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The Protective Role of Hydrogen Sulfide on Skeletal Muscle in Cast Immobilization Model of Hindlimbs in Rats

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- Hydrogen sulfide
- Cast
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- Tibialis anterior
- Glibenclamide

Abstract

Aim: Investigate the possible effect of H2S on skeletal muscle in cast immobilization model of hindlimbs in rats and the role of ATP sensitive K channels in the mechanism of action of H2S on skeletal muscle. **Methodology**: This study was conducted on 40 adult male rats weighing 180-250 grams. Rats were divided into 4 groups: Group (I) (Immobilized group) (I group) Group (II) (Immobilized and H2S group) (IH group) Group (III) (Immobilized with combined H2S and ATP sensitive K channels blocker group) (IHG group). All the three groups were maintained with plaster cast for two weeks. A group of negative control rats that not immobilized, not receiving any drugs were used Group (IV) (negative control group) (C group). At the end of the 2 weeks, the rats were sacrificed. Then, casts in immobilized groups were removed to obtain tibialis anterior muscles from immobilized and contralateral limbs. Biochemical, histopathological, and immunohistopathological examination were studied. Results: The immobilized group showed significant deterioration in the contractile parameters and significant atrophic changes in tibialis anterior muscles. NAHS supplementation in (IH gp) can help in protection against myopathy as it showed significant alleviation of atrophic changes in tibialis anterior muscles. Addition of glibenclamide to NAHS treatment to immobilized rats (IHG gp) led to similar changes in tibialis anterior muscles as the immobilized group. **Conclusion** It is possible to conclude that H2S has a protective role in the cast immobilization rat model and it exerts its actions mainly through opening the ATP sensitive K+ channels

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INTRODUCTION

It has been recently recognized that skeletal muscle is an endocrine organ that can express, synthesize and secrete a variety of bioactive molecules which exert significant regulatory effects (1).

Skeletal muscle is an essential regulator of energy homeostasis and a potent coordinator of exercise-induced adaptations in other organs including brain and liver (2). Recent observations in human and rodents have demonstrated the ability of contracting myofibers to release cytokines and other peptides (myokines) such as; myostatin, irisin, interleukin-6 (IL-6), brain-derived neurotrophic factor (BDNF), interleukin-15 (IL-15), myonectin (CTRP15), decorin, fibroblast growth factor (FGF) 21 and hydrogen sulfide (H_2S) (3).

Endogenous H_2S is mainly generated from L-cysteine by the catalysis of cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (4). H_2S can be produced exogenously from a number of H_2S-releasing compounds such as calcium sulfide, sodium hydrosulfide (NaHS) and sodium sulfide (Na2S) (5).

Skeletal muscle atrophy or wasting represents an important problem (6). Muscle atrophy results from disuse, metabolic disease, malnutrition and aging. It is associated with a variety of conditions such as bed rest, limb immobilization, myopathies and space flight (7). It is characterized by enhanced reactive oxygen species, increased mitochondrial apoptosis and reduced mitochondrial function (8). It is believed that the process of muscle atrophy is mediated via the activation of several proteolytic systems like muscle specific RING finger 1 (MuRF-1) and muscle atrophy F-box (MAFbx) mainly (9).

A number of studies have demonstrated that supplementation of various types of antioxidants is one of the effective countermeasures against disuse muscle atrophy (10). Recently, H_2S is proved to be important in abroad range of physiological and pathophysiological functions (11). Being a gas, H_2S travels freely across cell membranes activating various molecular targets such as activation of K_{ATP} channels which are directly sensitive to cell metabolism (12). They are called so, because they open when cellular ATP levels falls. The channels are widely distributed in a number of tissues (13). In the skeletal muscle sarcolemma, K_{ATP} channels are among the most abundantly expressed K+ channels. Their densities reaching of 10 channels per mm^2 of surface membrane (14). It accounts for different biological functions of H_2S in the cardiovascular, respiratory, gastrointestinal, nervous and endocrine systems (15). The physiological role played by K_{ATP} channels in skeletal muscle is not clear (16).

Thus, we aimed in this work to: investigate the possible effect of H_2S on skeletal muscle in cast immobilization model of hindlimbs in rats. Also, to clarify the role of ATP sensitive K channels in the mechanism of action of H_2S on skeletal muscle.

