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Effect of some chemical compounds on the survivability and physiology of *Channa punctatus* (Bloch) and *Anabas testudineus* (Bloch)

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Abstract: *Channa punctatus* and *Anabas testudineus* of different size-groups were exposed to table salt, urea, formalin, clove oil and folic acid for >72 hours to study the percentage survivability, tolerance time and physiological stress. Doses of compounds except table salt and urea were used as same for both the species. *A. testudineus* was more susceptible to table salt and urea compared to *C. punctatus*. All the chemicals affected survivability, tolerance time and produced stress in both species; effect of formalin and clove oil were greater than that of other compounds. Survivability and tolerance time were negatively related with the doses of the compounds, and positively related with the size of fishes. The highest dose (0.5 mg/l) of folic acid showed mortality in both species. The stress characters were secretion of excess mucous, imbalanced movements and respiratory problems were observed in both the species. These experiments were carried in the Aqua Lab (fisheries field lab), Department of Zoology, University of Rajshahi, during a period of three months from February to end of April, 2016.

Key words: Chemical compounds, survivability, tolerance, physiological stress, *C. punctatus*, *A. testudineus*

Introduction

Various chemicals are used in fisheries sector as anaesthetics, analgesics and sedatives to minimize stress in fish due to handling in live condition (Wurts, 1995). The mentioned chemicals are able to calm the excitable fish that might injure themselves during netting, handling and transportation (Berka, 1986; Davis & Griffin, 2004; Wurts, 1995). A number of these chemical anaesthetics or sedatives are used in pisciculture to prevent pathogenic and ectoparasitic diseases (Chinabut *et al.,* 1988; Swan & Fitzjerald, 1993 and Francis-Floyd, 1996; Peake, 1998; Woody *et al*., 2002). Such chemicals play important role in both fisheries research and aquaculture, and being used to facilitate various handling procedures (Summerfelt & Smith, 1990; Kazun & Swicki, 2001).

Fisheries scientists have been using quite a good number of chemical compounds as anaesthetics or sedatives. Among the commonly used effective sedatives or anaesthetics in aquaculture and fish handling, or as therapeutics and prophylactics are the clove oil, formalin, food grade salts (calcium sulfate, sodium chloride, sodium bicarbonate). Use of salts is prescribed by the scientists to maintain osmoregulation in transporting water and to manage a variety of disease in culture water (Burgdorf-Moisuk *et al*., 2011).

The chemical effect may vary with the fish species, size and weight of the fish, doses of the chemical, and the exposure time (Wurts, 1995). Before introducing an anaesthetic or a sedative, screening test must be run to explore effective doses and exposure time, against the species which are going to be treated. After treatment quick recovery of the fish is vital factor for grading a chemical as effective anaesthetic/sedative agent.

This research was aimed to study the survival percentage, the tolerance time and the stress effects of table salt, urea, formalin, clove oil and folic acid against two freshwater hardy fish, the snake head (*Channa punctatus* Bloch) and the climbing perch (*Anabas testudineus* Bloch).

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Materials and Methods

Compounds used: Five compounds such as commercial table salt, urea, formalin, clove oil and folic acid (iron tablet) were used in this experiment. Among these compounds table salt, formalin and clove oil are widely used as anaesthetics in fisheries sector for different purposes. Urea is used as fertilizer in the fish pond. Vitamin like ascorbic acid is provided as supplemental feed to the fish for the development of immune system. In these contexts the above mentioned compounds were chosen to observe their effects on fish.

Preparation of doses: To determine the sublethal doses of the compounds pilot experiments were conducted separately for two species of fish. In case of table salt and urea the doses were different for the two species, as *A. testudineus* was more tolerant to these salts than that of *C. punctatus*. The doses of other three compounds were the same for both the species. Five doses of each compound were used for the experiments, which were:

i) table salt and urea: 5, 10, 15, 20 and 30 mg/l of water (equivalent to 0.1, 1, 1.5, 2 and 3%) for *C. punctatus*; and 10, 20, 30, 40 and 50 mg/l of water (equivalent to 1, 2, 3, 4 and 5%) for *A. testudineus*.

ii) formalin and clove oil: 0.025, 0.05, 0.075, 0.1 and 0.5 ml/l of water for both the species.

iii) folic acid: 1, 2, 3, 4 and 5 mg/l of water (equivalent to 0.02, 0.04, 0.06, 0.08 and 0.1%) for both the species.

