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STOMACH AND HEART MUSCLES NATURAL RESPONSES. 2- IN
DIFFERENT PHYSIOLOGICAL SOLUTIONS.**

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ABSTRACT: The present investigations were conducted to study the effect of fungal culture broths of both *Aspergillus flavus* (*A. flavus*) and *Aspergillus niger* (*A. niger*) on the toad *Bufo tibiamicus* stomach and heart muscles spontaneous activity (Sp.Act) in different physiological solutions. All tested solutions and fungal growth medium (G.M.) have different ionic compositions which affected the responses of tissues. In additions, both muscles suspended in Zamzam water (Z.W.) clearly showed muscle contractures and increased heart responses, while the effect of other solutions on both muscles was not strong as that of Z.W. and they are as follows: distilled water (D.W.), tap water (T.W.) and Ca^{2+} -free saline (No/Ca) respectively. On the other hand, both fungal culture broths exerted toxic effect on toad stomach and heart muscles. Addition of *A. flavus* culture broth was more effective on the heart muscles as an inhibitory agent except those suspended in D.W., whereas it was without any effect on the stomach muscles. However, addition of *A. niger* culture broth induced contracture of the stomach muscle except those suspended in Z.W.. In addition, *A. niger* culture broth induced muscle contractures and it showed a transitional stimulatory effect on the heart muscles except those immersed in Ca^{2+} -free saline. The present studies also showed that the transient actions of *A. niger* culture broth additions on both muscle responses can be noticed in solutions having Mg^{2+} -ions and low or free Ca^{2+} -ions. This action indicates that this fungal broth may affect both extracellular and intracellular environments especially on stomach muscles. However, the effect of both G.M. and *A. flavus* culture broth additions on both muscle responses may indicate that they are Ca^{2+} -dependent. Moreover, these results are in agrrement with those previously obtained by Mutwally and Mahmoud (Under publication). In conclusion, the different actions of various physiological solutions, G.M. and both fungal culture broths on both muscles toad suspended in those various solutions may indicate that

they may have affected different ion-channels and / or different receptors due to their differences in ionic compositions of the tested solutions and G.M., as well as muscular and innervation systems of both tissues.

INTRODUCTION

Most of the studies on the amphibian muscles were concentrated on skeletal muscles (Lorkovic, 1967; Stuesse, et al, 1974; Frank , 1979) and cardiac muscles (Tjioe & Bianchi, 1969; Hussein, et al, 1992). However, little work was carried on the smooth muscles (Kitazawa, et al, 1986). When moulds such as *Aspergillus* are grown on relatively rich media in laboratory culture it is frequently observed that they produced a range of metabolites, some of which are retained in the mycelium and fruiting structures, but the majority of which are secreted into the media. *A. niger* was found to produce malformin- A_1 , which have been shown to be toxic to mammals (Anderegg, et al, 1976; Moss, 1977). They reported that the isolation and characterisation of malformin from *A. niger* with a significantly higher malforminic toxicity (i.p. LD₅₀ 0.9mg/Kg b.w. newborn rats and 0.87mg/Kg b.w. in the case of 28 days).

Most of the work done on *A. flavus* was in the field of *Aflatoxin production*. However, to the best of our knowledge no previous work was obliged on the tremogenic effect exerted by *A. niger* extracts. In the course of screening several strains of *A. flavus* isolated from contaminated corn and other food stuffs such as: oat, millet and rice or potatoes, a peculiar toxin syndrome was seen in mice after oral administration of the crude fungus extract (Wilsonn, 1971). Mice-trembling was caused by substances extracted from the mycelial mat and sclerotia of *A. flavus* but not from spores or from the culture broth (Betina, 1984). Ikegwuonu (1983) reported that aflatoxin- B_1 produced by *A. flavus*, resulted in neurotoxicity in rats. *Citreoviridin* is produced by *Penicillium citreoviridea*, *P. citrinum* as well as *A. terreus*. Moreover, *citreoviridin* is neurotoxin to several animals causing the same symptoms as in cute cardiac beriberi (Engel & Teuber, 1980).

Previous study of Mutwally and Mahmoud (Under publication) showed that both *A. flavus* and *A. niger* culture broths have an inhibitory and excitatory effect on

the toad *Bufo tibiamicus* stomach and heart muscle natural activity, K^+ - and FS-responses in normal amphibian saline. Addition of *A. flavus* culture broth enhanced normal activities of toad stomach muscles, while it inhibited natural activities of the heart muscles. This broth slightly inhibited K^+ -contractures of the stomach muscle affecting the tonic response, but was without significant effect on heart muscles K^+ -contractures and FS-responses. On the other hand, *A. niger* culture broth inhibited normal activities and severely inhibited K^+ -contractures and both phasic and tonic responses of toad stomach muscles, while it enhanced natural activities of the heart muscles, but was without significant effect on heart muscles K^+ -contractures and FS-responses. In general, both fungal culture broths exerted toxic effect on toad stomach and heart muscles. *A. flavus* culture broth was more effective on the heart as an inhibitory agent, whereas it has an excitatory action on the stomach muscle. Whereas, *A. niger* culture broth has an inhibitory effect on the stomach muscle and an excitatory effect on the heart muscle. The present studies are conducted to investigate the effect of fungal culture broths on spontaneous activity (Sp.Act) of toad *Bufo tibiamicus* stomach and heart muscles in different physiological solutions.

MATERIALS AND METHODS

Mutwally (Under publication) studied the effect of different anions and cations of the present physiological solutions on the heart and stomach muscles response of *Bufo tibiamicus*, focusing on the elements which are known to be essential for muscle contractions and they are as follows: Cl^- , Na^+ , K^+ , Ca^{2+} and Mg^{2+} -ions, as they can be seen in Table 1. The elements were determined using flame photometer, spectrophotometer and titration methods following the methods of Belcher, et al (1970), Vogel (1971) and Badawy (Personal comunacations).