Materials and Methods:

This study was conducted on 40 adult male albino rats aged 6-8 weeks, weighing 180-250 grams. Animals were bred and housed in the animal house of Medical Experimental Research center (MERC), Mansoura University, at a

temperature of 20 ℃ , fed a standard laboratory chow and had free access to tap water. All experimental protocols were approved by our local ethics committee (approval no: MDP.18.04.4.R1).

Chemicals: Sodium hydrosulfide (NAHS) is available in the form of flakes in glass bottle 10 gm with molecular weight 56.06 (Acros organics © Beligum) and Glibenclamide (Daonil) (sensitive K_{ATP} channels blocker) is available in tablets contain 5mg. It is produced by SANOFI – SYNTHELABO Pharmaceuticals. Both were dissolved in distilled water in a concentration (1mg/1ml).

Experimental design: Rats were divided randomly into 4 groups (10 rats each): ***Group (I)*** (Immobilized group) (I group): with their left hindlimbs were immobilized by plaster cast ***(17)***. ***Group (II)*** (Immobilized and H_2S group) (IH group): in which they were injected intraperitoneally by sodium hydrosulfide (NAHS) at a dose of 2 mg/kg daily **(18)** combined with immobilization. ***Group (III)*** (Immobilized with combined H_2S and ATP sensitive K channels blocker group) (IHG group): in which they received glibenclamide (sensitive K_{ATP} channels blocker) at a dose of 5 mg/kg daily by gastric gavage ***(19)*** combined with sodium hydrosulfide (NAHS) and immobilization.

All the three groups were maintained with plaster cast for two weeks. The contralateral loose limb were used as a control for each group and to ensure that the contralateral hindlimb were an adequate control reference, a group of negative control rats that not immobilized, not receiving any drugs were used **Group (IV)** (negative control group) (C group).

At the end of the 2 weeks, the rats were anesthetized with thiopental (120mg/kg) injected intraperitoneally. Rats were sacrificed by cardiac puncture. Then, casts in immobilized groups were removed to obtain our preparation by isolation of tibialis anterior muscle in order to record the contractile parameters by computerized data acquisition system unit MP45(BIOPAC Student Lab 3.7.3.). Then, these tissue samples were used for biochemical and histopathological examination.

Hind limb immobilization procedure: Rats were anesthetized intraperitoneally with 90 mg/kg ketamine and 10 mg/kg xylazine. Then, were subjected to cast immobilization by application of the cast from anterior superior iliac crest to the lower part of the foot (pelvipedal cast) with ankle joint in neutral position to avoid the lengthening of tibialis anterior. The rats were checked on daily basis for chewed plaster, venous occlusion, abrasions and fecal clearance ***(20)***.

1.Calculation of the relative weight of tibialis anterior

At the end of study, the rats were weighed, then tibialis and contralateral tibialis were dissected out. The relative weight ratio of the muscle was calculated as the ratio of the wet weight of the muscle divided by the body weight at the end of the experiment ***(21)***.

2.Recording of the contractile parameters by BIOPAC

The preparation (tibialis anterior muscle) must be immersed in Krebs solution at 30° C. The Krebs solution had the following composition (mM): 120 NaCl; 25 $NaHCO_3$; 1.2 NaH_2PO_4; 1.2 $MgSO_4$; 5.0 KCI; 2.5 calcium gluconate; 11. 5 glucose. It was continuously bubbled with a mixture of 5 %$C0_2$;

95 % 0_2, and the pH maintained at 7.4. Before recording the contraction, Biopac apparatus must be adjusted by connecting BSLSTM stimulator to channel (1) and recording electrode (SS12LA) was connected to channel (2). Place the stimulating electrodes on the muscle for direct stimulation. Set up channels and adjust the baseline. At first, record maximal isometric twitch by applying single maximal stimulation (30 V) ***(22)***. Then, record the tetanic contraction by applying continuous stimulation. The tetanic frequency in tibialis anterior was 80 HZ ***(23)***.