Collection and acclimatization of fish: Live *C. punctatus* and *A. testudineus* were collected early in the morning from the fish landing centers of Rajshahi city, when the fish arrived from the harvesting area. Active and healthy live fishes were selected and kept in plastic buckets containing water. Two species were kept in separate buckets. The fishes were then taken to the Aqua Lab (fisheries field lab), Department of Zoology, University of Rajshahi. The fishes were released in cemented tanks inside the hatchery, keeping two species in two separate tanks. Each tank was filled with pond water keeping the water depth as 30 cm. For the first 2-3 hours air was supplied in the tanks using aerators. The fishes were provided food (balls of wheat flour mixed with fish meal in a ratio of 3:1) twice daily after 24 hours of release. Water of the tanks was changed every day with fresh pond water. Weak or dead fishes if any were discarded. The fishes were thus acclimatized for a weak. Feeding was with held 24h before commencement of the experiment. Air temperature of the hatchery during the whole experimental period was ranged from 20-25^{0}C.

Exposure protocol

Experiment I: For recording the percentage survivability and tolerance time (average time from when mortal effect started) live and healthy fishes of each species were selected. Fishes of both the species were grouped into three categories having total length ranging from 95-110 mm, 111-120 mm and 135-160 mm. Five identical aquaria containing 15 liters of pond water was taken. Required quantities of one compound were mixed with the aquaria water to obtain the selected five doses in five separate aquaria. A single fish of a size-group of one species was released in each aquarium. Feeding was stopped during the experimental period, but aeration was continued. The aquaria were covered by net to inhibit escape of the fish. Three replications were used for each size-group of each species of fish and each dose of each compound.

Table 1. Effect of table salt and urea on survivability and tolerance period in different size groups of *C. punctatus*

Doses (g/l)	Average Survivability (%) Size Groups (mm)			Average Tolerance period (h) Size Groups (mm)		
	95-110	111-120	135-160	95-110	111-120	135-160
Table salt						
5	40.00	100	100	70	>72	>72
10	37.50	100	100	69.42	>72	>72
15	0	60.00	70.00	8	62	60
20	0	5.00	50.00	3	20	21.30
30	0	2.00	15.00	3	8	12
Urea						
5	95.00	100	100	60	>72	>72
10	95.00	100	100	58.30	>72	>72
15	92.00	100	100	58.15	>72	>72
20	88.50	98.20	100	50	67.30	>72
30	67.33	90.00	90.00	36	60	61.40

Experiment II: To study the physiological stress on the fish produced by the compounds the similar exposure protocol (like experiment I) used, with an exposure period of 72h. In this experiment size-groups of *C. punctatus* were the same as used in Experiment I, but the sizes of *A. testudineus* used were 90-100, 101-110 and 120-130mm because unavailability of larger fishes of this species at that time. This experiment was also replicated for three times.

Study period: All these experiments were carried during a period of three months from February to end of April, 2016.

Results and Discussion

Experiment I: Percentage survivability and tolerance time

Effect of the studied compounds on the survivability of the fish species and the respective tolerance time were recorded (Tables 1-5).

Percentage survivability in table salt and urea: Survivability of *C. punctatus* was more affected by table salt than that of urea, and *A. testudineus* was found to be more tolerant to both the salts compared to *C. punctatus* (Tables 1 and 2). The smaller size-group (95-110 mm) of *C. punctatus* failed to survive in doses higher than 10g/l of table salt (Table 1), whereas, the same sized of *A. testudineus* was found to survive 100% even at the dose of 30 g/l (Table 2). At 15-30 g/l of table salt, the percentage of survivability of larger sized *C. punctatus* was decreased with the increase of doses. In case of *A. testudineus*, the larger sized fishes were succeeded 100% survival up to doses of 40 g/l.

At 20 and 30 g/l urea, the survival percentages of the small sized *C. punctatus* were 88 and 67.33, and the larger size-group succeeded 100% survivability at the same doses of urea (Table 1). Comparatively higher doses of urea (up to 50 g/l) did not affect survivable rate of any size of *A. testudineus* (Table 2).