Experimental animals: Sexually mature male and female toads *Bufo tibiamicus* collected from Makkah area, Saudi Arabia, were used throughout this study. They were transported to the laboratory and kept in large glass aquaria with small amount of tap water (50ml) which were changed twice daily. Each toad was kept separtly in 500ml glass jar containing 50ml of normal amphibian saline which has the following compositions (g/l) : NaCl (20.16), KCl (0.555), Na_2HP0_4 (0.915), NaH_2PO_4 (0.398) and $CaCl_2$ (0.6615) adjusted to pH 7.1 using NaOH at room temperature ($\sim 27^\circ C$).

However, MgCl_2 (0.4066) and EGTA (0.761) were added in replacement of Ca^{2+} to make Ca^{2+} -free saline. Both salines were adjusted to pH 7.1 using NaOH at room temperature ($\sim 27^\circ\text{C}$) while other test solutions were directly used without any modifications.

The animals were then divided into 5 groups, each group consisted of 10 toads (in 500ml glass jar containing 50ml of each solution), as follows: The first group (Control) was kept in normal Ca^{2+} -saline (N.S.); the second was kept in Ca^{2+} -free saline (No/Ca); the third was kept in Zamzam well water (Z.W.); the fourth was kept in distilled water (D.W.) and the fifth was kept in tap water (T.W.). Each toad of all groups was kept separately in 500ml glass jar containing 50ml of above tested solutions at room temperature around $\sim 27^\circ\text{C}$.

Table 1: shows the 5 essential elements composition of different physiological solutions as ions in g/l:

IONS® SALINE ⁻	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}
N. S.	12.915	8.402	0.291	0.239	0.000
No/Ca	12.915	8.402	0.291	0.000	0.082
Z.W.	0.0333	0.0274	0.1877	0.7000	0.1824
D. W.	0.0053	0.00292	0.00096	0.0000	0.0000
T.W.	0.0131	0.0152	0.0216	0.3000	0.1216

The methods to record tensions and normal activities were those adopted by Oldfield and Huddart (1982), Mutwally and Jamel Al-Layl (1992 & 1993) with little modifications to suite the present study. All drugs and chemicals used in this study were obtained from Sigma Chemical Co., and were added to the organ baths from standard laboratory concentrates and freshly made up in distilled water just before use.

Muscle preparations: A toad was stunned and bled, and then the whole stomach and heart were removed and immediately immersed in normal amphibian saline. Each

specimen was ligated and suspended vertically in an aerated organ bath (25ml) whose contents could be rapidly changed. To record the isometric contractions, both tissues were connected to an isotonic transducer whose output was fed into a Washington Instruments MD 400/2 ink-writing oscillograph via an FC 137 coupler, and these were adjusted to put a slight passive tension on the preparations.

For stomach muscle, the pharyngeal region was connected to the transducer while the proventricular ligature was vertically attached to the glass hook in an organ bath (25ml). Whereas, for heart muscle, the auricular region was ligated to the transducer and the ventricular region was connected vertically to the glass hook. All experiments were run at room temperature (~27°C). The preparations were then allowed to equilibrate for 30-60 minutes in the organ bath, while normal saline was changed every 10-20 minutes. Salines could be removed from the organ baths by vacuum and replaced by gravity feed in less than 5 seconds. The contents of the organ baths were constantly aerated. All preparations were left in normal amphibian saline (1.5mM / 0.6615g/l) (control) for 30-60 minutes to develop Sp.Act before introducing the test solutions. There was a 10 minutes washout interval, with normal saline between each experiment.

The test fungi: The test strains of *Aspergillus flavus* and *Aspergillus niger* were isolated from imported Pakistani and Indian rice samples respectively. The isolation medium was potato dextrose agar (Difco). It has the following composition (g/l): Potato infusion from 200g, dextrose 20, and agar 15, with a final pH 5.1. The purified strains were kept on slants of the same medium. Both *A. flavus* and *A. niger* were identified according to Domsch, et al (1980).

The two test fungi were grown on Czapek's Dox broth medium (G.M.) which had the following composition (g/l): sucrose (30), NaNO₃ (3), K₂HPO₄ (1), MgSO₄ (0.5), KCl (0.4) and FeSO₄ (0.01) with pH 7.3. The test fungi were incubated at 25°C for 10 days; then only the filtrate of culture broths were used.

Table 2: Shows the elements composition in Czapek's Dox broth medium as ions in g/l:

IONS ®	Cl ⁻	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
MEDIA ⁻					
G.M.	0.190	0.812	0.659	0.000	0.101

To study the effect of G.M. before and after fungal growth (culture broths) on normal activities of toad stomach and heart muscles, they were separately added to the organ bath 3-5 minutes on both tissues. There was a 10 minutes washing interval between each response with normal saline.

RESULTS

Results presented in Figure 1 showed that the toad stomach muscle contracted naturally in normal saline, these contractions were inhibited in Ca²⁺-free saline. Addition of 1ml G.M. and *A. flavus* cultural broths were without any effect on the stomach muscles. However, addition of 1ml *A. niger* cultural broths induced muscle contractures. Stomach muscles suspended in Z.W. induced clear contracture, however, additions of G.M., *A. flavus* and *A. niger* culture broths were without any effect on this muscle (Figure 2). In Figure 3, the toad stomach muscle produced small contracture due to immersing in D.W., whereas addition of 1ml of G.M. and *A. flavus* were without any effect. However, addition of 1ml of *A. niger* culture broth induced large muscle contracture. Stomach muscles suspended in T.W. induced small contracture. However, only the addition of 1ml of *A. niger* induced large muscle contracture (Figure 4). In all cases, after washing with normal saline, the muscle tension returned to normal level.