To test the fatigability, a low frequency muscle fatigue protocol (stimulation at 4HZ for 5 min) was applied. The decrease in the tension after 5 min was expressed as a percentage of the initial tension, denoting the fatigue index (FI). An isometric single twitch was recorded 3 min after the completion of the fatigue protocol to estimate the recovery ability of the muscle. The tension recorded after the 3 min recovery period was expressed as a percentage of the initial tension, denoting the recovery index (RI). The force at 4HZ was considered the initial tension for both FI, RI ***(24)***. The contractile parameters: maximum isometric twitch force (P_t), maximum tetanic force (P_o) , maximum specific isometric tetanic force (sP_o), time to peak (TP), half relaxation time (1⁄2RT), fatigue index (FI) and recovery index (RI) were measured. The maximum specific force (sP_o), equals maximum tetanic force per cross-sectional area (CSA) and CSA were measured by scaling histopathological specimens ***(25)***.

3.Biochemical assessment: Determination of Total antioxidant capacity (TAC) was done by the method of ***(26)***.Determination of Malondialdehyde (MDA) was according to Urchiyama and Mihara method ***(27)***, utilizing kits purchased from Biodiagnostic, Egypt.

4. Histopathological examination

After recording the contractile parameters, tibialis anterior muscles were fixed in 10% neutral buffered formalin processed by standard procedure for paraffin embedding and serial sections were cut (5 μ). The sections were stained with hematoxylin and eosin.

5.Determination of MURF-1 by immunohistochemistry: Sections were stained with Polyclonal Anti-MuRF1/TRIM 63 antibody (MURF1) purchased from Biospes. Tibialis anterior was embedded in paraffin. They were cut into segments 8-10 mm in thickness. The samples were prepared following the manufacturer's recommendations ***(28)***. Staining was seen and photographed with microscope.

Computer Assisted digital image analysis (Digital morphometric study) Slides were photographed using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 20 X objective. The result images were analyzed on Intel® Core I5® based computer using VideoTest Morphology® software (Russia) with a specific built-in routine for area, % area measurement and object counting.

Statistical analysis: Data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 16. Quantitative data were described as means ± (SD) after testing for normality by Shapiro-Wilk test. One way ANOVA with LSD post-hoc multiple comparisons was used for comparison between groups. Independent samples t-test was used for comparison between sides of each group. "p value ≤0.05" was considered to be statistically significant.

Results

The relative muscle weight ratio of the tibialis anterior muscle: Table (1) showed significant reduction in the relative muscle weight ratio of tibialis (T) in immobilized group (I gp). On the other hand, sodium hydrosulfide (NAHS) treated immobilized group (IH gp) showed significant increase in relative weight ratio of tibialis, but with addition of glibenclamide to NAHS treated group (IHG gp), there were no significant change. By comparing contralateral tibialis (TC) in different groups to the control ones, there were no significant difference except in (IHG) group, that showed significant decrease in the relative weight ratio of TC.

Table (1): Comparison of the relative muscle weight ratio of tibialis anterior among the different experimental groups:

N=10 / Measure	Group C	Group I	Group IH	Group IHG
Relative wt T	0.2±0.0355	0.09±0.009^{*a}	0.2±0.013^{*ab}	0.08±0.009^{*ac}
Relative wt TC	0.2±0.035	0.2±0.022	0.2±0.035	0.2±0.009^{ac}

(C): control group , (I) Immobilized group, (IH) Immobilized treated with H_2S, (IHG) Immobilized treated with combined H_2S and Glibenclamide .
T (tibialis),TC (contralateral tibialis).
One way ANOVA with LSD post-hoc multiple comparisons was used for comparison between groups. Independent samples t-test was used for comparison between each muscle and its contralateral. "p value ≤0.05" was considered significant.
a=any significance with C group
b=any significance with I group
c=any significance with IH group
*=any significance between Tibialis & its contralateral within the same group.

Analysis of the contractile parameters of tibialis anterior muscle in the different experimental groups:

By measuring the contractile parameters of the tibialis muscle and its contralateral in different experimental groups, figure (1) showed the Biopac recording of these parameters.

By comparing the significance of the contractile parameters in different experimental groups, figure (1) showed significant changes in immobilized group (I gp) expressed in significant decrease in maximum isometric twitch force (P_t) and maximum tetanic force (P_o). In addition, there was a significant decrease in maximum specific isometric tetanic force (sP_o) and recovery index (RI). In the contrary, there was a significant increase in fatigue index (FI), a significant prolongation of time to peak (TP) and half relaxation time (1⁄2RT) of tibialis muscle.

