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Research Article

Effects of Synthetic Hormones on Fertilization, Fecundity, Hatching and Gonado-Somatic Index of Giant Snakehead (*Channa Marulius*) in Captivity

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Abstract

The effects of a crude protein (40% CP) diet on the spawning performance of giant Snakehead (*Channa marulius*) broodstock were assessed in this study through a three-week feeding trial. Mature *C. marulius* brood stock (N = 36) was taken from the brooders pond and stocked into nine small experimental breeding ponds, each measuring 4mx2mx1.5m (LxWxD). Following acclimatization, all male and female *C. marulius* broodstock with an average weight of 1.5–2.5 kg BW and a length of 70 cm were removed from the small breeding ponds. The current study's objective was to induce breeding in *C. marulius* by injecting synthetic hormones intramuscularly and monitoring fertilization, fecundity, hatching, and gonado-somatic index. Using a completely randomized design (CRD), nine fishponds were split into three treatments (T_1, T_2, and T_3) during the study period, each with three replicates. In addition to evaluating the effects of artificially applied hormones, Conceptal® (T_1), Suprefact® (T_2), and Ovaprim® (T_3), this study aimed to comprehend the reproductive biology of *C. marulius*. The hormones were injected into the test fish at the following proportion (0.3ml, 0.4ml and 0.5ml to male and 0.8ml, 0.9ml and 1.0ml to female's ml/kg body weight) and then released into the earthen ponds for large scale production of seed. Two-way analysis of variance (ANOVA) was used. The results showed no successful spawning on Conceptal®. The gonado-somatic index (GSI) estimates for the study period showed that Suprefact had the highest average GSI values for both males and females (3.32±1.62% and 1.67±0.18%, respectively), followed by Ovaprim (1.13±0.56 and 1.22±0.68, respectively). Absolute fecundity was also estimated. The result showed that fish stimulated with suprefact (T_2) obtained the highest average fecundity (3079.3±100.7%), fertilization rate (96.33±1.20%), hatching rate (94.67±2.40%), and survival rate (95.75±1.51%), then in ovaprim fecundity rate (1669.3±836.5%), fertilization rate (58.00±29.02), hatching rate (61.27±30.65), survival rate (64.67±32.34%), respectively. In conclusion, the use of Suprefact® and Ovaprim® can optimise the results of *C. marulius* breeding induction in small experimental breeding ponds, ensuring higher-quality eggs and a greater number of normal larvae.

Keywords: *Channa marulius*, induced breeding, synthetic hormones, fecundity rate, hatching rate

1. Introduction

The snakehead *Channa marulius* is widely distributed in the natural water bodies of Pakistan, India, Bangladesh, Thailand, and Vietnam. With its high market demand as a food source and its delectable flavor, it is a fish of substantial economic value, boasting fewer intramuscular spines than most fish and

rich nutritional content [1,2]. Its preferred habitat is still, muddy bodies of water like lakes, ponds, marshes, canals, rivers, and marshes. These aggressive predators mostly hunt live prey; adult snakeheads eat invertebrates, small fish, and frogs, while hatchlings and fry eat zooplankton and small insect larvae.

The nutritional aspect plays a pivotal role in the realm of intensive aquaculture, significantly impacting production costs, fish growth, overall health, and waste management. Throughout the various stages of fish development, including, gonadal development, spawning, fecundity, hatching, and larval growth, the provision of optimal nutrition to broodstock is paramount for success [3,4,5,6,7,8].

The most vital nutrients for fish reproduction are proteins, amino acids, fats, carbohydrates, vitamins, and minerals, all of which are found in fish diets [9,10,11,12]. Dietary protein levels and feeding rates are receiving more attention as important aspects of broodstock nutrition [13, 14]. Reproductive performance can be negatively impacted by food restriction and nutritional deficiencies [15]. The effects of dietary protein on the reproductive parameters of female broodfish in a variety of fish species, such as Nile tilapia (*Oreochromis niloticus*), swordtails (Xiphophorus helleri), bagrid catfish (Mystus nemurus), and rohu (Labeo rohita), have been the subject of numerous studies [13,16,17, 18]. Notably, the main components of egg yolk which are essential for fish embryonic development are proteins and lipids. For this reason, maintaining proper broodstock nutrition is essential to obtaining high seed and ideal breeding performance.

