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Antibacterial and Irritant Activities of Organic Solvent Extracts of *Agave americana* Linn., *Albizzia lebbek* Benth. *Achyranthes aspera* Linn. and *Abutilon indicum* Linn - A Preliminary Investigation

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Abstract.- Hexane, chloroform and ethanol extracts of leaves of *Agave americana* L., seeds of *Albizzia lebbek* Benth and *Achyranthes aspera* L., and the whole plant of *Abutilon indicum* were tested for the antibacterial activity against Gram positive and Gram negative bacteria. Ethanol and chlorofonn extracts of *Agave americana* and *Achyranthes aspera* and ethanol extract of *Albizzia lebbek* exhibited mild to moderate antibiotic activity. Vacuum liquid chromatography isolated fractions from chloroform extract of *Achyranthes aspera* displayed antibacterial activity against *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.* The antibacterial range of ethanol extract of *Abutilon indicum* was prominent than those of other organic extracts. Chloroform and ethanol extracts of *Agave americana* exhibited acute as well as chronic irritant activity on applying to the inner ear of male albino rabbits.

Key words: *Agave americana* L., *Albizzia lebbek* Benth, *Achyranthes aspera* L. *Abutilon indicum* L., antibacterial activity, irritant activity, herbal drugs.

The herbal drugs used throughout the world have received greater attention in recent times because of their diversity of curing diseases, safety and well tolerated remedies when compared to the conventional medicines. Development of resistant against antibiotics has further emphasized the necessity of research for new antimicrobial agents (Bax and Mullan, 2000).

Agave americana L. (Family Agavaceae) has been reported to have molluscicidal (Sukumaran and Parasher, 1994), antifungal (Sharma and Ahmad, 1998), mosquitocidal (Dharmshaktu and Prabhakaran, 1987), antibacterial (Akhtar *et al.*, 1997), anti-inflammatory (Peana and Moretti, 1997) and acute irritant contact dermatitis (Ricks and Voget, 1999) effects. Homoisoflavanoids (Tinto *et al.*, 2005), steroidal saponin (Yokosuka and Mimaki, 2000), metal content (Parmar and Gupta, 1993), flavanone (Parmar and Jha, 1992), fructosyl transferase (Bhatia and Nandra, 1979), and aminopeptidase protease (Du Toit *et al.*, 1978), have been reported from *Agave americana* L.

Allbizzia lebbek Benth (Family Leguminosae (Fabaceae) is known for its antiallergic action (Baruah *et al.*, 1996), antiviral activity (Misra *et al.*, 1995), anticonvulsant response (Kasture *et al.*, 1996), anti-inflammatory activity (Tripathi and Sadhra, 1999), antidiarrhoeal action and antibacterial activity. Saponins, sapogenin glycosides alkaloids, flavonoids and tannins have been reported from *Albizzia lebbek*.

Achyranthes aspera (Family Arnaranthaceae) is used as astringent, diuretic, antiperiodic and purgative. It cures vomiting, cough, pain, itching, flatulence and piles. The seeds have remedy for insects and reptile bites, are used as expectorant (Nadkarni and Chopra, 2002), bleeding piles and rabies (Ravendra and Martin, 2006). The seeds of *Achyranthes aspera* contains steroids (Wang and Sheng, 1991), oleic acid, linoleic acid, linolenic acid (Rawat and Singh, 2002), hydrocarbons (Muhammad, 1994), saponins (Michel *et al.*, 2000), alkaloids and amino acids (Noor, 1983).

Abutilon indiclim L. (Family Ma1vaceae) is known for antimalarial (Beha *et al.*, 2004), analgesic and antimicrobial (Parekh *et al.*, 2006) activity. It contains essential oils (Jain *et al.*, 1982), sesquiterpene lactones (Sharma *et al.*, 1989), flavonoids (Matlawska and Sikorska, 2005), water soluble galactomannan (Singh *et al.*, 1997), 3-sitosterol and D-amyrin, eugenol and gallic acid.

The present work was aimed at screening

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leaves of *Agave americana* L., seeds of *Albizzia lebbek* Benth, *Achyranthes aspera* L. and whole plant *Abutilon indicum* L. for antibacterial and irritant activities.

Materials and methods

Collection and extraction of plant material

Agave americana, Albizzia lebbek, Achyranthes aspera and *Abutilon indicum* were collected from the Herbarium of Government College University, Lahore.

Distilled 5 l each of hexane, chloroform and ethanol (E.Merck, Germany) were used successivety for extraction of active principles from the (1Kg) dried and pulverized plant parts using Soxhlet extraction method. The extraction was continued for 72 hours, till the extraction became colourless. The extracts were concentrated on vacuum rotary evaporator (Rikakikai Co. Ltd., Tokyo) and their percentage yields were calculated.