Noteworthy, treated group with NAHS (IH gp) showed significant increase in (P_t), (P_o), (sP_o), (RI), significant decrease in (FI) and significant shortening in (TP) and (1⁄2RT). But, adding glibencalimde to treated group (IHG gp) showed similar results to immobilized group (I gp) except for FI which was significantly increased in (IHG) group.

In addition, by comparing TC in different groups to the control rats, there were no significant difference except in IHG group, show significant reduction in (P_t), (P_o), (sP_o), (RI) and significant increase in (FI) and (1⁄2RT).

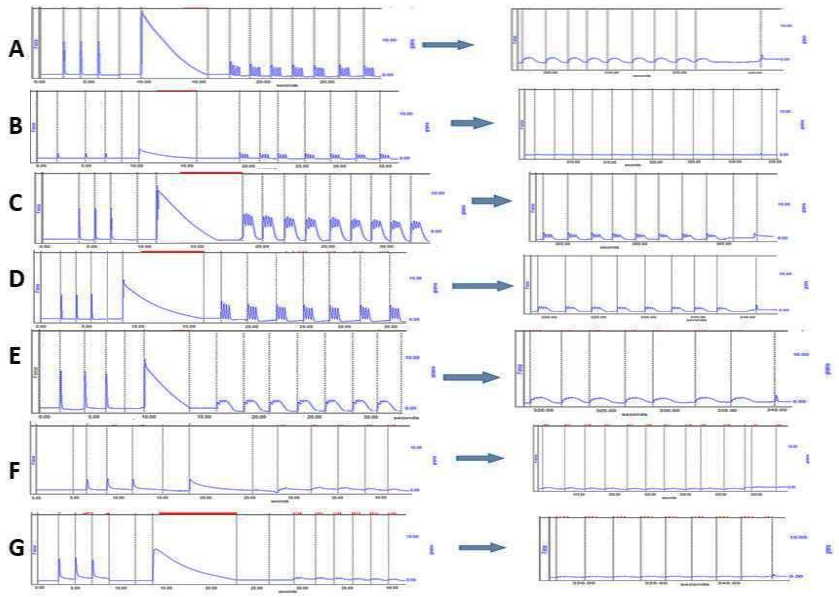

Figure (1): Biopac record of tibialis anterior muscle in different experimental groups: (A) control group(C gp) (B) immobilized group (I gp) (C) contralateral tibialis in immobilized group (I gp) (D) H_2S treated immobilized group (IH gp) (E) contralateral tibialis in H_2S treated immobilized group (IH gp) (F) combined glibenclamide and H_2S treated immobilized group (IHG gp) (G) contralateral tibialis in combined glibenclamide and H_2S treated immobilized group (IHG gp).

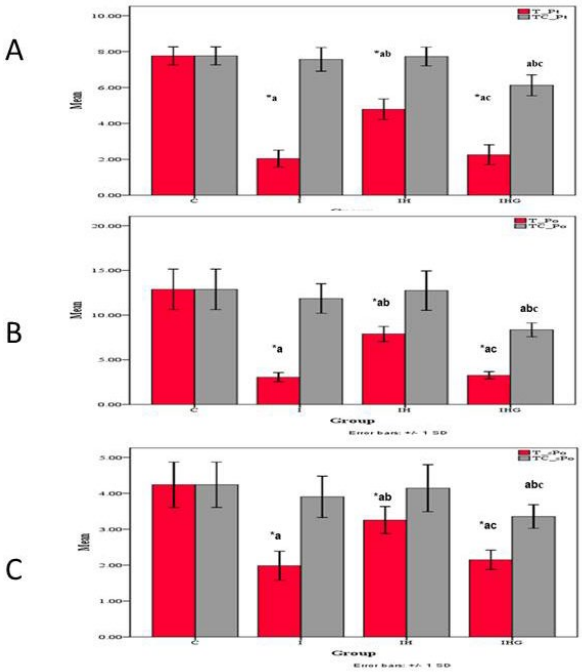

Figure (2): Comparison of contractile parameters recorded: (A)maximum isometric twitch force (P_t) (B) maximum tetanic force (P_o) (C)maximum specific isometric tetanic force (sP_o))in tibialis anterior muscle among different experimental groups.