For aquaculture production to be sustained through the domestication of wild fish, a thorough understanding of fish reproduction in captivity is essential. When used properly, treatment with synthetic hormones can improve spermiation and ovulation as well as increase hatchery productivity. In the context of induced breeding, synthetic hormones are administered to stimulate ovulation, enhance spawning responses, and achieve higher fertilization and hatching rates [19,20]. Commercially available synthetic inducing hormones, such as Ovaprim®, Suprefact®, Ovopel®, and Aquaspawn®, are gaining popularity due to their proven effectiveness in fish spawning. Ovaprim, ovatide, suprefact, and HCG have all been effectively used as inducing agents in *Channa striatus* [21, 22, 23], *C. marulius* [24], and *Channa punctatus* [25] to effectively induce ovulation and spawning in murrels. A synthetic form of salmon GnRH, the natural peptide present in most teleost fish, is found in Ovaprim®. It has a dopamine inhibitor as well. By considerably accelerating maturation without compromising viability or fecundity, this can enhance and synchronise maturation in treated fish. A synthetic peptide analogue of the luteinizing hormone-releasing hormone (LHRH) agonist, buserelin is found in Suprefact®, a commercial product that stimulates the pituitary gland's production of the gonadotrophin-releasing hormone receptor [26,27,24]. To optimize the adoption of breeding techniques, the hormone needs to be effective, affordable, and easily accessible in an acceptable quantity.

There is limited research on induced breeding and seed production of *C. marulius*, which hinders its cultural potential in Pakistan. Developing region-specific techniques for this commercial fish species would benefit farmers. Due to the lack of data on *C. marulius* captive breeding, this study aims to improve understanding of the reproductive biology of *C. marulius* and investigate their response to different inducing agents (Ovaprim®, Suprefact®, and Conceptal®), used independently or in manipulated ratios. To direct future research and support the development of induced spawning methods and conservation strategies, the research focuses on obtaining vital data, such as the gonadosomatic index (GSI). The present study assesses the efficacy of these three spawning induction agents for the induced breeding and spawning performance of *C. marulius* brooders in captive conditions in Pakistan. The specific research objectives are to investigate the effects of synthetic hormones on the reproductive biology of giant snakehead fish, assess their breeding and spawning responses, and develop effective region-specific breeding techniques. The hypotheses guiding this investigation include the effectiveness of synthetic hormones (Ovaprim®,

Suprefact®, and Conceptal®) in improving breeding and spawning performance, the enhancement of reproductive success through region-specific techniques, and the significant impact of these hormones on the reproductive biology of the species.

2. Materials and Methods

2.1. Ethics statement

The protocols and procedures of this study were accepted by the animal use and animal care committee of the University of Veterinary and Animal Sciences, Lahore, Pakistan (DR/163, 26-04-2021).

2.2. Fish, experimental design, and diets

The experiment was carried out at the Department of Fisheries & Aquaculture, UVAS, Ravi Campus, Pattoki in the spring 2020. Fish were acclimatized in brooders pond and fed twice a day with a commercial fish feed in (6mm floating pellet) containing 40% crude protein in (Table 1) at @3% of their body weight (BW) daily for three months, until reaching sexual maturity. Poultry viscera was also given to all the brood fishes at regular intervals as supplementary feed regularly. The mature brood stock of *C. marulius* was collected from the brooders pond and stocked in small experimental breeding ponds 4mx2mx1.5m (LxWxD at the Department of Fisheries and Aquaculture UVAS. Nine small ponds (P) with one pair of brooders in each, each with an average weight of 1.5–2.5 kg BW and a length of 70 cm, were present. Nine fishponds were divided into three treatments (T1, T2, and T3) during the study period, each with three replicates, using a completely randomized design (CRD). They were again acclimatized for one week. For hiding purposes, aquatic macrophytes (*Echornea crassipes* and *Hydrilla verticillata*) were added to the breeding ponds. Temperature, dissolved oxygen content, and pH were among the water quality parameters that were observed throughout the experiment.

Table 1. Commercial feed fed to *Channa marulius* for breeding biology at UVAS.