The toad heart muscles contracted normally in both normal and in Ca²⁺-free salines, while the addition of 1ml G.M. slowed these responses. However additions of 1ml of both fungal culture broths severely inhibited these responses. The heart muscles resumed their activities after washout with normal saline (Figure 5). Water of Zamzam well stimulated heart muscles and increased their responses rate, while the addition of G.M. (1ml) slowed these responses, although, the addition of 1ml *A. flavus* cultural broths slightly inhibited these responses. However, addition of 1ml of *A. niger* culture broths only delayed heart responses for short period but the heart muscle

responses were not affected. The responses of the toad heart muscle were increased after washout with normal saline (Figure 6). The replacement of normal amphibian saline with D.W. increased heart muscle responses. Moreover, additions of 1ml of G.M., *A. flavus* and *A. niger* cultural broths caused more acceleration to heart muscle responses. In addition, *A. niger* culture broths also induced small contracture. After washing with normal saline the heart muscle responses returned to normal level (Figure 7). Immersing in T.W., the heart responses were increased and the addition of 1ml of G.M. and *A. flavus* culture broths inhibited these responses. However, addition of 1ml of *A. niger* culture broths induced small contracture and delayed heart responses for short period but were without significant effect on heart responses. After washout with normal saline the heart muscles resumed their activities (Figure 8).

DISCUSSION

The present investigations were conducted to study the effect of fungal culture broths of both *A. flavus* and *A. niger* on the Sp.Act of toad *Bufo tibiamicus* stomach and heart muscles suspended in different physiological solutions. The acute dependence of the fast twitch activity upon $[Ca^{2+}]_o$ for both initiation and maintenance of the response clearly shows that influx of $[Ca^{2+}]_o$ underlies the development of this response similar to that was seen in rat smooth muscles and locust visceral muscles respectively (Langton & Huddart, 1987; Mutwally & Jamel Al-Layel, 1992 & 1993). The absence in smooth muscle of the early fast Na^+ -channel has led to the spike-generating myocardial type slow-channel taking up the mantle of the fast rapidly-inactivating Ca^{2+} -channel in smooth muscle (Sperelakis, 1984), which determines transmembrane Ca^{2+} -influx during sustained depolarization, so maintaining the tonic component of the K^+ -response (Brading, et al, 1983; Huddart, et al, 1984).

In this study, further confirmation has been obtained by the observation that Ca^{2+} -free saline can lead to a drastic fall of muscle contraction in toad muscles, this occurs somewhat more rapidly when EGTA is present. The greater ease with which the effects of Ca^{2+} -depletion may be reversed if the depletion time is brief and this may be related to metabolic deterioration. Jenden and Reger (1963) reported that it is possible that Ca^{2+} -ions or other bivalent metal ions are required in the extracellular

fluid for the maintenance of some property upon which the inward transmission of excitation from the cell membrane depends upon $[Ca^{2+}]_o$.

The effect of changes in the $[Na^+]_o$ or the $[Ca^{2+}]_o$ on the contractile responses of the heart can be interpreted in terms of Na^+ - Ca^{2+} exchange across the sarcolemma (Chapman, 1979). This dependence of the Ca^{2+} -movements on $[Na^+]_o$ and $[Na^+]_i$, has been examined by Reuter (1974). He found that in guinea pig atria the movement of Na^+ and Ca^{2+} are coupled to yield an exchange ratio of two Na^+ -ions for each Ca^{2+} -ion. These cation fluxes are probably not directly dependent upon metabolism, but could be due to the activity of the ATP-dependent Ca^{2+} -pump (Jundt & Reuter, 1974). In frog ventricular muscle, Goldman and Morad (1977) suggested that an involvement of K^+ -ions rather than Na^+ -ions in an exchange for Ca^{2+} -ions. There are two systems which operate to relax amphibian atrial trabeculae: One is slow, unaffected by either membrane potential, the $[Ca^{2+}]_o$ or the $[Na^+]_o$ and requires the integrity of the cell metabolism and is probably the SR; the other which requires little energy, is affected by the membrane potential, $[Ca^{2+}]_o$ and $[Na^+]_o$, therefore most probably represents the activity of Na^+ - Ca^{2+} exchange. This shows that when the Na^+ - Ca^{2+} exchange is inactive, repolarization-induced relaxation is also blocked (Chapman, 1973 & 1979). In an amphibian heart, the intracellular stores, which are probably the SR and other intracellular loci, may contain sufficient Ca^{2+} to activate contraction but the discharge of this Ca^{2+} into the sarcoplasm is only effective in initiating contraction when the activity of the Na^+ - Ca^{2+} exchange is reduced (Chapman, 1979).

The strong dependence of visceral muscles on $[Ca^{2+}]_o$ for the maintenance of activity has led to the view that influx of Ca^{2+} to the cells is an essential requirement for the excitation-contraction (EC) coupling mechanisms (Cheng, 1976; Huddart, et al, 1984). Previous study of Mutwally and Mahmoud (Under publication) showed that the stomach muscles of the toad responded to K^+ -additions and the responses were dose-dependent. However, above $80mMK^+$, the relation-ship between excitation-contraction was uncoupled.

Hurwitz, et al (1980) and Hurwitz (1986) reported that many smooth muscles, can be stimulated to contract by exposing them to a membrane-depolarizing high K^+ -

bathing medium, which activated at least two different types of voltage-dependent (or K^+ -activated) Ca^{2+} -channels in the smooth muscle cell. Ratz and Flaim (1982) concluded that Ca^{2+} -channels in a high K^+ -medium may play an important role in smooth muscle cells that normally contract in response to graded depolarizations of the plasma membrane. Batra (1976) reported that the final outcome in the contractile activity of the muscle will be governed by the level of the free Ca^{2+} in the myoplasm.

