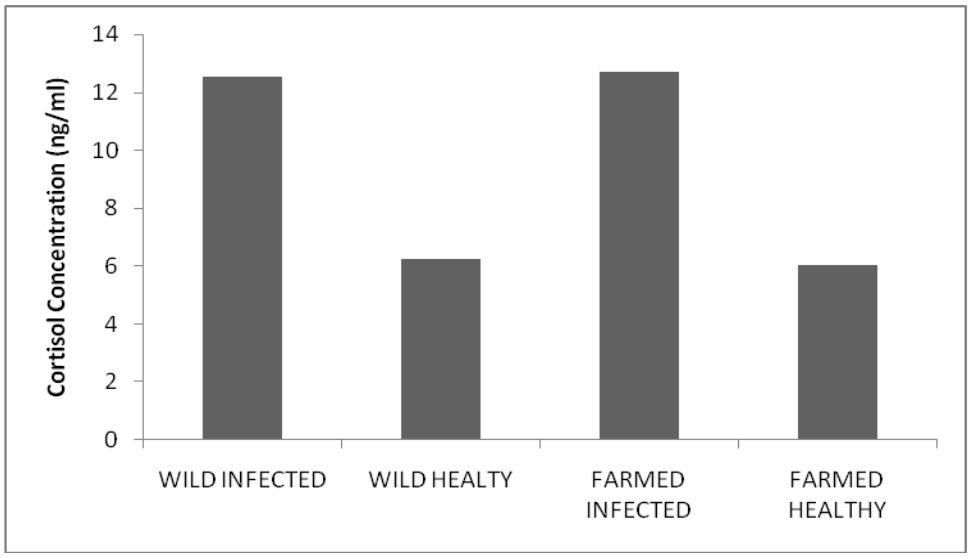

Figure 2: Comparison of cortisol concentration between healthy and infected wild and farmed *Bangana Dero.*

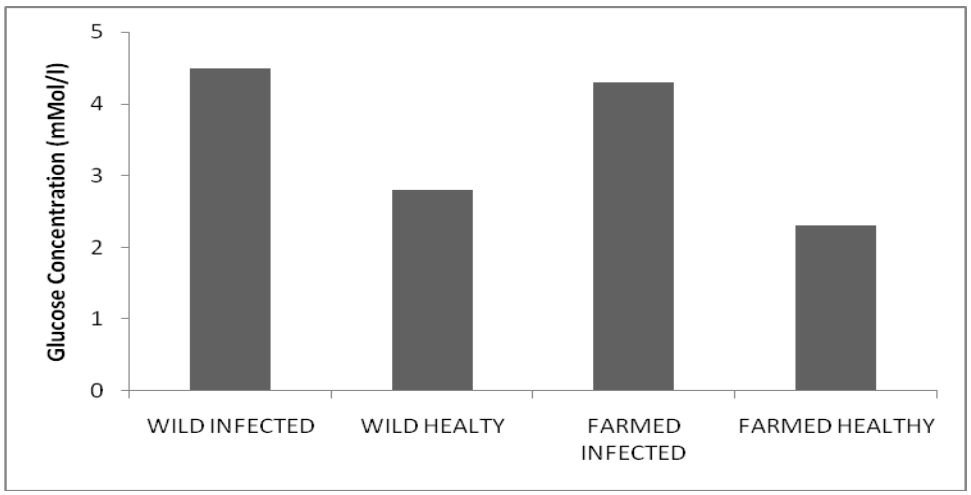

Figure 3: Comparison of glucose concentration between healthy and infected wild and farmed *Bangana dero.*

The quality and quantity of leukocytes are generally used to determine immune reactions and diseases (Cagirgan and Yildirim, 1990). The immune systems of fish display a similar response to unfavorable conditions (Palikova and Navratil, 2001) and findings were similar in Caspian salmon (*Salmo trutta caspius*) infected with *Saprolegnia* (Jamalzadeh *et al*., 2009). Leukocytes are important cells in the immune system because of their main defensive function. Leukocytes are normally lower in healthy fishes than in infected fish, and can be used as an indicator of infectious disease. In present study, the increasing trend of leukocytes supports previous findings having significant lower values in non infected healthy fish of wild and cultured stocks. Due to the fungal infection, the leukocyte counts were enhanced, indicating that fish can develop a defensive mechanism to overcome the stress caused by infection. Hence, the increase in leukocytes from the normal range ie. 16.33±0.45 is an indicator of fungal infection.