Ingredients	Commercial diets
	Commercial Feed (40% crude protein)
Fish Meal	28
Poultry Meal	23
Corn	21
Soybean meal	22
Fish Oil	2.5
Lysine sulphate 55%	2
DL Methionine	1
Dicalcium phosphate	0.135
Vitamin min. premix	0.34
Phytase	0.02
Total	100
Nutrients composition	
Crude protein%	40.46
Metabolizable energy (kcal/kg)	3200
EE%	11.45
Crude fiber%	1.43
Ash%	8.82
Ca%	1.97
Phosphorus%	1.24
Lysine%	3.43
Methionine%	1.74

Table 2. Synthetic hormones used as stimulators, their dosage concentrations for *Channa marulius.*

Groups/Treatments	Hormones used	Ponds	Dosage concentrations (ml/kg BW)		Time Interval of second dose (days)
			Male	Female	
T1	Conceptal	P1	0.3	0.8	15 Days
		P2	0.4	0.9	
		P3	0.5	1.0	
T2	Suprefact	P4	0.3	0.8	15 Days
		P5	0.4	0.9	
		P6	0.5	1.0	
T3	Ovaprim	P7	0.3	0.8	15 Days
		P8	0.4	0.9	
		P9	0.5	1.0	

The average water temperature of 28.3 to 30°C, pH 7-8, and dissolved oxygen (DO) 5-6 mg/l were measured by using digital meters such as YSI Model 55 Dissolved Oxygen and Temperature System, Ohio, 4387, USA.

2.3. Selection of broodstock

The selected broodstock could be identified sexually. The genital opening is located behind the genital papilla on the slender male body. The genital opening is located above the genital papilla and the female body is chubby. The mature males were selected based on pressing on the male's belly, a white color milt oozes out from the egential papila. While a mature female was identified by a soft, swollen, yellow belly that was protruding. One male and one female make up each pair (ratio: 1:1).

2.4. Preparation of superfact-20 hormonal solution for10 kg biomass of fish

Ten (10) tablets of Motilium-V have been taken, thoroughly ground, and added to a small petridish with 10 ml of distilled water to form a solution. With the help of a 1ml syringe, 0.3ml suprefact® hormone was then added, and the motilium solution was thoroughly mixed [24].

2.5. Broodstock selection, induced breeding, conditioning, spawning, and hatching of eggs

A total of 18 pairs of sexually mature healthy broodstock of males and females of *C. marulius* were collected from the small breeding ponds. For the induced breeding trial, three different hormones Ovaprim® (Syndel Laboratories, Vancouver, BC, Canada) and Suprefact-20 Hormone (Sanofi Aventis, Germany), Conceptal injection 5 ml (Star Laboratories (Pvt.) Ltd. was used in triplicate. Suprefact® and Motilium-V tablets were combined, and the mixture was diluted with distilled water to make a solution. The solution was then injected into males and females at varying concentrations (0.3, 0.4, and 0.5 ml for males, and 0.8, 0.9, and 1.0 ml for females/kg BW), respectively. The same dosages of Ovaprim® and Conceptal hormones (0.3, 0.4, and 0.5 ml for males and 0.8, 0.9, and 1.0 ml for females/kg BW) were also injected. The hormonal doses were injected into the recipient fish intramuscularly. After the hormonal injections of three stimulatory hormones, each pair of brooders was transferred back into their small experimental ponds. After 24 hours of injection of synthetic hormones, the response of brooders was observed in experimental ponds.

2.6. Data collection

During the first week of January, a dragnet was used to remove 3-5 fish samples from the pond to examine reproductive performance. Then, 3-5 samples were selected and dissected once more in March after the mature brood stock became available. Department of Fisheries & Aquaculture, UVAS, Laboratory received fresh test fish, which were measured and recorded to the nearest millimeter and gram, respectively, for their length and total body weight. Using scissors, the ventral sides of the fish were cut longitudinally from the anus to the lower jaw, and the gonad was removed for the gonado-somatic index. The gonads, or the ovaries and testes, were removed after dissection. The weight of the gonads was measured using

a digital balance (Model NBL 254e, 250 g × 0.0001 g), following the complete removal of moisture from the ovaries using filter paper [28]. The formulas that were used are given below.

$$\text{Gonado-Somatic Index (GSI)} = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

$$\text{Fecundity (F)} = \frac{\text{N x Gonad weight}}{\text{Sample weight}}$$

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs collected}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatched eggs.}}{\text{Number of fertilized eggs}} \times 100$$

$$\text{Larval survival rate (\%)} = \frac{\text{No. of actual fish survived}}{\text{No. of actual fish stocked}} \times 100$$

2.7. Statistical analysis

Two-way analysis of variance (ANOVA) was used to examine data on hormones and fertilization rate, fecundity rate, hatching rate, and larval survival rate from various treatments using SPSS-22 software. The Tukey's test ($p \leq 0.05$) was used to compare the treatment means across different experimental groups to determine their significance.