Determination of antibacterial activity

For determining antibacterial activity concentrated extracts of leaves of *Agave americana* in hexane (5.53%), chloroform (6.87%) and ethanol (6.37%); seeds extracts of *Albizzia lebbek* in hexane (l0%),chloroform (6.67%) and ethanol (12.61%); and whole plant extracts of *Abutilon indicum* in hexane (8%), chloroform (5.90%) and ethanol (11.80%) were tested against three Gram positive *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 9341), *Staphylococcus aureus* (ATCC 25923) and three Gram negative bacteria *viz., Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella cholerae-suis* (ATCC 13312) by using the hole plate diffusion method (Haavic and Jhonasson, 1973). Vacuum liquid chromatography (VLC) of isolated fraction from chloroform extract of *Achyranthes aspera* was carried out by using the method of Homans and Fuchs (1970).

Determination of irritant activity

Six male albino rabbits (*Oryctolagus cuniculus*) weighing 1.0 - 1.3kg were used to test the irritant activity of hexane, chloroform and ethanol extracts of leaves of *Agave americana* L, seeds of *Albizzia lebbek* Benth and *Achyrangthes aspera* L, and whole plant of *Abutilon indicum* L. Two concentrations of extracts *viz*. 1 and 2 mg/ml in acetone were used. For testing the irritant activity, hair on the inner surface of rabbit ear were shaved off and divided into three portions with the help of black marker. Ten µl of different concentrations of each extract was applied on the three portions. The other ear was used as vehicle control. The ear was observed for redness after every 15 minutes then after every 30 minutes. The maximum irritancy on rabbit's ear that corresponded to the + + scale of Hecker (1971) after 24 hours were recorded. The data was presented as Mean±S.E.

Results and discussion

Table I shows that the chloroform extract of *Agave americana* L. displayed antibacterial activity against *B. subtilis* and *S. aureus,* and *S. choleraesuis.* Ethanolic extract exhibited the antibacterial activity against *S. aureus.* The intensity of antibacterial response seems to be somewhat greater in ethanolic extract than in the chloroform extracts.

Antibacterial activity in chloroform and ethanol extracts of leaves of *Agave americana* may be attributed to the presence of an alcoholic component such as tetratriacontanol derivatives (Virinder *et al.,* 1992) and homoisoflavanoids (Tinto *et al.,* 2005). Our finding of antibacterial activity also correlates to the work done by Akther and co-workers (1997).

The ethanol extracts of *Albizzia lebbek* exhibited antibacterial activity against *Micrococcus luteus, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Only 100mg/ml concentration or ethanol extract of *Albizzia lebbek* displayed antibacterial activity against *E. coli.* This antibacterial activity may be due to presence of flavone derivatives.

Antibacterial activity of chloroform extract of *Achyranthes aspera* was more prominent than the ethanol extract. None of the 'extracts demonstrated antibacterial activity against *M. luteus* and S. *cholerae-asuis* except chloroform extracts with the concentration of 100 mg/ml. VLC isolated fraction of chloroform extract containing alkaloid and triterpenoid (Bisht and Sandhu, 1991) showed antibacterial activity against *B. subtilis, S. aureus,*

Table I.- Effect of different concentrations (5, 50 and 100 mg/ml) of hexane, chloroform, ethanol and aqueous extracts on three gram positive and three gram negative bacteria. The values represents the Mean±SE of diameter of zone of inhibition (mm) in replicates. All values were not significantly different at P>0.05; - no effect.