In the present study, *B. dero* blood cells were characterized microscopically and hematological indices were analyzed. The mature erythrocytes of *B. dero* show an average size and ultrastructural features similar to those described for mature erythrocytes of other fish species (Watson *et al*., 1963; Hartman and Lessler, 1964; Conroy, 1972; Blaxhall and Daisley, 1973; Javaid and Akhtar, 1977; Nakamura and Shimozawa, 1984; Rowley *et al*., 1988; Groff and Zinkl, 1999; Esteban *et al*., 2000; Hrubec *et al*., 2000; Ueda *et al*., 2001). Thrombocytes are the most abundant blood cells after erythrocytes, representing more than 50% of circulating leucocytes (Ueda *et al*., 1997). However, some authors do not include thrombocytes within leucocytes (Hrubec *et al*., 2000, 2001; Orun and Erdemli, 2002; Ranzani-Paiva *et al*., 2003).

The ranges of serum biochemistry vary from species to species and can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food feed, age and sex of the fish (Jawad *et al*., 2004). The lower erythrocyte counts in the infected *B. dero* were similar in Caspian salmon (Jamalzadeh *et al*., 2009). This reduction can be caused by a lack of sufficient oxygen due to fungal infestation of the gills and can lead to greater destruction of erythrocytes or a lower rate of formation of erythrocytes because of a lack of Hb in the cellular medium (Chen *et al*., 2004).

The higher values of cortisol and glucose were observed in infected fish compared to healthy individuals which indicates the stressful condition to infected fish and are in the conformity of the previous report (Tekmedash *et al*., 2014).

The significance of observing PCV is to evaluate the effect of stressors on fish health and to determine the oxygen carrying capacity of the fish blood. MCV, MCH, and MCHC are completely dependent on the levels of PCV, TEC, and Hb in the blood. In the present study, PCV, TEC, and Hb

concentrations were altered due to fungal infection and calculated values are comparatively lower in the infected fish. These measurements clearly reflect the diseased condition of fish in wild as well as in pond environment. The increasing trend of leukocytes and decrease in other parameters could be a useful diagnostic tool for saprolegniosis infection in cultivable and wild fish species.

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Reference

Bahmani, M., Kazemi, R. and Donskaya, P., 2001. A comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). *Fish Physiology* and *Biochemistry*, 24, 135-140.

Blaxhall, P.C. and Daisley, K.W., 1973. Routine haematological methods for use with fish blood. *Fish Biol*ogy, 5, 771-781.

Cagirgan, M.I. and Yildirim, M.B., 1990. An application of factor analysis to data from control and macro mutant populations of 'Quantum' barley. *Journal Faculty Agric*ulture, 4, 125-138.

Chen, X., Yin, D., Hu, S. and Hou, Y., 2004. Immunotoxicity of penta chlorophenol on macrophage immunity and IgM secretion of the crucian carp (*Carassius auratus*). *The Bulletin of Environmental Contamination* and *Toxicology*, **73**, 153-160.

Chen, Y.E., Jin, S. and Wang, G.L., 2005. Study on blood physiological and biochemical indices of *Vibrio alginolyticus* disease of *Lateolabrax japonicas*. *Journal of Ocean Tai. Str*, 24, 104-108.

Cnaani, A., Tinman, B., Advidar, Y., Ron, M. and Hulate, G., 2004. Comparative Study of Biochemical parameters in response to stress in *O. aureus*, *O. Mossambicus* and two strains of *O. Silaticus. Aquaculture*, 35, 1434-1440.

Conroy, D.A., 1972. Studies on the haematology of the Atlantic salmo (*Salmo salar*). *Zoological Society of London*, 30, 101–127.