3. Results

3.1. Reproductive performance/Induced spawning

Synthetic hormones and their dosage concentrations for male and female *C. marulius* are presented in Table 2. Table 3 displays the outcome of *C. marulius* artificial breeding performance. After 10 hours of post-injection, the fish that had been exposed to hormones exhibited aggressive behavior. The courtship behavior of the breeding pairs, which began 1-2 days before spawning, was observed as well as their mutual roaming, nudging, and splashing in the water. Fish breeding behavior was closely monitored for the entire 48-hour spawning process. *C. marulius* formed a floating nest of weeds for the deposition of eggs. After 48 hours, the eggs hatch, and fry can see, and parents guard the fry for about a month. *C. marulius* did not spawn in treatment T1 (Conceptal®). Gonado-Somatic Index did not appear to be significantly ($p \geq 0.05$) affected by hormone doses.

Table 3. Reproductive performance of *Channa marulius* treated with Conceptal®, (GnRH Analogue), Suprefact® (LHRH) and Ovaprim® (GnRH+dopamine inhibitor). (Means±SE, N = 3).

Parameters	T_1(Conceptal)	T_2 (Superfact)	T_3 (Ovaprim)
Male body weight (kg)	1.345 ± 0.04^{b}	1.11 ± 0.03^{b}	1.500 ± 0.05^{a}
Female body weight (kg)	2.100 ± 0.05^{b}	2.5 ± 0.05^{a}	2.800 ± 0.06^{a}
Body length of Male (cm)	58 ± 3.51^{a}	55 ± 3.50^{a}	60 ± 3.54^{a}
Body length of Female (cm)	62 ± 3.58^{b}	70 ± 3.60^{a}	72 ± 3.60^{a}
GSI % (Female)	Nil	3.32 ± 1.62^{a}	1.13 ± 0.56^{b}
GSI % (Male)	Nil	1.67 ± 0.18^{a}	1.22 ± 0.68^{b}
Fecundity rate %	Nil	3079.3 ± 100.7^{a}	1669.3 ± 836.5^{ab}
Fertilization rate %	Nil	96.33 ± 1.20^{a}	58.00 ± 29.02^{b}
Hatching rate %	Nil	94.67 ± 2.40^{a}	61.27 ± 30.65^{ab}
Survival rate %	Nil	95.75 ± 1.51^{a}	64.67 ± 32.34^{ab}
Time interval (response after 2nd dose)	Nil	15.00 ± 0.00^{a}	10.00 ± 5.00^{ab}

In a row or column, means that have the different superscripts are statistically significant ($p \leq 0.05$). Means ± SD comparison (n =3)