Plant / drug (Conc.)	*Bacillus subtilis*	*Micrococcus luteus*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella cholerae-suis*
***Agave americana* L.**						
Chloroform extract						
5 mg/ml	-	-	10.80±0.40*	-	-	-
50 mg/ml	15.80±0.40	-	11.20±0.98	-	-	12.80±0.40
100 mg/ml	15.80±0.40	-	12.80±0.40	-	-	18.40±0.80
Ethanol extract						
5 mg/ml	-	-	13.20±0.40	-	-	-
50 mg/ml	-	-	13.80±0.40	-	-	-
100 mg/ml	-	-	16.60±0.49	-	-	-
***Albizzia lebbek* B.**						
Ethanol extract						
5 mg/ml	-	10.80±0.40	16.60±0.49	10.40±0.49	-	-
50 mg/ml	-	12.00±0.62	13.20±0.40	13.80±0.40	-	-
100 mg/ml	-	19.80±0.90	13.80±0.40	17.40±0.49	12.00±0.63	-
***Achyranthes aspera* L.**						
Chloroform extract						
5 mg/ml	18.89±0.45	-	20.80±0.33	21.70±0.42	16.90±0.52	-
50 mg/ml	19.92±0.36	-	21.65±0.40	21.80±0.35	18.47±0.26	-
100 mg/ml	20.92±0.40	-	22.25±0.34	22.97±0.46	19.97±0.22	15.10±0.58
Ethanol extract						
5 mg/ml	-	-	-	-	-	-
50 mg/ml	8.05±0.32	-	8.50±0.15	8.08±0.16	8.48±0.51	-
100 mg/ml	9.35±0.19	-	9.18±0.19	9.76±0.07	8.85±0.27	-
VLC fraction	10.98±0.21	-	10.00±0.21	11.45±0.15	9.68±0.33	-
1 mg/ml	8.10±0.80	-	-	7.36±1.59	8.70±2.06	-
***Abutilon indicum* L.**						
Hexane extract						
5 mg/ml	-	10.80±0.40	16.60±0.49	-	-	-
50 mg/ml	-	12.00±0.63	13.20±0.40	-	-	-
100 mg/ml	-	19.80±0.97	13.80±0.40	-	12.00±0.63	-
Chloroform extract						
50 mg/ml	-	-	11.20±0.98	-	-	-
100 mg/ml	-	-	12.80±0.40	18.80±0.80	-	-
Ethanol extract						
5 mg/ml	12.00±0.63	11.80±0.40	13.00±1.26	12.60±0.49	14.20±2.23	11.80±0.98
50 mg/ml	12.60±0.49	16.20±1.47	16.80±1.47	17.00±0.89	15.60±1.63	13.60±0.20
100 mg/ml	20.20±1.16	21.40±2.80	19.20±0.98	21.60±0.49	20.20±2.72	17.60±0.49
Aqueous extract						
50 mg/ml	15.60±1.49	-	13.00±0.63	-	14.00±0.63	13.00±0.63
100 mg/ml	19.00±1.26	-	15.40±0.80	16.80±0.40	16.00±0.63	15.60±1.02
Ampicillin (1 mg/ml)	34.60±0.50	34.40±0.50	35.20±0.40	37.80±0.75	34.00±1.09	36.60±0.49
Streptomycin (1 mg/ml)	19.60±0.50	22.60±0.50	22.60±0.40	24.80±0.75	29.20±0.98	21.80±0.98

E. coli, P. aeruginosa. Maximum desired response has been observed by *E. coli* and *P. aeruginosa. B. subtilis, S. aureus, E. coli* and *P. aeruginosa* were susceptible to different concentrations of chloroform and ethanol extracts of *Achyranthes aspera*.

Hexane extract of *Abutilon indicum* L. exhibited moderate antibacterial activity against *M. luteas* and *S. aureus* whereas at 100mg/ml concentration the hexane extract showed noteable antibacterial activity against *E. coli.* Chloroform extracts (50 and 100mg/ml) exhibited antibacterial activity against *P. aeruginosa.* The antibacterial effect of chloroform extract of *Abutilon indicum* was progressive with the increase in concentration.

Ethanol extracts of *Albutilon indicum* L. exhibited antibacterial effect against *M. luteus.* This effect when compared with the standard antibiotics such as Ampicillin and Streptomycin, the measured diameter of zone of inhibition was closer to the Streptomycin than that of Ampicillin. Ethanol extracts at 5, 50 and 100mg/ml displayed maximum antibacterial activity against all the microorganisms used in the present investigation but still it was not equal or higher when compared with that of standard antibiotics ampicillin (1 mg/ml) and Streptomycin (l mg/ml).

The aqueous extract showed antibacterial activity against two Gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and two Gram negative bacteria (*E. coli, Salmonella choleraesuis*).

It is postulated that antibacterial activity in all extracts of *Abutilon indicum* L. except hexane could be due to the presence of any polar component like flavoniods and any of the flavonoid derivatives (Matalawska and Sikorska, 2002).

The acute and chronic irritant response was exhibited by the chlorofonn and ethanolic extracts of leaves of *Agave americana* L. Hexane extract did not exhibit any irritation. This irritant-effect is in accordance with the work carried out by Ricks and Voget (1999).

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Squamous Cell Carcinoma of the Penis and Prepuce with Pulmonary Metastasis in a Horse

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Abstract.- Squamous cell carcinoma of penis and prepuce is a fairly common and a potentially life threatening genital tumor of the horse. Metastasis of squamous cell carcinoma of penis and prepuce to the lungs, however, is relatively rare. This study describes a case of squamous cell carcinoma of penis and prepuce with pulmonary metastasis in a horse. This appears to be the first report of squamous cell carcinoma of penis and prepuce in horse from Pakistan.