De Pedro, N., Guijarro, A.E., Lopez-Patino, M.A., Marinez-Alvarez, R. and Delgado, M., 2005. Daily and seasonal variation in hematological and blood biochemical parameters in touch, *Tinca Yince. Aquaculture,* 36, 85-96.

Eroh, P.O., Adonye, C.C. and Okhamafe, A.O., 2003. Response of trypanosome brucei brucei– induced anaemia to a commercial herbal prepration. *African Journal of Biotechnology*, 2(**9**). **307-311.**

Esteban, M.A., Munoz, J. and Meseguer, J., 2000. Blood cells of sea bass (*Dicentrarchus labrax* L.). Flowcytometric and microscopic studies. *The Anatomical Record*, 258(**1**), 80-89.

Groff, J.M. and Zinkl, J.G., 1999. Hematology and clinical chemistry

of Cyprinid fish. Common carp and goldfish. *Veterinary Clinics of North America: Exotic Animal Practice*, 2(**3**), 741–746.

Grover, S.P., 1971. Some biological notes on *Barilius bendelisis* (Ham); *Indian Journal of Fisheries*, 18, 182-183.

Hamilton, F., 1822. An account of the fishes found in the river Ganges and its branches. Edinburgh and London.: i-vii + 1-405, pp. 1-39.

Hartman, F.A. and Lessler, M.A., 1964. Erythrocyte measurements in fishes amphibia, and reptiles. *Biological Bulletin*, 126, 83-88.

Hayatbakhsh, M.R., Khara, H., Movahed, R., Sayadborani, M., Rohi, J.D., Ahmadnezhad, M., Rahbar, M. and Rad, A.S., 2014. Haematological characteristics associated with parasitism in bream, *Abramis brama orientalis*. *Journal of Parasitic Diseases*, 38, 383-388.

Hora, S.L. and Mukherjee, D.D., 1936. Fishes of the Eastern Doons, United Provinces; *Records of the Indian Museum*, 38, 133-146

Hrubec, T.C., Cardinale, J.L. and Smith, S.A., 2000. Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* hybrid). *Veterinary Clinical Pathology*, 29(**1**), 7-12.

Hrubec, T.C., Smith, S.A. and Robertson, J.L., 2001. Age related in haematology and chemistry values of hybrid striped bass chrysops *Morone saxatilis*. *Veterinary Clinical Pathology*, 30(**1**), 8–15.

Jamalzadeh, H.R., Keyvan, A., Ghomi, M.R. and Gherardi, F., 2009. Comparison of blood indices in healthy and fungal infected Caspian salmon (*Salmo trutta caspius*). *African Journal of Biotechnology*, 8(**2**), 319-322.

Javaid, M.Y. and Akhtar, N., 1977. Haematology of fishes in Pakistan. II. Studies on fourteen species of teleosts. *Biologia*, 23, 79–90.

Jawad, L.A., Al-Mukhtar, M.A. and Ahmad, H.K., 2004. The relationship between haematocrit and some biological parameters of the Indian shad, *Tenualosa ilisha* (Family Clupeidae). *Animal biodiversity and conservation*, 27(**2**), 47-52.

Lal, M.B. and Chatterjee, P., 1962. Survey of Eastern Doon fishes with certain notes on their biology*; Journal Zoological Society of India*, 14, 203-243.

Lerman, C.L., Lappe, R., Crestani, M., Vieira, V.P., Gioda, C.R., Schetinger, M.R.C., Baldissertto, B., Moraes, G and Morsch, V.M. 2004. Effect of different temperature regimes on metabolic and blood parameters of silver catfish Rhamdia quelen. *Aquaculture*, 239, 497-507.

Masuda, K. and Karki, K. B., 1980. Fish and fisheries of the river. A report on the survey of the Trisuli River conducted in 1979. *Fisheries Development Section*, HMG, Nepal.

Mulcahy, M.F., 1975. Fish blood changes associated with diseases. A haematological study of Pike lymphoma and salmon ulcerative dermal necrosis. In: Ribelin, W. E and Migaki, G (ed). The *Pathology*

of Fishes, **Univ Wisconsin Press, Madison. 925-944.**

Nakamura, H. and Shimozawa, A., 1984. Light and electron microscopic studies on the leucocytes of the medaka. *Medaka*, 2, 15–22.