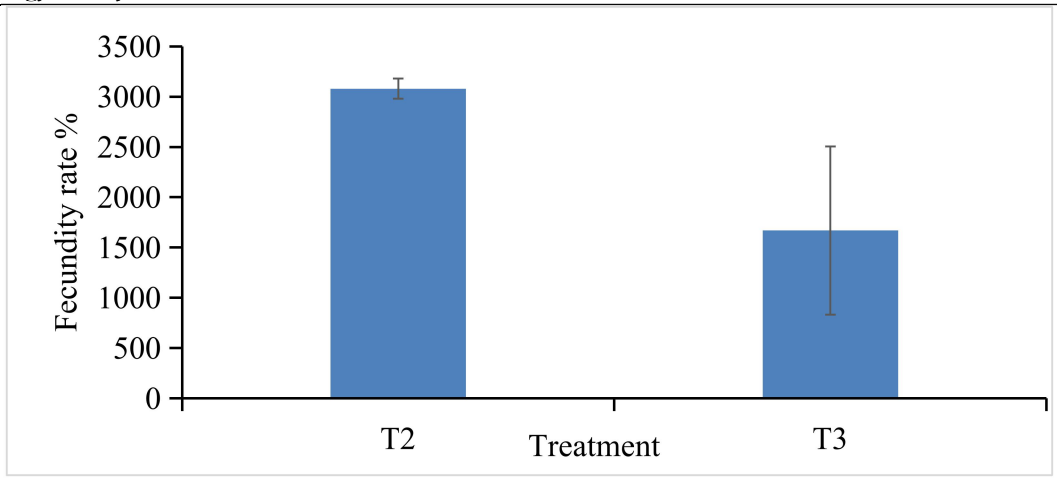

Figure 1. Absolute fecundity of *C. marulius with Superfact* (T_2) *and Ovaprim hormone* (T_3).

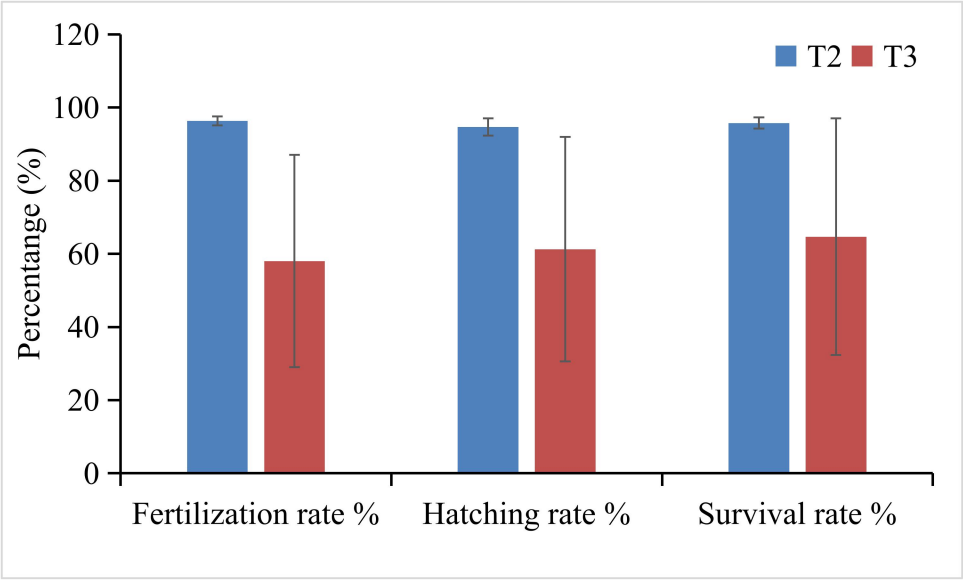

Figure 2. Induced breeding parameters of *C. marulius with Superfact* (T_2) *and Ovaprim hormone* (T_3).

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Overall, the average higher values for female and male GSI (3.32±1.62 and 1.67±0.18, respectively) appeared in treatment T2 (Suprefact®). The highest ($p<0.05$) average fecundity (3079.3±100.7%) was observed in treatment T2 (Suprefact®) while treatment T3 (Ovaprim®) had comparatively low fecundity (1669.3±836.5%) as shown in Figure 1. In snakehead, hormone doses had a significant ($p\leq0.05$) impact on the fertilization rate, hatchibility, and survival rate with higher average values (96.33±1.20, 94.67±2.40 and 95.75±1.51) appeared in treatment T2 (Suprefact®) as compared to T3 (Ovaprim®), Figure 2.

4. Discussion

In the present study, a single intramuscular injection of the synthetic hormones Suprefact® (LHRH), Ovaprim® (GnRH + dopamine inhibitor), and Conceptal caused the air-breathing fish, *C. marulius*, to successfully spawn and undergo changes in its gonadal development in small experimental breeding ponds. In the case of *C. marulius*, a very similar observation was made by [24], who employed a unique combination of synthetic hormones Suprefact® (LHRH) and Ovaprim® (GnRH + dopamine inhibitor). Following the first hormonal dosages of Motilium-V mixed Suprefact (0.3, 0.4, and 0.5 ml