Key words: Histopathology, tumor, lungs.

In equids squamous cell carcinoma (SCC) of the eye, penis and prepuce, vulva and perianal region and skin have been reported (Valentine, 2006). Squamous cell carcinomas of penis and prepuce are the most important and commonly diagnosed genital tumors of horses (Valentine, 2006; Brinkso, 1998; Howarth *et al.,* 1991; Fortier and Mac Harg, 1994; Mair *et al*., 2000). The exact aetiology of SCC of penis and prepuce in horse is uncertain. In human beings, phimosis, chronic inflammatory conditions (balanitis), ultraviolet light irradiation, smoking and infection with Human papilloma virus have been identified as the key risk factors for the development of SCC of penis (Dillner *et al.,* 2000). Smegma is believed to contribute to the development of SCC in equids (Plaut and Kohn-Speyer, 1947). Persistent phimosis or repeated injury to the penis and prepuce may also play a role in the development of this lesion (MacFadden and Pace, 1991). Although genital SCC is often a very locally invasive disorder in equids, its metastasis to the local lymph nodes (Murray, 1978; Fortier and MacHarg, 1994) and even rarely to the lungs has been reported (Murray, 1978; Moulton, 1978; Philson, 2006). A survey of equine cutaneous tumors in the Pacific Northwest in various horse breeds has indicated that the incidence of SCC of penis and prepuce is highest in Appaloosa horses followed by Paint and American Quarter Horse breeds. Older horses are more susceptible to SCC of penis and prepuce (Valentine, 2006). SCC of penis and prepuce has responded well to the topical application of 5-fluorouracil and radiotherapy (Fortier and MacHarg, 1994; Pizzocaro *et al*., 1997). Surgical treatment (*e.g*. penile amputation and urethrostomy) of SCC lesions on the penis and prepuce, if diagnosed early in the course of disease, provides satisfactory results (Howarth *et al.,* 1991). Recurrence of SCC lesions on the penis and/or prepuce has been reported in 19% of horses after surgical treatment (Mair *et al*., 2000). This short communication describes a SCC of the penis and prepuce with pulmonary metastasis in a horse (Moulton, 1978; Philson, 2006). This appears to be the first report of squamous cell carcinoma of the penis and prepuce in a horse from Pakistan. SCC has been reported in horses from the USA (Philson, 2006), United Kingdom (Mair *et al*., 2000), The Netherlands (Top *et al*., 2008), and Australia (Kerr and Alden, 1974).

Materials and methods

An eleven year old stallion was presented to the Indoor Hospital (Surgery Section), University of Veterinary and Animal Sciences, Lahore, Pakistan for the evaluation of a swelling on the penis of

approximately 10 days duration. However, the penis was not routinely examined and the lesion may have been of longer duration. When first observed, the skin over the lesion was ulcerated, bleeding, and infested with maggots (Fig. 1). The ulcerative lesion was approximately 10 cm in length and 3 inches in width. Physical examination revealed a temperature of 101°F, heart rate of 38 beats per minute, and respiratory rate of 10 breaths/minute. There was slight bilateral nasal discharge with no other respiratory abnormalities. Abnormal posture while urinating was consistent with stranguria. On palpation, the penis was from hard to firm but not painful. The horse was initially treated with systemic antibiotics, corticosteroids, and an antiseptic dressing. The lesion was treated with topical povidone iodine as an antiseptic and copper sulphate solution to cauterize the area. Parenteral treatment included antibiotics and non-steroidal anti-inflammatory drugs. Over two weeks time, the lesion increased in size until it extended proximally to the area of the brisket. Due to rapid progression of the lesion, failure to respond to treatment and poor prognosis, the horse was euthanised.

Results and discussion

Postmortem examination revealed a fibrous lesion that extended on the ventral midline from pelvis to brisket. An area of chronic inflammation was observed extending from the cranial side of the prepuce to the brisket over the midline. The lungs contained nodular lesions.

Histopathological evaluation of the lesion on the penis and prepuce revealed marked epithelial disruption, hyperaemia, leukocytic infiltration, early fibrosis and horn pearls of squamous cells (Fig. 2), consistent with the diagnosis of SCC. Metastasis of the tumor was observed in the lung tissue in the form of horn pearls, most often near the bronchioles. Non-specific changes including sloughing of bronchiolar epithelium, severe haemorrhage, and transudate (Fig. 3) were also observed (Moulton, 1978).