(C): control group, (I) Immobilized group, (IH) Immobilized treated with H_2S, (IHG) Immobilized treated with combined H_2S and Glibenclamide .

T (tibialis),TC (contralateral tibialis).

One way ANOVA with LSD post-hoc multiple comparisons was used for comparison between groups. Independent samples t-test was used for comparison between each muscle and its contralateral. "p value ≤0.05" was considered significant.

a=any significance with C group

b=any significance with I group

c=any significance with IH group

*=any significance between T & its contralateral within the same group.

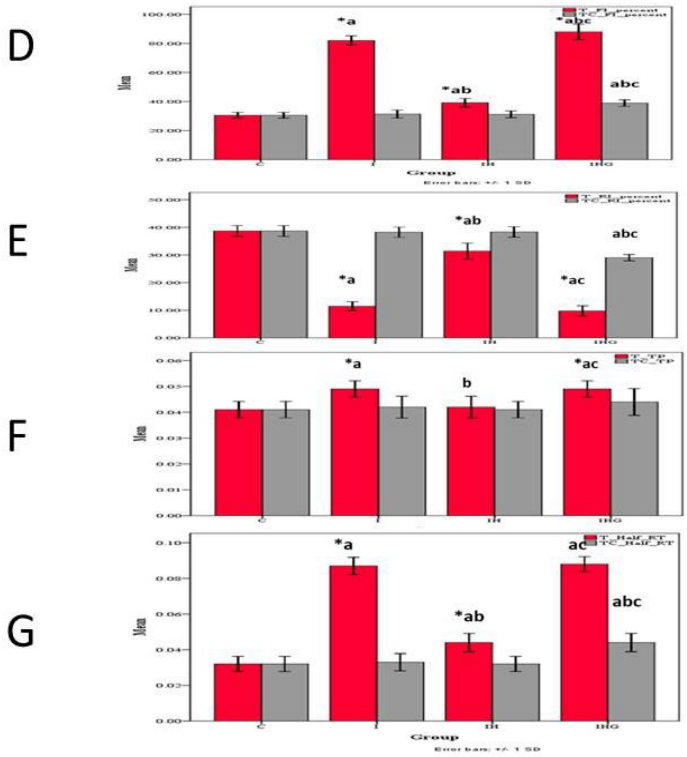

Figure (3): Comparison of contractile parameters recorded: (D) fatigue index (FI%) (E) recovery index (RI%) (F) time to peak (TP) (G) half relaxation time (1/2 RT) in tibialis anterior muscle among different experimental groups.

(C): control group, (I) Immobilized group, (IH) Immobilized treated with H_2S, (IHG) Immobilized treated with combined H_2S and Glibenclamide

T (tibialis),TC (contralateral tibialis).

One way ANOVA with LSD post-hoc multiple comparisons was used for comparison between groups. Independent samples t-test was used for comparison between each muscle and its contralateral. "p value ≤0.05" was considered significant.

a=any significance with C group

b=any significance with I group

c=any significance with IH group

*=any significance between T &its contralateral within the same group.

Assessment of total antioxidant capacity and malondialdehyde levels in tibialis anterior muscles

Figure (4) showed significant increase in malondialdehyde (MDA) together with significant reduction in total antioxidant capacity (TAC) in tibialis muscle in (I group). Noteworthy, NAHS treatment in (IH) group showed significant decrease in MDA and significant enhancement in TAC. By adding glibenclamide to treated group, there were no significant changes. In addition, by comparing the contralateral muscle TC in different groups to the controls, there were no significant differences, except in IHG group, that showed significant increase in MDA with significant reduction in TAC.

Results of histopathological and immunohistochemistry examination (MURF-1)

A-Analysis of histopathological studies:

Immobilized group (I gp) showed significant atrophic changes in tibialis anterior muscles in as shown in figure (5B,C). These atrophic changes were in form of marked separation of the muscle fibres, marked increased fibrous tissue and severe infiltration by inflammatory cells. Separation of the muscle fibers (pointed by blue arrows) and

infiltration by inflammatory cells (pointed by red arrows). Treatment of immobilized group with NAHS (IH gp) in tibialis anterior muscle showed significant alleviation of atrophic changes as shown in figure (5D). By adding of glibenclamide to treated group (IHG gp), there were no significant changes as compared to (I) group as shown in figure (5E,F). In addition, by comparing TC in different groups to control, there were no significant differences except in (IHG) group, show some atrophy as compared to the control rats as shown in figure (5G).