Tolerance time against table salt and urea: The tolerance time was decreased with the increase dose levels of table salt at all size-groups of *C. punctatus*; and the tolerance time was positively related with the size of the fish (Table 1). At 20 g/l of table salt, the tolerance periods were recorded as 3, 20 and 21.30h for 95-110, 111-120 and 135-160 mm size-group respectively. The tolerance time in *A. testudineus* for table salt was more than 72h up to a dose level of 40 g/l, which was about the same for each size-group (Table 2).

Table 2. Effect of table salt and urea on survivability and tolerance period in different size groups of *A. testudineus*

Doses (g/l)	Average Survivability (%) Size Groups (mm)			Average Tolerance period (h) Size Groups (mm)		
	95-110	111-120	135-160	95-110	111-120	135-160
Table salt						
10	100	100	100	>72	>72	>72
20	100	100	100	>72	>72	>72
30	100	100	100	>72	>72	>72
40	66.12	100	100	71	>72	>72
50	34.20	92.00	96.00	55.30	61	62.10
Urea						
10	100	100	100	>72	>72	>72
20	100	100	100	>72	>72	>72
30	100	100	100	>72	>72	>72
40	96.00	100	100	65	>72	>72
50	96.00	98.00	100	61.25	67.35	>72

Tolerance period for urea was comparatively longer for both species. In *C. punctatus* the tolerance time for urea was decreased with the increase of dose levels, and it was increased with the increase of fish size (Table 1). At highest dose of urea (30 g/l) the tolerance periods were recorded as 36, 60 and 61.4h in *C. punctatus* having respective sizes of 95-110, 111-120 and 135-160 mm respectively. *A. testudineus* at 50 g/l of urea showed tolerance for 55.30, 61 and 62.10h in the respective size-group of 95-110, 111-120 and 135-160 mm (Table 2).

Percentage of survivability in formalin: The percentage of survivability in formalin was more or less similar in both fish species, *A. testudineus* being a little bit more susceptible than that of *C. punctatus*. In both cases the percentage of survivability was increased with the increased dose of formalin, and decreased with the increase size of fish (Table 3). In three size-groups (95-110, 111-120 and 135-160 mm) of *C. punctatus*, the percentages of survivability were observed as 85, 92.15 amd100% respectively at the highest dose of formalin (0.5 ml/l). In the same dose the percentage of survivability of *A. testudineus* was 82, 90 and 95% for 95-110, 111-120 and 135-160 mm size-group respectively (Table 3).

Tolerance time in formalin: For both species, the tolerance period was decreased with the increased dose of formalin, and increased with the increase size-groups of fish (Table 3). At 0.5 ml/l the tolerance times were recorded as 54.25, 58.30 and >72h in *C. punctatus* for the respective size-groups of 95-110, 111-120 and 135-160 mm., in the same size- groups of *A. testudineus* the tolerance times for the same dose of formalin were 58.30, 65.15 and 60.30h (Table 3).

Percentage of survivability in clove oil: At 0.025 and 0.05 ml/l, the survivability rate of *C. punctatus* was 100% (Table 4). The lowest survivability rate of *C. punctatus* was observed in 135-160 mm size-group at 0.5 ml/l dose, but in the size-group 111-120 mm the percentage survivability was 90% at same dose. Whereas *A. testudineus* was found to be more susceptible to the clove oil treatments with compared to *C. punctatus*. The survivability rate of *A. testudineus* was only 40% in 95-110 mm size group at the lowest dose (0.025 ml/l), and the highest survivability rate of this species was recorded as 66.50% in the size-group 135-160 mm at the same dose. In both species the percentage survivability was decreased with the increase of dose of clove oil and size of the treated fish (Table 4).

Table 3. Effect of formalin on survivability and tolerance period in different size groups of *C. punctatus* and *A. testudineus*

Doses (ml/l)	Average Survivability (%) Size Groups (mm)			Average Tolerance period (h) Size Groups (mm)		
	95-110	111-120	135-160	95-110	111-120	135-160
C. punctatus						
0.025	100	100	100	>72	>72	>72
0.05	100	100	100	>72	>72	>72
0.075	98.00	100	100	64.15	>72	>72
0.1	97.00	99.20	100	62.50	60.15	>72
0.5	85.00	92.15	100	54.25	58.30	>72
A. testudineus						
0.025	100	100	100	>72	>72	>72
0.05	100	100	100	>72	>72	>72
0.075	97.50	100	100	69	>72	>72
0.1	94.00	98.00	100	63.25	67.30	>72
0.5	82.00	90.00	95.00	58.30	65.15	60.30

Tolerance time in clove oil: *C. punctatus* treated in clove oil for 72h up to doses of 0.05 ml/l for 95-110 and 111-120 mm size-groups, and 0.075 ml/l for 135-160 mm size-group (Table 4). Whereas, *A. testudineus* showed minimum tolerance time against clove oil as 8.30 h at 0.5 ml/l dose for 95-110 mm size-group, and the maximum time was recorded as 52.30h at a dose of 0.025 ml/l for 135-160 mm size-group (Table 4).