There was no evidence of concurrent habronemiasis. Moreover, acid fast staining of impression smears from lung tissues revealed no evidence of tuberculous bacteria.

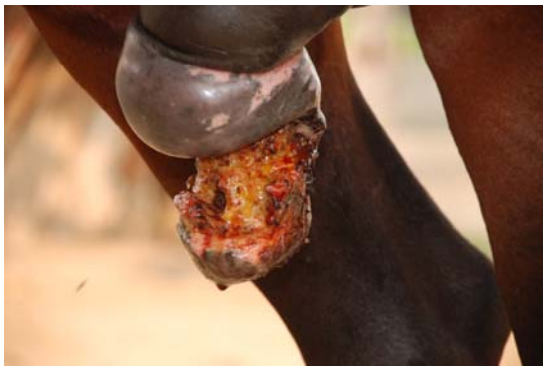

Fig. 1. Squamous cell carcinoma of penis and prepuce in a horse.

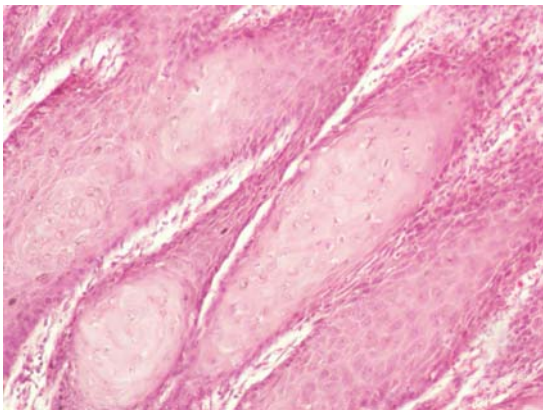

Fig. 2. Histological structure of squamous cell carcinoma showing epidermal cells are concentrically arranged leading to horn pears formation. (Hematoxylin and eosin stain; X100).

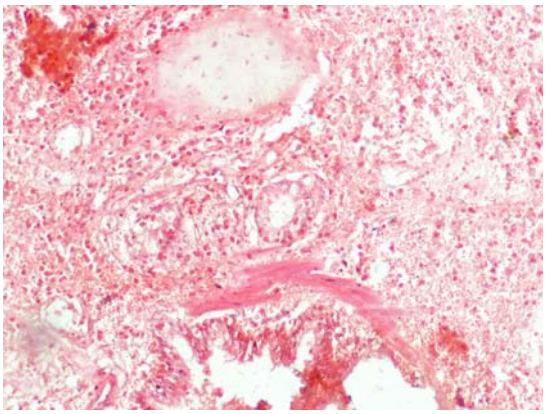

Fig. 3. Histology structure of lung from a horse showing metastasis from squamous cell carcinoma of preputial skin (Hematoxylin and eosin stain; X100).

This case is typical of SCC of the penis and prepuce which is fairly common in older horses that accumulate excess smegma and is a potentially life threatening condition (Valentine, 2006; Moulton, 1978; Philson, 2006). However, pulmonary metastasis with SCC in horses is relatively uncommon (Top *et al.,* 2008). Recent studies on penile and preputial tumors of equids included radiographic evaluation of the subjects and have suggested that pulmonary metastasis is uncommon with such tumors (Philson, 2006; Top *et al.,* 2008). Moreover, penile SCC in younger geldings (<10 years of age) is more likely to be malignant than in older geldings (Knottenbelt, 2003). The present case, therefore presents a rare picture in terms of metastasis of SCC of penis and prepuce to lungs in an elderly horse.

Proper care, regular examination and cleaning of the equine penis, especially removal of semegma, may help in preventing development of SCC of penis and prepuce (MacFadden and Pace, 1991). Timely diagnosis often results in successful treatment, prolonging the horse's life (Brinkso, 1998; Howarth *et al.,* 1991). Early detection and treatment are therefore of utmost importance.

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Anax indicus Lieftinck, 1942 (Odonata: Anisoptera: Aeshnidae) an Addition in the Fauna of Pakistan

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Abstract.- *Anax indicus* Lieftinck is recorded for the first time from Pakistan; it is the fourth species of the genus to be recorded from the country. A key to all species of *Anax* known from Pakistan is presented.

Key words: Lectotype, odonate anatomy, dorsolateral spot, standing water, Khasala Dam.