The action potential of the frog heart was reported to be affected by alterations of external K^+ (Pappano & Rembish, 1970). At low concentration of $[K^+]_o$, the normal heart activities were inhibited while at $80mMK^+$ -induced large contraction (Mutwally & Mahmoud, Under publication). It was reported that the total Ca^{2+} -content of heart muscle is close to that found in the plasma (Sands & Winegrad, 1970). It is this Ca^{2+} which were ionized and free in the sarcoplasm due to K^+ -depolarization and field stimulation, the contractile apparatus would be fully activated (Chapman, 1979). There is evidence of two mechanisms which operate to maintain a low $[Ca^{2+}]_i$; an active Ca^{2+} -pump and an ionic exchange system in the cardiac system which are: (a) The membrane Ca^{2+} -pump, and (b) The Na^+ - Ca^{2+} exchange (Chapman, 1973 & 1979).

Betina (1984) reported that mice-trembling was caused by substances extracted from the mycelial mat and sclerotia of *A. flavus* but not from spores or from the culture broth. The present study also showed that both preparations contracted naturally in normal saline, but the addition of the G.M. slightly slowed the rhythm of these normal activities. This action may be due to differences in ionic composition between G.M. and normal amphibian saline as well as to substances secreted in the culture broths.

Mutwally and Mahmoud (Under publication) showed that in normal amphibian saline, the addition of *A. flavus* culture broth on stomach muscle accelerated the Sp. Act and inhibited heart muscle responses. On the contrary, *A. niger* culture broth inhibited Sp. Act and accelerate the heart beats. They indicated that both tissues may depend on $[Ca^{2+}]_o$ and may also suggest that the G.M. may slow ions-exchange through cell membrane, while the substances secreted in both fungal culture broths may affect cell membrane and other cellular loci. Moreover, Lancaster, et al

(1961) speculated that *A. flavus* culture broth may contain one or more factors (may be aflatoxin-B₁), which may activate intracellular Ca²⁺-stores of the toad stomach muscle, whereas, it may inactivate these intracellular Ca²⁺-stores of the toad heart muscle. However, Anderegg, et al (1976) concluded that *A. niger* culture broth may contain one or more factors (may be malformin-A₁), which may affect Ca²⁺-channels, cellular membrane, cellular loci, and / or other ion-channels. Results obtained in the present studies are in agreement with the previous results.

In this present studies, it could be concluded that the cultural broths of both fungi exerted toxic effect on toad stomach and heart muscles. *A. flavus* culture broth was more effective on the heart as an inhibitory agent, whereas it has an excitatory action on the stomach muscle. On the other hand, *A. niger* culture broth inhibited stomach muscle responses but it increased heart muscle responses. These two different actions of both fungal culture broths on both muscle preparations may indicate that they may affect different ion-channels and / or different receptors which may confirm previous studies of Young (1933) and Tjioe and Bianchi (1969).

In addition, when the toad stomach muscles were immersed in D.W. and T.W. they produced slight contractions, while when they were in Z.W. they evoked larger contractions. However, the heart tissues natural activities were not affected by the absence of [Ca²⁺]_o and these normal activities were clearly increased in Z.W. and D.W. solutions. In the present study, the stomach muscle natural responses disappeared in Ca²⁺-free saline and both G.M. and *A. flavus* cultural broths (1ml) were without any effect, while *A. niger* culture broth (1ml) induced muscle contraction. This action may indicate that stomach muscle responses, and the effect of G.M. and *A. flavus* cultural broths are Ca²⁺-dependent. However, heart muscles retained their normal activities and even additions of G.M. and fungal culture broths were without effect in Ca²⁺-free saline, while additions of both fungal cultural broths inhibited heart responses. These actions may indicate that heart muscle responses were not Ca²⁺-dependent. Moreover, the inhibitory effect of cultural broths may affect both cellular membrane and SR. In amphibians the vagus and sympathetic nerves had motor effects on the organs which they innervated such as the stomach and the heart (Young, 1933, Burnstock, 1969). However, the present studies showed that both tissues responded

differently. This could be due to differences in muscular structures and innervation system orientations.

A number of diverse cellular function contractions are known to be regulated by fluctuations in the free Ca^{2+} -ions concentrations in the cytosol (Huddart, 1975). Hurwitz (1986) reported that the ventricular muscles from guinea pig heart appeared to possess multi ion Ca^{2+} -channels in its pore. Monovalent cations, such as Na^+ and K^+ also exhibit an affinity for the Ca^{2+} -channel binding site and can penetrate its aqueous pore (Reuter & Scholz, 1977). In frog skeletal muscles, there are various types of K^+ -channel, one of them is Ca^{2+} -dependent K^+ -channel, which opens following increases in $[\text{Ca}^{2+}]_i$ (Stefani & Chiarandini, 1982). Elevated external K^+ -stimulated active Na^+ -transport of frog sartorius muscles (Hays & Connett, 1978). In Ca^{2+} -free saline, the K^+ -contracture of frog skeletal muscle was reduced (Frank, 1960). The final outcome in the contractile activity of the muscle will be governed by the level of free Ca^{2+} in the myoplasm (Batra, 1976).

Worcel, et al (1976) and Mutwally (1994) respectively reported that both rat uterine smooth muscles and locust visceral muscles, when Ca^{2+} -ions were suppressed from the bathing solutions in the presence of Mg^{2+} , spontaneous contractions disappear. In addition, Doge and Rahamimoff (1967) and Balnave and Gage (1973) concluded that Mn^{2+} -ions and Mg^{2+} -ions might inhibit secretion of transmitter by 'competing' with Ca^{2+} -ions so that the influx of Ca^{2+} -ions in response to membrane depolarization of excitation-secretion coupling is reduced. Moreover, Lorkovic (1967) concluded that in the presence of $5\text{mM}\text{Mg}^{2+}$ and Sr^{2+} the area under the contracture curves and the mechanical threshold were decreased, i.e., these two ions acted like Ca^{2+} at a lower concentration. In the present study Ca^{2+} -free saline, T.W. and G.M. decreased muscle responses. This could be due to presence of Mg^{2+} -ions and absence of Ca^{2+} -ions. On the contrary, T.W. contains Mg^{2+} -ions (0.1216g/l) and Ca^{2+} -ions (0.3g/l), this difference in ionic composition did not prevent Mg^{2+} -ions inhibitory effect. On the otherhand, Z.W. contains Mg^{2+} -ions (0.1824g/l) and also Ca^{2+} -ions (0.7g/l), but the difference in ionic composition here may abolish the inhibitory effect of Mg^{2+} -ions.