Percentage of survivability in folic acid: The survivability rate of *C. punctatus* was more than that of *A. testudineus*. The survivability was found as 88 and 90% at 0.5 mg/l of folic acid for the size-groups 95-110 and 111-120 mm (Table 5). In case of *A. testudineus* percentage for survivability was noted in fishes of 95-110 mm as 60-68% at 0.5 and 0.075 mg/l of folic acid; and 100% fish were survived in the largest size-group in 0.25 and 0.05mg/l (Table 5).

Table 4. Effect of clove oil on survivability and tolerance period in different size groups of *C. punctatus* and *A. testudineus*

Doses (ml/l)	Average Survivability (%) Size Groups (mm)			Average Tolerance period (h) Size Groups (mm)		
	95-110	111-120	135-160	95-110	111-120	135-160
C. punctatus						
0.025	100	100	100	>72	>72	>72
0.05	100	100	100	>72	>72	>72
0.075	96.10	98.00	100	62.15	65.25	>72
0.1	80.50	98.00	68.00	60.40	65	70
0.5	68.00	90.00	58.00	58	65	49
A. *testudineus*						
0.025	40.00	68.00	66.50	39	48.50	52.30
0.05	32.50	68.00	68.00	39	42.20	52
0.075	20.00	45.00	38.30	32.15	40	51
0.1	15.10	38.00	12.15	31	38	39.50
0.5	00	00	10.50	8.30	18	29

Tolerance time in folic acid: The smaller sized *C. punctatus* found to tolerate folic acid treatment only for 3h (0.1 and 0.5 mg/l) and 8h (0.075 mg/l), otherwise the larger fishes tolerated the treatment from 67.40h (0.5 mg/l) to >72h (Table 5). The 95-110 mm sized *A. testudineus* tolerated the treatment for 50-60h at 0.5-0.075 mg/l doses. The larger fishes tolerated all doses of folic acid from 70.35 - >72h (Table 5).

Experiment II: Physiological stress

The experimental compounds produced stress effects on the fish, which were indicated excess mucous secretion, sluggish or rapid movements, respiratory stress, unbalanced movement of fish, discoloured gills, etc. The dose and the time of attaining stress characters in the fish are mentioned in Tables 6-10. Stress started soon after treatment in smaller size-group of both species, and *A. testudineus* was found to be more susceptible than *C, punctatus*.

Among the five compounds used in the present experiments all four except the folic acid are used in different steps of pisciculture, handling and transportation of fry and brood fish. As analgesic and anaesthetic clove oil, formalin and table salt are widely used in different countries including Bangladesh.

Table 5. Effect of folic acid on survivability and tolerance period in different size groups of *C. punctatus* and *A. testudineus*

Doses (mg/l)	Average Survivability (%)			Average Tolerance period (h)		
	Size Groups (mm)			Size Groups (mm)		
	95-110	111-120	135-160	95-110	111-120	135-160
C. punctatus						
0.025	100	100	100	>72	>72	>72
0.05	100	100	100	>72	>72	>72
0.075	99.00	100	100	8	>72	>72
0.1	100	100	100	3	>72	>72
0.5	88.00	95.00	100	3	67.40	>72
A. testudineus						
0.025	100	100	100	>72	>72	>72
0.05	100	100	100	>72	>72	>72
0.075	68.00	100	100	60	>72	>72
0.1	67.00	95.00	99.00	52	70.50	71
0.5	60.00	92.00	99.00	50	70.35	71