The genus *Anax* Leach has a cosmopolitan distribution (Fraser, 1936). Six species of *Anax* are known from India; five were listed by Fraser (1936), Lieftinck later added *A. indicus* Lieftinck, 1942 to the known fauna. Three species of *Anax* have been reported from Sri Lanka (Bedjanič *et al.,* 2007) and three species from Pakistan (Yousaf, 1972; Kanth, 1985; Ahmad, 1994; Rehman, 1994; Jehangir, 1997): *A. immaculifrons* Rambur, 1842, *A. nigrofasciatus nigrolineatus* Fraser, 1935 and *Anax*

parthenope Selys, 1839. In this paper we present the first record of a fourth species of *Anax* from Pakistan, discovered in District Rawalpindi in 2006.

District Rawalpindi (Fig. 1) is situated in the lowlands of the Southern Himalayan slope (Faisel, 2006). The district lies between 33^{O} and 34^{O} N and 72 ^{O} and 74 ^{O} E, and shares boundaries with Hazara division to the north, Poonch to the east, Jhelum district to the south and Attock district to the west. The altitude in the district ranges from 1,500 ft. at Gujar Khan and 1750 ft. at Rawalpindi to 7,500 ft. at the hill station of Murree (Zafar *et al.,* 2006).

Materials and methods

A single male *Anax* (Fig. 2) was collected while perched on vegetation at Khasala dam, Rawalpindi, Pakistan on 14 July 2006 by the first author. The Khasala dam is 27 km from Rawalpindi on a stream called Khasala Kas. The coordinates of the site are 33° 20′N, 72° 58′E. The elevation of the watershed ranges from 381 to 427 m above sea level (Ashraf *et al.,* 2007).

The specimen was identified as *Anax indicus* by comparing it with the descriptions in Lieftinck (1942, 1955); a photograph of the specimen was compared with material, including the lectotype of *A. indicus* and material of the similar *A. guttatus* Burmeister, 1839 in the collection of National Museum of Natural History, Leiden, the Netherlands, by R.A. Dow. This species has not previously been recorded from Pakistan.

Terminology for odonate anatomy used here follows that of Fraser (1936).

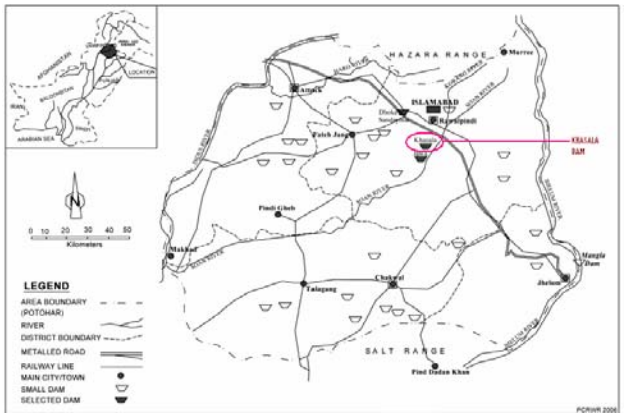

Fig. 1 Map of Pothwar region showing Rawalpindi. (Ashraf *et al.,* 2007)

Fig. 2. *Anax indicus.*

Key to *Anax* species of Pakistan

1 Sides of thorax with broad black markings..... .....*immaculiforns*
Sides of thorax without broad black markings..... 2
2 Abdomen with large orange coloured dorsal-lateral markings .....*indicus*
Abdomen without large orange coloured dorsal-lateral markings 3
3 Frons with a T shaped dark mark, superior anal appendages sharp at the end.....*nigrofasciatus*
Frons without T shaped dark mark, superior anal appendages rounded at the end ..... *parthenope*

Descriptive note

Abdomen: 61mm (anal appendages included), forewing: 53mm, hindwing: 50mm

Labium black. Labrum, face and frons light brown. Pterostigma of all wings dark brown. 18-19 antenodal, 8-9 postnodal and 11 antenodal, 11 postnodal nerves present in fore-wings and hind-wings respectively. Discoidal cells in forewings made up of six cells and in hindwings made up of five cells. Four cubital nerves are present in forewings and three in hindwings. Membrane dark brown or blackish. Segment 2 of the abdomen has a blue dorsolateral spot. Segment three without blue markings. Segments 4-10 have large, bright dorsolateral orange markings. A large pale brown patch is present on the hind-wings. The anal appendages are reddish brown.

Remarks

Anax indicus is the fourth species of the genus to be recorded in Pakistan. It is a poorly known species, but it has previously been recorded from India (Lieftinck, 1942, 1955), Sri Lanka (Lieftinck, 1955), Nepal (*e.g*. Vick, 1989) and Thailand (*e.g.* Hämäläinen, 2002). As per key of Fraser (1936), this species was under-recorded in India.

Anax species typically breed in standing waters (Fraser, 1936), so it is not surprising to find this species at the Khasala Dam.