Adrian (1956 & 1969) reported that at rest, Cl^- and other K^+ channels are responsible for the dominant conductance. They also proposed the existence of aqueous channels for Cl^- in *Xenopus*. Moreover, Adrian (1969) and Hodgkin and Horowicz (1960a & b) suggested that in mammalian and frog muscle fibers, Cl^- -ions contribute the major share to the resting membrane conductance. In frog skeletal muscle Cl^- -channels are mainly located on the surface membrane. On the other hand, at least 60% of Cl^- -conductance which is time-dependent, is located in the TS in mammalian muscle fibers and is markedly dependent on external pH which increases in alkaline pH and decreases at acid pH. They are about 7.0 pH in frog and 5.5 pH in mammals (Adrian, 1969; Huddart, 1975; Stefani & Chiarandini, 1982). These chloride conductance may be inhibited by aromatic carboxylic acids by binding to a specific intramembrane site and altering the selectivity of the channel (Stefani & Chiarandini, 1982).

All tested solutions and fungal growth medium (G.M.) have different ionic compositions which affected both tissues responses. In addition, both muscles suspended in Zamzam water (Z.W.) clearly induced muscle contractures and increased heart responses, while the effect of other solutions on both muscles were not strong as Z. W. and they are as follows: distilled water (D.W.), tap water (T.W.) and Ca^{2+} -free saline (No/Ca) respectively. Results obtained in this present study and from Table 1 and 2, indicated that the stomach muscles clearly showed slight contraction in presence of Z.W., while, the heart muscles clearly responded to the presence of all the used physiological solutions. However, they were strongly affected in the presence of both Z.W. and T.W..

Previous studies of Mutwally and Mahmoud (Under publication) showed that additions of both G.M., *A. flavus* and *A. niger* culture broths affected both tissues suspended in N.S.. They concluded that this action could be due to ionic differences. Although, the present results are in agreement with previous studies, yet the addition of G.M. and both fungal cultural broths were without effect on stomach muscles responses, although, they exerted a remarkable excitatory effect on the heart muscles suspended in D.W., Ca^{2+} -free, Z.W. and T.W. respectively. However, the addition of *A. niger* culture broths induced stomach muscle contractions which disappeared when

muscles were suspended in Z.W.. Moreover, the addition of *A. niger* culture broths stimulated the contraction of the heart muscles suspended in D.W. and T.W.. In all cases the effect of *A. niger* culture broth was transitional, i.e. it showed an initial inhibitory effect followed by a gradual increasing excitatory effect. This transient action was not seen with muscles suspended in Ca²⁺-saline. The present results are in agreement with those previously obtained by Mutwally and Mahmoud (Under publication). They also showed that the transient actions of *A. niger* culture broth additions on both muscle responses can be noticed in solutions have Mg²⁺-ions and low or free Ca²⁺-ions. This action indicate that this fungal broth may affect both extracellular and intracellular environments especially on stomach muscles. However, the effect of both G.M. and *A. flavus* culture broth additions on both muscle responses may indicate that they are Ca²⁺-dependent.

The different actions of various physiological solutions, G.M. and both fungal culture broths on both toad muscles suspended in those various solutions may indicate that they may affect different ion-channels and / or different receptors due to their differences in ionic compositions of the tested solutions, G.M., and muscular and innervation systems of both tissues.

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REFERENCES

ADRIAN, R.H. (1956) The effect of internal and external potassium concentration on the membrane potential of frog muscle. *J. Physiol.*, Vol. 133: 631.

ADRIAN, R.H. (1969) Rectification in muscle membrane. *Prog. Biophys. Molec. Biol.*, Vol. 19: 341-369.

ANDEREGG, R.J., Biemann, K., Buchi, G. and Gushman, M. (1976) Malformin-c, a new metabolite of *Asprgillus niger*. *J. Am. chem. soc.*, Vol. 98: 3365-3370.

BALNAVE, R.J. and GAGE, P.W. (1973) The inhibitory effect of manganese on transmitter release at the neuromuscular junction of the toad. *Br. J. Pharmacol.*, Vol. 47: 339-352.

BATRA, S. (1976) Mitochondrial calcium release as a mechanism for quinidine contracture in skeletal muscle. *Biochem. Pharmacol.*, Vol. 25: 2631-2633.

BELCHER, R., NUTTEN, A.J. and MACDONALD, A.M.G. (1970) Quantitative inorganic analysis. Butter Worths, London.

BETINA, V. (1984) Mycotoxins, production, isolation, separation and purification. Elsevier Science Pub., New York.

BURNSTOCK, G. (1969) Evolution of the autonomic innervation of visceral and cardiovascular systems in vertebrates. *Pharmacol. Rev.*, Vol. 21 no. (4): 247-324.

CHAPMAN, R.A. (1973) The ionic dependence of the strength and spontaneous relaxation of the potassium contracture induced in the heart of the frog. *J. Physiol.*, Vol. 231: 209-232.

CHAPMAN, R.A. (1979) Excitation-contraction coupling in cardiac muscle. *Prog. Biophys. Molec. Biol.*, Vol. 35: 1-52.