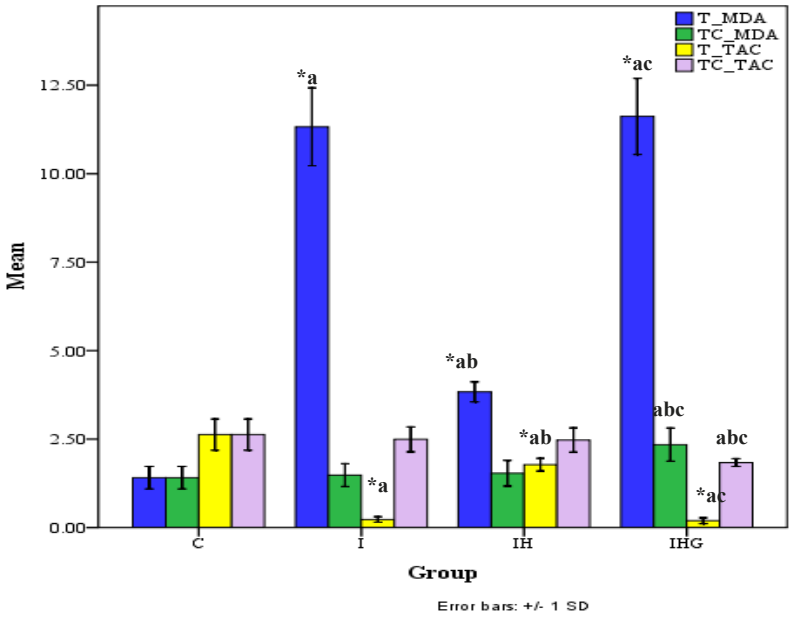

Figure (4): Assessment of of total antioxidant capacity (TAC) and malondialdehyde (MDA) levels in tibialis anterior muscle in different experimental groups

(C): control group , (I) Immobilized group, (IH) Immobilized treated with H_2S, (IHG) Immobilized treated with combined H_2S and Glibenclamide , S (soleus) &SC (contralateral soleus) ,T (tibialis),TC (contralateral tibialis).

One way ANOVA with LSD post-hoc multiple comparisons was used for comparison between groups. Independent samples t-test was used for comparison between each muscle and its contralateral. "p value ≤0.05" was considered significant.

a=any significance with C group , b=any significance with I group , c=any significance with IH group

*=any significance between T & its contralateral within the same group.

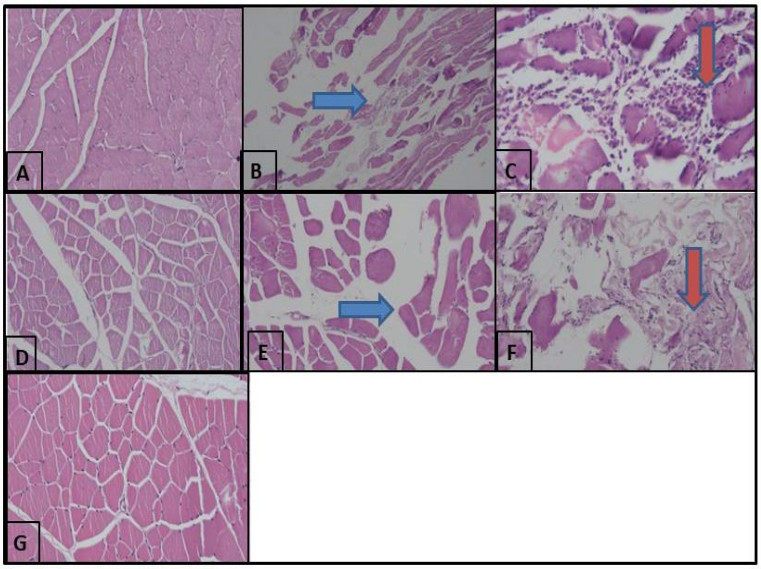

Figure (5): show the histopathological in the tibialis anterior muscle stained with Hx & E in magnification x 200 (A) control group (B,C) the immobilized group (I gp) (D) NAHS treated immobilized group (IH gp) (E,F) combined glinenclamide and NAHS treated immobilized group (IHG gp) (G) contralateral tibialis muscle of the combined glinenclamide and NAHS treated immobilized group (IHG gp). Separation of the muscle fibers (pointed by blue arrows) and infiltration by inflammatory cells (pointed by red arrows).