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Morphometric Characters and Their Relationships in *Gudusia chapra* (Hamilton) from Keenjhar Lake (Distt: Thatta), Sindh, Pakistan

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Abstract.- Morphometric characters and their relationships in *Gudusia chapra* (Hamilton) have been described. The fork length, dorsal fin length, pectoral fin length, body depth and head length of the fish were found to be highly correlated with its total length, whereas the eye diameter, snout length and post orbital head length were highly correlated with the head length of the fish.

Key words*:* Morphometric characters, *Gudusia chapra*, Keenjhar Lake.

Measurements of the various morphological body parts have been extensively used in the identification of the species of fishes (Lashari *et al*., 2004; Hoque and Rahman, 1985). The present study was undertaken to observe various morphometric characteristics and their relationships in *Gudusia chapra* (Hamilton), a common freshwater fish of Pakistan. The present paper describes the rate of growth of different body parts of the fish in relation to its total length.

No published information related to present aspect is available from Pakistan. Various workers from India and Bangladesh like (Day, 1878, 1889; Chonder, 1977; Shafi and Quddus, 1982; Hoque and Rahman, 1985) have reported morphometric measurements of this important fish.

Materials and methods

For the present study a total number of 189 specimens of *G. chapra* with total length ranging from 110 mm to 185 mm were collected by random sampling from Keenjhar lake (Distt: Thatta), Sindh during the months of July through November 2006. All the measurements, except eye diameter, were taken on a millimeter scale. The eye diameter measurement was taken with the help of vernier calipers. The following measurements were made from each fish: (1) Total length (TL): the greatest length from the anterior most extremity of the fish to the end of the caudal fin; (2) Fork length (FL): length from the anterior most extremity of the fish to the tip of the median ray of the caudal fin; (3) Dorsal fin length (DFL); (4) Pelvic fin length (Pel. FL); (5) Pectoral fin length (Pec. FL); (6) Body depth (BD); (7) Head length (HL): from the tip of the snout, when the mouth is closed, to the posterior edge of the opercular bone; (8) Eye diameter (ED): the greatest distance between the free orbital rims; (9) Snout length (SL): from the anterior most extremity of the fish to the hind margin of the orbit; and (10) Post orbital head length (POHL): the greatest distance between the hind margin of the orbit and the bony opercular margin as suggested by Lowe McConnell (1951). For the convenience of statistical analysis the fish samples were divided into 10 mm length groups. To explain the relationship between various body measurements least square methods given by LeCren (1951) was followed. The rate of growth of different morphological body parts is given in percentage (%).

Results and discussion

Data related to the various body measurements of the fish (*Gudusia chapra*) are presented in Table I. The following mathematical equations were obtained in regression analysis between:

TL and FL:	FL = 0.00952 + 0.92443 TL, and r = 0.992
TL and DFL:	DFL = 0.90012 + 0.77821 TL, and r= 0.998
TL and Pec. FL:	Pec. FL=0.99481+1.04111 TL, and r=0.996
TL and Pel. FL:	Pel. FL=9.21834+4.79213 TL, and r=0.982
TL and BD:	BD = 0.59264 + 0.99216 TL, and r = 0.994
TL and HL:	HL = 0.34821 + 0.98316 TL, and r = 0.996
HL and ED:	ED=63.30921+29. 88714 HL, and r=0.974
HL and SL:	SL = 3.99722 + 3.90218 HL, and r = 0.992
HL and POHL:	POHL=0.26159+1.10001 HL, and r=0.989

Table II.- The rate of growth of different body parts in *Gudusia chapra* (Hamilton) from Keenjhar lake (Distt: Thatta), Sindh, in relation to the total length of fish.

TL Group	Growth of different variables at different length groups (%)							
10 mm	DFL	Pec. FL	Pel. FL	BD	HL	ED	SL	POHL
110	14.54	15.54	10.90	25.45	24.54	6.0	4.09	14.54
120	14.16	15.0	10.83	25.0	24.16	6.0	4.16	14.16
130	13.84	14.61	10.0	24.61	23.84	6.53	4.23	13.07
140	13.57	14.28	9.28	24.28	23.57	6.42	4.28	12.85
150	13.33	14.66	9.33	24.0	23.33	6.33	4.33	12.66
160	13.12	14.37	9.37	23.75	23.12	6.25	4.37	12.50
170	13.52	14.70	9.41	24.11	22.94	6.17	4.41	12.35
180	13.33	14.44	9.44	23.88	23.33	6.11	4.44	12.22
190	13.15	14.37	9.47	22.63	22.63	6.05	4.47	12.10

BD, body depth; DFL, dorsal fin length; ED, eye diameter; FL, fork length; HL, head length; Pec. FL, pectoral fin length; Pel. FL, pelvic fin length; POHL, post orbital head length; SL, standard length; TL, total length.