for males and 0.8, 0.9, and 1.0 ml for females per kilogramme BW), each pair of brooders was then moved to blue-colored fibreglass drums. The experimental fish were given a second dose of the second hormone (Ovaprim®) at the same concentration after 24 hours. *C. marulius* was spawned successfully after 48-hours and fertilized eggs were moved with the help of plastic bowls into a circular cemented tank that had a gentle flow of aerated water where eggs were incubated. In the current study, three different hormones Conceptal®, Suprefact®, and Ovaprim® were also used, without combination but with the same hormonal dosage concentrations (0.3, 0.4, and 0.5 ml for males and 0.8, 0.9, and 1.0 ml for females per kilogram BW) and each pair was then released back into the small experimental earthen ponds. From these three hormones, no induced spawning was observed with Conceptal®.

In our study, both the male and female gonadosomatic index (GSI), which indicates gonadal development and maturation, was higher and peaked in April [29] observed normal fish ovarian development in *L. dyocheilus* kept in captivity and observed a similar trend in GSI. In addition to predicting the breeding season, GSI can also show a fish's maturity level and frequency of spawning [30]. In additional study, [31] observed *C. bleheri's* highest GSI value occurred between April and July. Similarly, [32] confirmed maximum GSI value in the rainy season during May and August.

Fecundity is frequently used to measure the capacity of a species to reproduce; in the current study, broodstock that received the highest protein diet produced the most eggs overall, which is in line with findings in female swordtail (Xiphophorus helleri) [33]. In the current study, treatment T2 (Suprefact®) exhibited higher fecundity rate, fertilization, and hatchability than treatment T3 (Ovaprim®). Our results are in line with those of Maradun [34], who reported that the fertilization rate in *C. gariepinus* ranged from 72 to 88%. Contrary to the findings of [35], where a higher dietary protein intake was associated with a higher fertilization rate, the fertilization rate was not influenced by dietary protein. Ovaprim® 1.0–2.0 ml/kg BW was utilised by [36] in Asian catfish, *Clarias batrachus*, when the doses proved effective. In a different study, it was found that female *Mystus gulios* injected with 2.5 ml/kg BW had maximal ovulation and an 80% hatching rate [37]. *Clarias batrachus* spawned 21–22 hours after pituitary extract injection [38] and 24 hours after receiving an ovaprim injection [39]. In contrast, [40] found that African catfish, *C. gariepinus*, with Ovaprim® induction had the maximum fertilization rate of 87.34%.

The temperature variation in this study was between 28.3 and 3 0°C, and the two treatment eggs hatched in 48 hours, which is n ormal for *C. marulius* breeding conditions in contrast to [41], temperature variation ranged from 25 to 27°C, and all the treatment eggs hatched in 72 to 75 hours for koi carp (*Cyprinus carpio*).

Because many of these factors lead to larger eggs, better spawning performances, earlier oocyte maturation, and higher rates of vitellogenesis, there is a positive correlation between the optimal growth rates and reproductive performance in broodstock [42]. Protein in the oocytes is transported and accelerated during oocyte maturation [43]. As a result, the current study reveals that fecundity was significantly impacted, most likely because of feeding with 40% CP protein.

5. Conclusion

According to the study, feeding *C. marulius* protein can improve the number and quality of their larvae. To enhance both the quality of the larvae produced and the reproductive performance of the broodstock, future studies should concentrate on the function of amino acids in diets. Suprefact® (T2) and Ovaprim® (T3), which produce more mature gametes and have higher GSI values than T3, appear to be useful synthetic hormones for inducing breeding in *C. marulius*, according to the findings. Comprehending the reproductive biology of *C. marulius* is imperative for the implementation of conservation strategies, selective breeding, and sustainable fisheries management in Pakistan. These areas need to be further investigated to support the maturation and upbringing of *C. marulius* in captivity.

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Data Availability statement

The data used to support the outcomes of this study is available from the corresponding author on request.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Authors Contribution

Sadia Nazir conducted the research, collected, and analyzed the data, and wrote the manuscript. Noor Khan, supervised, edited, and reviewed. Dilawar Hussain edited and reviewed. Sheeza Bano, Moazama Batool, Muhammad Asghar, Zahra Hussain, Muhammad Adnan Ali, formal analysis, writing, editing, and review. Simon J. Davies, co-supervisor, writer, reviewer, and editor.

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