Analysis of immunohistochemistry studies:

Table (2) and figure (6B) showed intense staining and significant increase in percentage area stained with anti-Murf-1 in tibialis anterior muscle in the immobilized group. Surprisingly, NAHS treatment in (IH) group showed significant decrease in percentage area stained with anti-Murf-1 in tibialis as compared to (I) group as shown in figure (6C). Moreover, adding glinenclamide to NAHS treatment in (IHG) group caused no significant changes as compared to (I) group as shown in figure (6D).

In addition, by comparing the contralateral muscles in different groups to the control group, there was a significant increase in percentage area stained with anti-Murf-1 of tibialis anterior muscle of (IHG) group as compared to the control group as shown in figure (6E).

Table (2): Comparing of percentage area stained with anti-Murf-1 in tibialis anterior muscles among different experimental groups

N=10 / Measure	Control group	Group I	Group IH	Group IHG
T area%	0.197±0.02	30.2±0.76^{*a}	10.9±0.454^{*ab}	31.3±0.474^{*abc}
TC area%	0.197±0.02	0.221±0.043	0.202±0.026	9.5±0.662^{abc}

(C): control group , (I) Immobilized group, (IH) Immobilized treated with H_2S, (IHG) Immobilized treated with combined H_2S and glinenclamide

T (tibialis),TC (contralateral tibialis).

One way ANOVA with LSD post-hoc multiple comparisons was used for comparison between groups. Independent samples t-test was used for comparison between each muscle and its contralateral. "p value ≤0.05" was considered significant.

a=any significance with C group , b=any significance with I group, c=any significance with IH group

*=any significance between between T & its contralateral within the same group.

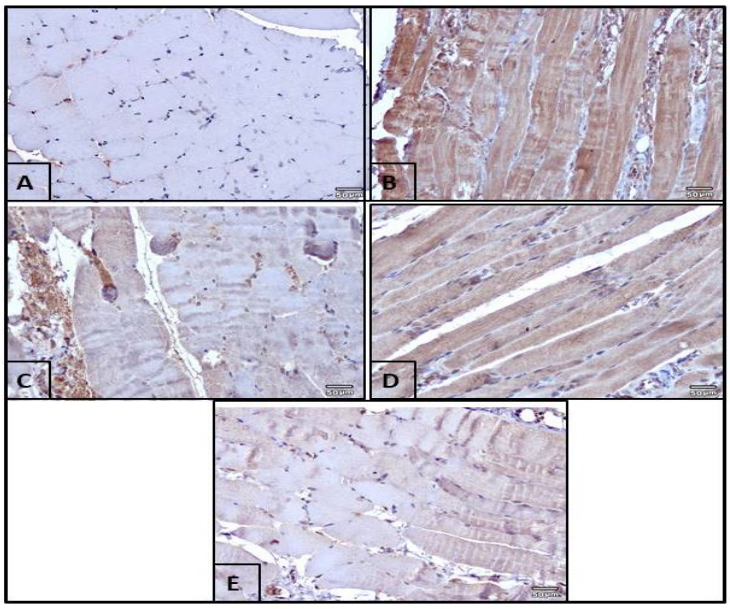

Figure (6): show the immunological findings in the tibialis muscle stained with anti-Murf-1 antibody in magnification x 200 A) control group B) the immobilized group (I gp) C) NAHS treated immobilized group (IH gp) D) combined glinenclamide and NAHS treated immobilized group (IHG gp) E) contralateral tibialis muscle of the combined glinenclamide and NAHS treated immobilized group (IHG gp) (black arrows refers to atrophy in the muscle fibers).

Discussion

In the present work, we aimed to study the possible effect of H_2S on cast immobilization rat model of myopathy and investigate the role of ATP sensitive K^+ channels and its possible mechanisms. Previous studies demonstrated that H_2S has a protective effect in many health problems, but the possible protective role of H_2S in skeletal muscle diseases still is not sufficient (29).