The reliability of the above equations would be seen to be high from the co-efficient of correlation (r) values in all cases. From the regression equations it is evident that FL, DFL, Pec. FL, Pel. FL, BD and HL are highly correlated with TL, while, ED, SL and POHL are highly correlated with HL and the relationships in between body measurements are linear. Similar linear relationship was also obtained by Ganguly *et al*. (1959) in *Lates calcarifer,* Mehta and Bapat (1977) in *Ophicephalus gachua,* Prakash and Verma (1982) in *Notopterus notopterus,* Chonder (1977) and Hoque and Rahman (1985) in *Gudusia chapra,* and Lashari *et al*. (2004) in *Cirrhinus reba*.

The rate of growth of different morphological body parts of the fish in relation to the total length are presented in percentage and shown in Table 2. From the table it is evident that in *G. chapra* the growth rate of DFL, BD and HL decreased gradually till 160 mm length group, for both DFL and BD, and till 170 mm length group for HL were reached and then increased suddenly, which was immediately followed by a decreasing rate of growth with the increase in TL. The growth rate of Pec. FL and Pel. FL decreased gradually till 140 mm length group was reached after which they showed an increasing rate of growth and POHL showed a decreasing rate of growth with the increase in TL. On the other hand growth rate of ED increased suddenly in the 130 mm length group followed immediately by a decreasing rate of growth with the increase in TL. Literature available regarding the growth of the various variables in relation to the total length indicates that in fishes the growth of various morphological body parts varies from species to species. In case of *Gudusia chapra* Hoque and Rahman (1985), Chonder (1977) and in *Notopterus notopterus* Prakash and Verma (1982) stated that the growth rate of the pectoral fin first increases with the increase in total length and later decreases with the increase in total length. On the other hand, growth rate of the dorsal fin first decreases with the increase in total length and later increases with the increase in total length which accords with the present findings in *Gudusia chapra* from Keenjhar lake (Distt: Thatta), Sindh, Pakistan.

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Table I.- **Morphometric measurements of various body parts of *Gudusia chapra* (Hamilton) from Keenjhar Lake (Distt: Thatta), Sindh.**

TL (mm)	No. of fish	FL	DFL	Pec. FL	Pel. FL	BD	HL	ED	SL	POHL
110	2	91± 1.01	16 ± 0.25	17 ± 0.45	12 ± 0.05	28 ± 0.50	27 ± 0.55	7 ± 0.50	4.5± 0.25	16 ± 0.15
120	9	100± 0.50	17 ± 0.55	18 ± 0.22	13 ± 0.15	30 ± 0.20	29 ± 0.40	8 ± 0.33	5 ± 0.50	17 ± 0.20
130	38	106±1.09	18 ± 0.65	19 ± 0.33	13 ± 0.25	32 ± 0.33	31± 0.95	8.5 ± 0.45	5.5 ± 0.20	17 ± 0.55
140	63	112±0.20	19 ± 0.10	20 ± 0.20	13 ± 0.33	34 ± 0.55	33 ± 0.85	9 ± 0.90	6 ± 0.80	18 ± 0.45
150	46	119±0.90	20 ± 0.33	22 ± 0.55	14 ± 0.20	36 ± 0.15	35 ± 0.50	9.5± 0.55	6.5 ± 0.55	19 ± 0.50
160	17	128± 1.02	21 ± 0.45	23 ± 0.44	15 ± 0.12	38 ± 0.25	37 ± 0.65	10± 0.80	7 ± 0.65	20 ± 0.40
170	10	137±0.77	23 ± 0.55	25 ± 0.60	16 ± 0.15	41± 0.33	39 ± 0.85	10.5 ±0.20	7.5 ± 0.55	21 ± 0.20
180	2	145±0.33	24 ± 0.20	26 ± 0.10	17 ± 0.35	43 ± 0.10	42 ± 0.33	11± 0.60	8 ± 0.65	22 ± 0.15
190	2	157±0.55	25 ± 0.10	28 ± 0.20	18 ± 0.10	45 ± 0.15	43 ± 0.65	11.5± 0.20	8.5 ± .20	23 ± 0.15

BD, body depth; DFL, dorsal fin length; ED, eye diameter; FL, fork length; HL, head length; Pec. FL, pectoral fin length; Pel. FL, pelvic fin length; POHL, post orbital head length; SL, standard length; TL, total length.