CHENG, J.T. (1976) Calcium-induced release of calcium in rectal smooth muscle of mice. *Jap. J. Pharmacol.*, Vol. 26: 73-78.

DODGE, F.A. and RAHAMIMOFF, R. (1967) Co-operative action of calcium ions in transmitter release at the neuromuscular junction. *J. Physiol., London*, Vol. 193: 419-432.

DOMSCH, K.H., GAMS, W. and ANDERSON, T.H. (1980) "Compendium of soil fungi". Academic Press (Publ.), London.

ENGEL, G. and TEUBER, M. (1980) In "Mycotoxins, production, isolation, separation and purification". Elsevier Science Pub., New York, pp. 306.

FRANK, G.B. (1960) Effects of changes in extracellular calcium concentration on the potassium-induced contracture of frog' skeletal muscle. *J. Physiol.*, Vol. 151: 518-538.

FRANK, G.B. (1979) Surface membrane bound calcium is the main source of trigger calcium for excitation-contraction coupling in vertebrate skeletal muscle fibers. *Proc. West. Pharmacol. Soc.*, Vol. 22: 309-319.

GOLDMAN, Y. and **MORAD, M.** (1977) Ionic membrane conductance during the time course of the cardiac action potential. *J. Physiol.*, Vol. 268: 655-695.

HAYS, E.T. and **CONNETT, R.J.** (1978) Effect of methylxanthines and elevated external potassium on high energy phosphate content in frog skeletal muscle. *Biochem. Pharmacol.*, Vol. 27: 2965-2968.

HODGKIN, A.L. and **HOROWICZ, P.** (1960a) Potassium contractures in single muscle fibres. *J. Physiol.*, Vol. 153: 286-403.

HODGKIN, A.L. and **HOROWICZ, P.** (1960b) The effect of nitrate and other anions on the mechanical response of single muscle fibres. *J. Physiol.*, Vol. 153: 403-412.

HUDDART, H. (1975) The comparative structure and function of muscle. Pergamon Press. Oxford.

HUDDART, H., LANGTON, P.D. and **SAAD, K.H.M.** (1984) Inhibition by papaverine of calcium movements and tension in the smooth muscles of rat vas deferens and urinary bladder. *J. Physiol.*, Vol. 349: 183-194.

HURWITZ, L. (1986) Pharmacology of calcium channels and smooth muscle. *Ann. Rev. Pharmacol. Toxicol.*, Vol. 26: 225-258.

HURWITZ, L., MCGUFFEE, L.J., LITTLE, S.A. and **BLUMBERG, H.** (1980) Evidence for two distinct types of potassium-activated calcium channels in an intestinal smooth muscle. *J. Pharmacol. Exp. Ther.*, Vol. 214: 574-580.

HUSSEIN, M.F., AHMED, N.A., ABOU EL-ALA, K.S. SALLAM, A.H. and **SHEHATA, Z.H.** (1992) Effect of temperature acclimatization on heart rate and

electrocardiogram of toad's heart (*Rana ridibunda*). *J. Egypt Ger. Soc. Zool. Comp. Physiol.*, Vol. 7 (A): 297-314.

IKEGWUNU, F.I. (1983) The neurotoxicity of aflatoxin-B₁, in the rat. *Toxicology*, Vol. 28: 247-250.

JENDEN, D.J. and **REGER**, R.F. (1963) The role of resting potential changes in the contractile failure of frog sartorius muscles during calcium deprivation. *J. Physiol. (London)*, Vol. 169: 889-901.

JUNDT, H. and **REUTER**, H. (1974) Is sodium-activated calcium efflux from mammalian heart dependent on metabolic energy?. *J. Physiol.*, Vol. 266: 78.

KITAZAWA, T., **FURUHASHI**, H. **UMEZAWA**, K., **MORIOKA**, M., **TEMMA**, K. and **KONDO**, H. (1986) Is there functional cholinergic innervation in the frog duodenum (*Rana catesbiana*)?. *Comp. Biochem. Physiol.*, Vol. 85C no. (2): 275-282.

LANCASTER, M.C., **JENKINS**, F.P. and **PHILIP**, J. (1961) Toxicity associated with certain samples of ground nuts. *Nature*, Vol. 192: 1095-1096.

LANGTON, P.D. and **HUDDART**, H. (1987) The involvement of fast calcium channel activity in the selective activation of phasic contractions by partial depolarization in rat vas deferens smooth muscle. *Gen. Pharmacol.*, Vol. 18 (1): 47-55.

LORKOVIC, H. (1967) Effect of some divalent cations on frog twitch muscles. *Amr. J. Physiol.*, Vol. 212 no. (3): 623-628.

MOSS, M.O. (1977) Aspergillus mycotoxins: In "Genetic and physiology of Aspergillus". (Smith, J.E. and Pateman, J.A., ed.), Academic Press, London, pp 499-524.

MUTWALLY, H.M.A. (1994) The effect of Mg²⁺ on the spontaneous activity of foregut and hindgut muscles of *Locusta migratoria*. *J. Fac. Edu. Ain Shams Univ. Cairo*, Vol. 19 (No. 1): 1-12

MUTWALLY, H.M.A. and Jamel Al-Layl, K.S. (1992) The effect of cyanobacterial neurotoxin on the locust *Locusta migratoria* foregut and hindgut visceral muscles. *J. Egypt Ger. Soc. Zool. Comp. Physiol.*, Vol.9 (A): 203-220.

MUTWALLY, H.M.A. and Jamel Al-Layl, K.S. (1993) The effect of cyanobacterial neurotoxin on field stimulation (FS) responses of locust *Locusta migratoria* foregut and hindgut visceral muscles. *J. Egypt Ger. Soc. Zool. Comp. Physiol.*, Vol.11 (A): 19-31.