Previous works with clove oil, formalin and table salt against *A. testudineus* and *C. punctatus* and some other species, proved these compounds are good anaesthetics fish species (Alam *et al*., 2012; Ahsan *et al*., 2014 and Parween *et al*., 2015). However, the time that the fish species could tolerate these chemicals were not mentioned in those studies. The smaller sizes fish of both the species failed to tolerate the treatments as the larger sized fish could. Moreover, the fishes survived treatments when kept in fresh water and provided with oxygen they recovered soon within 4-6 hours. Which revealed that except folic acid the other compounds are good anaesthetic to fish. To reduce mortality rate (Murai *et* al., 1979) and physiological stress in transporting fish (Davis & Griffin, 2004; Chen *et al*., 2004; Morales *et al*., 2005), mild sedation were suggested by Radull *et al*. (2002), Koeypudsa & Jongjareanjai (2011); Wurts (1995) and Davis & Griffin (2004) prescribed the food grade salts as sedatives in the mentioned cases. Wurts (1995) reported that traditionally 0.5 – 2.0g/l sodium chloride is used in fish transpotating water to minimize dehydration. The author also mentioned that agricultural gypsum (calcium sulfate) at a rate of 125-250 mg/l is also used in these cases.

Table 6. Stress characters observed in *C. punctatus* and *A. testudineus* in table salt treatment, exposed for 72 hrs.

C. punctatus				***A. testudineus***			
Doses (g/l)	**Size Groups (mm)**	**Stress characters**	**Time after exposure (hr)**	**Doses (g/l)**	**Size Groups (mm)**	**Stress characters**	**Time after exposure (hr)**
5	95-110	Excess mucous	24	10	90-100	Normal with slow movement	>50
	111-120	Excess mucous with sluggish movement	40		101-110		
	135-160	Normal	>72		120-130		
10	95-110	Stressed	30	20	90-100	Normal with rapid movement	>50
	111-120	Excess mucous, rapid movement	12		101-110	Normal but sluggish	
	135-160	Normal	>72		120-130		
15	95-110	Died in stressed condition	8	30	90-100	Excess mucous with rapid movement	>32
	111-120	Excess mucous, stressed	25-28		101-110		37-40
	135-160	Excess mucous, stressed	30		120-130		
20	95-110	Died in stressed condition	3	40	90-100	Stressed	12
	111-120	Excess mucous, stressed	1		101-110	Sluggish movement	37-40
	135-160	Excess mucous, stressed	4		120-130		
30	95-110	Died in stressed condition	3	50	90-100	Died in stressed condition	65
	111-120	Excess mucous, stressed	1		101-110	Stressed + unbalanced	12
	135-160	Excess mucous, stressed	4		120-130		40-50

Overdosing of a sedative compound or retaining fish too long in the treatment leads to the fading of ventilation, hypoxia and finally respiratory-cardiaccollapse (Tytler & Hawkins, 1981). Again, the optimum dose of such compounds especially salts vary with the intrinsic factors of fish i.e., species, size and weight of fish (Newman & Aplin, 1992; Koeypudsa & Jongjareanjai, 2011; Ahsan *et al*., 2015) and extrinsic factor like water temperature (Wurts, 1995). So, selection of suitable doses of the chemicals is a very vital point. Before using any additive, analgesic or anaesthetic, screening should be done against the fish that would be treated. Both tolerance time and recovery rate against the treatment should be monitored.

Conclusion: Except clove oil other chemicals used in the study produced little stress at low doses and against the larger size groups of the fish species. Among the two species *A. testudineus* was found to be comparatively susceptible to the chemicals. According to Dabrowski *et al.* (2004) addition of ascorbic acid with supplement food reduced vitamin deficiency in culturing ponds. From the present study it can be suggested that folic acid can also be used as supplement with the food to develop strong immune system of the fish especially in the nursery ponds and where stocking density is high.

Table 7. Stress characters observed in *C. punctatus* and *A. testudineus* due to urea treatment, exposed for 72 hrs.

C. punctatus				***A. testudineus***			
Doses (g/l)	**Size Groups (mm)**	**Stress characters**	**Time after exposure (hr)**	**Doses (g/l)**	**Size Groups (mm)**	**Stress characters**	**Time after exposure (hr)**
5	95-110	Normal, slight increase of mucous	48	10	90-100	Normal	>72
	111-120	Normal	>72		101-110		
	135-160				120-130		
10	95-110	Slightly stressed	37	20	90-100	Normal	>72
	111-120	Normal with mucous secretion	>72		101-110		
	135-160				120-130		
15	95-110	Slightly stressed	32-35	30	90-100	Slight increase of mucous	>50
	111-120	Normal with increased mucous secretion	>50		101-110	Normal	>72
	135-160				120-130		
20	95-110	Stressed	12	40	90-100	Fully stressed	48
	111-120		28		101-110	Stressed	55-57
	135-160		36		120-130		
30	95-110	Stressed and unbalanced	12	50	90-100	Fully stressed	>25
	111-120	Fully stressed	30-36		101-110	Stressed	>30
	135-160				120-130		

Table 8. Stress characters observed in *C. punctatus* and *A. testudineus* due to formalin treatment, exposed for 72 hrs.