Orun, I. and Erdemli, A.U., 2002. A study on blood parameters of *Capoeta trutta* (Heckel, 1843*). Journal of Biological Science,* 2(**8**), 508–511.

Palikova, M. and Navratil, S., 2001. Occurrence of *Anguillicola crassus* in the water reservoir Korycany (Czech Republic) and its influence on the health condition and hematological indices of eels. *Acta Veterinaria Brunensis*, 70, 443-449.

Ranzani-Paiva, M.J.T., Rodrıguez, E.L., Veiga, M.L., Eiras, A.C. and Campos, B.E.S., 2003. Differential leucokocyte counts in "dourado", *Salminus maxillosus* Valenciennes, 1840, from the Mogi-Guac¸u River, Pirassununga, SP. Braz. *Journal of Biology*, 63(**3**), 517–525.

Reddy, M.P. and Bashamohideen, M., 1989. Fenvalerate and cypermethrin induced changes in the haematological parameters of Cyprinus carpio. *Acta Hydrochimica et Hydrobiologica*, 17, 101-107.

Rowley, A.F., Hunt, T.C., Page, M. and Mainwaring, G., 1988. Fish. In: Rowley, A.F., Ratcliffe, N.A. (Eds.), Vertebrate Blood Cells. Cambridge University Press, Cambridge, pp. 19–127.

Sampath, T.K., Rasha, K.E., Doctor, J.S., Tucker, R.F. and Hoffman, F., 1993. Drosophilia transforming growth factor beta superfamily proteins induces endochondral bone formation in mammals. *Proceedings of the National Academy of Sciences, USA*, 90, 6004–6008.

Shah, A.W., Praveen, M., Mir, S.H., Sarwar, S.G. and Yousuf, A.R., **2009.** Imapact of helminth parasitism on fish haematology of Anchar Lake, Kashmir. *Pakistan Journal of Nutrition,* 8(**1**)**,** 42-45.

Singh, P.P., 1964. Fishes of the Doon vally; *Ichthyologica,* 3, 86-92.

Skjervold, P.O., Fjæra, S.O., Østby P.B. and Einen, O., 2001. Live-chilling and crowding stress before slaughter of Atlantic Salmon (*Salmo salar*). *Aquaculture*, 192, 267-282.

Svobodova, Z., Kroupova, H., Modra, H., Flajshans, M., Randak, T., Savina, L.V. and Gela, D., 2008. Haematological profile of common carp spawners of various breeds, *Journal of Applied Ichthyol*ogy, 24, 55-59.

Tekmedash, F.S., Hemmatzadeh, M. and Khara, H., 2014. In press. Stress indices of Grass carp, *Ceteopharyngodon idella*, (Cuvier and Valenciennes, 1884) change in response to monogenean parasites pollution, *Gyrodactylus* spp. and *Dactylogyrus* spp. *Journal of Parasitic Disease*, pp. 1–3.

Ueda, I.K., Egami, M.I., Sasso, W.S. and Matushima, E.R., 1997. Estudos hematol´ogicos em *Oreochromis* (*Tilapia*) *niloticus*. (Linnaeus, 1758) (Cichlidae, Teleostei)—Part I. Braz. *Journal* of *Veterinary Research and Animal Science,* 34(**5**), 270–275.

Ueda, I.K., Egami, M.I., Sasso, W.S. and Matushima, E.R., 2001. Cytochemical aspects of the peripheral blood cells of *Oreochromis* (Tilapia) *niloticus*. (Linnaeus, 1758) (Cichlidae, Teleostei)—Part II. Braz. *Journal* of *Veterinary Research and Animal Science*, 38(**6**), 273–277.

Watson., Gordon, R.S., Karmen, A. and Jover, A., 1963. The absorption and excretion of castor oil in man. *Journal of Pharmacy* and *Pharmacology*, 15, 183-188.