MUTWALLY, H.M.A. (Under publication) Ecophysiological effect of different physiological solutions on the toad *Bufo tibiamicus* stomach and heart muscles responses. .

MUTWALLY, H.M.A. and Mahmoud, M.I. (Under publication) Effect of fungal culture broths on K^+ - and field stimulation-responses of *Bufo tibiamicus* stomach and heart muscles. 1- In normal amphibian saline.

OLDFIELD, A.C. and Huddart, H. (1982) Spontaneous activity of foregut and hindgut visceral muscle of the locust *Locusta migratoria*. 1- Normal activity and the effect of KCL depolarization and glutamate. *Comp. Biochem. Physiol.*, Vol. 73 (C): 298-302.

PAPPANO, A.J. and Rembish, R.A. (1970) Nicotine-induced restoration of action potentials to cardiac tissue depolarized by potassium. *Life Sciences*, Vol. 9: 1381-1388.

RATZ, P.H. and FLAIM, S.F. (1982) Species and blood vessel specificity in the use of calcium for contraction. In "*Calcium blockers*, Ed. S.F., Flaim and R. Zelis, Baltimore: Urban and Schwarzenberg. Vol.16: 77-98.

REUTER, H. (1974) Exchange of calcium ions in mammalian myocardium. Mechanisms and functional significance. *Circulation Res.*, Vol. 34: 599-605.

REUTER, H. and SCHOLZ, H. (1977) A study of the ion selectivity and the kinetic properties of the calcium dependent slow inward current in mammalian cardiac muscle. *J. Physiol.*, Vol. 264: 17-47.

SANDS, S.D. and **WINEGRAD, S.** (1970) Treppe and total calcium content of the frog ventricle. *Am. J. Physiol.*, Vol. 218: 908-910.

SPERELAKIS, N. (1984) Hormonal and neurotransmitter regulation of Ca^{2+} -influx through voltage-dependent slow channels in cardiac muscle membrane. *Membrane Biochem.*, Vol. 5: 131-166.

STEFANI, E. and **CHIARANDINI, D.J.** (1982) Ionic channels in skeletal muscle. *Ann. Rev. Physiol.*, Vol. 44: 357-372.

STUESSE, S.C., LINDLEY, B.D. and **KIRBY, A.C.** (1974) Potassium contractures of frog single denervated muscle fibers: time course and central spread. *Amr. J. Physiol.*, Vol. 227 no. (1): 200-208.

TJIOE, S. and **BIANCHI, P.** (1969) Effects of local anesthetics on muscarinic sites of the isolated frog ventricle. *Eurp. J. Pharmacol.*, Vol. 7: 143-151.

VOGEL, A.I. (1971) A text-book of quantitative inorganic analysis including elementary instrumental analysis. Longman Group Lt., London.

WILSON, B.J. (1971) "Fungal toxins". Vol. VI "Microbial toxins", A. CIEGLER, S. KADIS and S.J. AJL (Edd.). Academic Press, New York.

WORCEL, M., PAPADIMITRIOU, A., HAMON, G. And **RANGACHARI, P.K.** (1976) Role of transmembrane Ca^{2+} movements and Ca^{2+} binding in the activation of rat uterus smooth muscle contraction. *Smooth muscle Pharmacol. And Physiol.*, Vol. 50: 353-362.

YOUNG, J.Z. (1933) The autonomic nervous system of selachians. *Quart. J. Microscop. Sci.*, Vol. 75: 571-624.

FIGURE LEAGENDS

Figure 1: The effect of Ca^{2+} -free saline (No/Ca) (1st-point) and additions of 1ml of growth medium (G.M.) and both fungal culture broths of *A. flavus* (A.F.) and *A. niger* (A.N.) (2nd-point) on toad stomach muscle spontaneous activity (Sp.Act). Addition of A.N. culture broth induced muscle contracture. After washout (W, 3ed-point) with normal saline the stomach muscles resumed their normal activities. Calibration and time scales apply to all traces.

Figure 2: The effect of Zamzam water (Z.W.) (1st-point) and additions of 1ml of growth medium (G.M.) and both fungal culture broths of *A. flavus* (A.F.) and *A. niger* (A.N.) (2nd-point) on toad stomach muscle spontaneous activity (Sp.Act). Stomach muscles suspended in Z.W. induced muscle contracture and the addition of G.M. and both fungal culture broths were without effect on the same muscle. After washout (W, 3ed-point) with normal saline the stomach muscles resumed their normal activities. Calibration and time scales apply to all traces.

Figure 3: The effect of distilled water (D.W.) (1st-point) and additions of 1ml of growth medium (G.M.) and both fungal culture broths of *A. flavus* (A.F.) and *A. niger* (A.N.) (2nd-point) on toad stomach muscle spontaneous activity (Sp.Act). Stomach muscles suspended in D.W. induced small contracture and the addition of G.M. and A.F. fungal broths were without effect on the same muscle. However, addition of A.N. cused large contracture. After washout (W, 3ed-point) with normal saline the stomach muscles resumed their normal activities. Calibration and time scales apply to all traces.

Figure 4: The effect of tap water (T.W.) (1st-point) and additions of 1ml of growth medium (G.M.) and both fungal culture broths of *A. flavus* (A.F.) and *A. niger* (A.N.) (2nd-point) on toad stomach muscle spontaneous activity (Sp.Act). Stomach muscles suspended in T.W. induced tiny contracture and the addition of G.M. and A.F. fungal broths were without effect on the same muscle. However, addition of A.N. cused large contracture. After washout (W, 3ed-point) with normal saline the stomach muscles resumed their normal activities. Calibration and time scales apply to all traces.