Doses (ml/l)	***C. punctatus***			***A. testudineus***		
	Size Groups (mm)	**Stress characters**	**Time after exposure (hr)**	**Size Groups (mm)**	**Stress characters**	**Time after exposure (hr)**
0.025	95-110	Respiratory stress with excess mucous	>2	90-100	Normal	>72
	111-1520	Stressed movement	>35	101-110		
	135-160	Stress level increased	>40	120-130		
0.05	95-110	Respiratory stress with excess mucous	2-3	90-100	Slightly stressed	36
	111-1520	Stressed movement	32-34	101-110	Normal with mucous secretion	>50
	135-160	Stress level increased	>35	120-130		>60
0.075	95-110	Stressed and sluggish	10	90-100	Slightly stressed	18
	111-1520		20	101-110		40-42
	135-160			120-130		>55
0.1	95-110	Fully stressed	7-8	90-100	Slightly stressed	>15
	111-1520		14	101-110		>40
	135-160		17	120-130		40-41
0.5	95-110	Stressed and unbalanced	0.30	90-100	Stressed and unbalanced	12-14
	111-1520		4	101-110	Stressed with rapid movement	35-52
	135-160		4-5	120-130	Slow and sluggish	35-38

Table 9. Stress characters observed in *C. punctatus* and *A. testudineus* due to clove oil treatment, exposed for 72 hrs.

Doses (ml/l)	*C. punctatus*			*A. testudineus*		
	Size Groups (mm)	**Stress characters**	**Time after exposure (hr)**	**Size Groups (mm)**	**Stress characters**	**Time after exposure (hr)**
0.025	95-110	Slight respiratory stress	40	90-100	Respiratory stress	4
	111-1520		60	101-110		>6
	135-160		>62	120-130		10-11
0.05	95-110	Respiratory stress with excess mucous	38	90-100	Slightly stressed	38
	111-1520		>48	101-110		>48
	135-160		48	120-130		48
0.075	95-110	Respiratory stress and unbalanced	20 - >24	90-100	Slightly stressed	8-12
	111-1520		<24	101-110		>10
	135-160		10-30	120-130		45-50
0.1	95-110	Fully stressed	>2	90-100	Stressed, gills discoloured	>4
	111-1520	Stressed and sluggish	14	101-110	Fully stressed	10-12
	135-160		15-16	120-130	Became sluggish	24
0.5	95-110	Stressed, gills discoloured, excessive mucous	17-18	90-100	Stressed	3-4
	111-1520	Fully stressed	1-2	101-110		>10
	135-160		>4	120-130		10

Table 10. Stress characters observed in *C. punctatus* and *A. testudineus* due to folic acid treatment, exposed for 72 hrs.

Doses (mg/l)	*C. punctatus*			*A. testudineus*		
	Size Groups (mm)	**Stress characters**	**Time after exposure (hr)**	**Size Groups (mm)**	**Stress characters**	**Time after exposure (hr)**
1	95-110	Normal	>72	90-100	Slightly stressed	>60
	111-1520			101-110	Normal	>72
	135-160			120-130		
2	95-110	Normal	>72	90-100	Slightly stressed with quick movement	55-57
	111-1520			101-110	Slightly stressed	>55
	135-160			120-130		>62
3	95-110	Slight uneasiness	>62	90-100	Stressed	45
	111-1520	Normal	>72	101-110	Stressed and sluggish	50-52
	135-160			120-130		>60
4	95-110	Uneasiness with slight mucous	62-65	90-100	Stressed,	>48
	111-1520	Normal	>72	101-110	Stressed and sluggish	50-52
	135-160			120-130		>60
5	95-110	Slightly stressed	>52	90-100	Stressed and unbalanced	30
	111-1520	Normal but with mucous	>68	101-110		28-29
	135-160	Normal	>72	120-130		>48

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