Figure 5: The effect of Ca^{2+} -free saline (No/Ca) (1st-point) and additions of 1ml of growth medium (G.M.) and both fungal culture broths of *A. flavus* (A.F.) and *A. niger* (A.N.) (2nd-point) on toad heart muscle natural activity. Heart muscles suspended in No/Ca were not largely affect with this immersion, whereas addition of both G.M. and A.F. fungal broths slowed heart responses. However, addition of A.N. cused severe inhibition of heart normal responses. After washout (W, 3ed-point) with normal saline the heart muscles resumed their normal activities. Calibration and time scales apply to all traces.

Figure 6: The effect of Zamzam water (Z.W.) (1st-point) and additions of 1ml of growth medium (G.M.) and both fungal culture broths of *A. flavus* (A.F.) and *A. niger* (A.N.) (2nd-point) on toad heart muscle natural activity. Heart muscles suspended in Z.W. clearly stimulated muscle responses. Moreover, additions of G.M. and both fungal broths delayed heart responses but after short period the muscles retained their activities. After washout (W, 3rd-point) with normal saline the heart muscles resumed their normal activities. Calibration and time scales apply to all traces.

Figure 7: The effect of distilled water (D.W.) (1st-point) and additions of 1ml of growth medium (G.M.) and both fungal culture broths of *A. flavus* (A.F.) and *A. niger* (A.N.) (2nd-point) on toad heart muscle natural activity. Heart muscles suspended in D.W. clearly stimulated muscle responses, and additions of G.M. and both fungal broths enhanced heart responses. Moreover, addition of A.N. culture broth induced muscle contracture. After washout (W, 3rd-point) with normal saline the heart muscles resumed their normal activities. Calibration and time scales apply to all traces.

Figure 8: The effect of tap water (T.W.) (1st-point) and additions of 1ml of growth medium (G.M.) and both fungal culture broths of *A. flavus* (A.F.) and *A. niger* (A.N.) (2nd-point) on toad heart muscle natural activity. Heart muscles suspended in T.W. stimulated and delayed the duration of the muscle responses, and additions of G.M. and both fungal broths inhibited heart responses. Moreover, addition of A.N. culture broth induced muscle contracture and gradually increased heart responses. After washout (W, 3rd-point) with normal saline the heart muscles resumed their normal activities. Calibration and time scales apply to all traces.

أثر الوسط الغذائي الفطري على الأنقباضات الطبيعية لعضلتي المعدة والقلب للضفدعة المصرية *بوفوق تيببيامكس*. ٢ - في محاليل فسيولوجية مختلفة.

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قسم الاحياء-كلية العلوم التطبيقية-جامعة أم القرى-ص ب ٣٧١١-مكة المكرمة -المملكة العربية السعودية.

الملخص:

هذا البحث أهتم بدراسة أثر الوسط الغذائي الفطري على الأنقباضات الطبيعية لعضلتي المعدة والقلب للضفدعة المصرية *بوفوق تيببيامكس* في محاليل فسيولوجية مختلفة. ركز البحث على دراسة أثر التركيب الأيوني لكل من المحلول الفسيولوجي الطبيعي، المحلول الفسيولوجي خالي الكالسيوم، ماء زمزم، الماء المقطر وماء الصنبور. وقد ركز البحث على ٥ عناصر أساسية هي: الكلور، البوتاسيوم، الصوديوم، الكالسيوم والمغنسيوم وذلك لأهميتها جميعا في أحداث واستمرار الانقباضة العضلية المختلفة. وجد أن هذه الأيونات تختلف تركيزاتها الأيونية في جميع المحاليل الفسيولوجية المستخدمة في هذه الدراسة. نتيجة هذا البحث أشارت الى أن كلا العضلتين المغمورة في ماء زمزم قد نشطت انقباضاتهما الطبيعية بشكل واضح مقارنة مع مثيلاتها المغمورة في المحاليل الفسيولوجية الأخرى. كما أن إضافة الوسط الغذائي الفطري الذي نمي عليه فطرة *أسبرجلس فلأفس* كان عديم الأثر على عضلات معدة الضفدعة في حين أنها قد تثبطت أنقباضات القلب الطبيعية، ما عدا التي غمرت في الماء المقطر. كما أن إضافة الوسط الغذائي الفطري الذي نمي عليه فطرة *أسبرجلس تيجر* قد أحدثت انقباضة لعضلة المعدة ما عدا التي غمرت في ماء زمزم. أيضا وجد أن الوسط الغذائي لنفس الفطرة قد أحدثت أنقباضة لعضلة قلب الضفدعة وكذلك سببت تثبيط أولي لفترة زمنية وجيزة أعقبها تنشيط تدريجي لنفس عضلة القلب، ما عدا تلك العضلة التي غمرت في المحلول الفسيولوجي خالي الكالسيوم. هذا الأثر كان واضحا في المحاليل التي تحتوي على أيونات المغنسيوم وعلى المحلول الفسيولوجي المحتوي على قليل من أيونات الكالسيوم أو المحاليل خالية الكالسيوم. هذه الآثار دللت على أن الوسط الغذائي الفطري والوسط الغذائي المنمى عليه الفطرتين لهما أثر على الغشاء الخلوي والوسط الخارجي والداخلي للعضلة خصوصا عضلة المعدة. أجمالا، يحتمل أن كلا وسطي النمو للفطرتين قد أحدثا أثرا سميا على انقباضات عضلات المعدة والقلب للضفدعة المصرية *بوفوق تيببيامكس*. لكن كان أثرهما متضادان على أنقباضات كلا العضلتين، يحتمل أن يكون سبب ذلك لتأثيرهما على قنوات أيونية مختلفة أو على نهايات المستقبلات العصبية المنتشرة على كلا العضلتين والتي يكون سببها هو الاختلافات الجوهرية في التركيب الأيوني لكل من الوسط الغذائي الفطري والمحاليل الفسيولوجية المستخدمة في هذه الدراسة وكذلك للاختلافات الجوهرية في التركيب العضلي والتوزيع العصبي لكلا العضلتين.