Potassium channels have been the first to be discovered to be the most important cellular targets of H_2S. It has been stated that H_2S can activate ATP-sensitive potassium (K_{ATP}) channels (30) through S-sulfhydration the sulfonylurea receptor 1 (SUR1) and SUR2B subunits of K_{ATP} channels leading to an increased K_{ATP} channel current (31).

Cast immobilization leads to many atrophic changes and attenuation in the muscle power that was confirmed by many findings in the present study. For instance, the present study showed significant reduction in the relative muscle weight ratio of tibialis anterior muscles in immobilized group (I gp). It can be explained by the decline in protein synthesis rate in the early state of unloading with dominant increase in protein degradation (32).

Also, the present results showed similar significant changes in the contractile parameters of tibialis anterior muscles in immobilized group (I gp) when compared to either the contralateral loose hindlimb muscles in the same group or control group (C gp). This confirms that the contralateral loose hindlimb was an adequate control reference as it had the same results as the negative control rats. The contralateral loose hindlimb served as a reliable control as it exposed to the same stress exerted on the immobilized limb. These results are in agreement with Marmonti et al. (33), who reported that there was significant decrease of muscle force after 7 and 14 days of physical inactivity. Giordano et al.,(34) claimed that the attenuation of muscle force may be due to reduced cross sectional area (CSA) of the muscle, increase the connective tissue between myofibrils, sarcomere dissolution, the skeletal muscle blood vessel endothelial degradation, reduction in capillary density and reduction in the number of mitochondria . The possible mechanisms of muscle atrophy are mediated via the activation of several proteolytic systems as calpain system, lysosomal protease system, caspase endoprotease system and ubiquitin–proteasome system (35).

Furthermore, the present experiment showed significant increase in malondialdehyde together with significant reduction in the total antioxidant capacity in the tibialis anterior muscles of immobilized group (I gp). This is in agreement with Mostafa and Samir, (36) who reported that hindlimb immobilization resulted in significant elevation of MDA levels with significant reduction in total antioxidant capacity in the soleus muscle as compared with controls. It may be explained by the exposure to stress is associated with the production of oxidative species, which overcome the oxidant capacity of the muscle, eventually leading to muscle atrophy.

All of these findings were confirmed by the histopathological examinations. The present work showed significant atrophic changes in tibialis anterior muscles in immobilized group (I gp). These atrophic changes were in form of marked separation of the muscle fibres, marked increased fibrous tissue and severe infiltration by

inflammatory cells. These results are consistent with Santos-Junior et al.,(37) who reported that hind limb immobilization in rats for two weeks leads to reduction in fiber size in the histopathological examination. Likewise, the current study showed diffuse marked positive staining and significant increase in percentage area stained with anti-Murf-1 in tibialis anterior muscles in the immobilized group.

Noteworthy, the present research illustrated that sodium hydrosulfide (NAHS) treated immobilized group (IH gp) showed significant increase in relative weight ratio of and tibialis anterior in relation to (I) gp (immobilized group). Likewise, we reported that NAHS treated group (IH gp) showed significant improvement in the contractile parameters of tibialis anterior muscles with reduction in fatigue index and better recovery. These results are consistent with Wetzel and Wenke, (38) who demonstrated that administration of H_2S once a day for 4 weeks following femoral artery ligation in rat model led to significantly increased capillary density, collateral vessel growth and regional tissue blood flow in ischemic hind limb muscles.

Remarkably, NAHS treatment in IH group showed significant decrease in MDA with significant enhancement in TAC level as compared to (I) group in the present study. This is in agreement with Shefa et al.,(39) who stated that H_2S protects the CNS by acting as an antioxidant thus protecting against central neurodegenerative diseases such as Alzheimer's disease.

The protective effect of hydrogen sulfide was confirmed by the results of the histopathological examinations. Interestingly, the present work showed significant alleviation of atrophic changes in tibialis anterior muscles in the treated group with NAHS (IH gp). As well, NAHS treatment showed less diffuse moderate staining and significant decrease in percentage area stained with anti-Murf-1 tibialis as compared to (I) group in the present study.

The possible protective effect of H_2S can be explained by activation of AKT pathway by H_2S resulting in enhancing the protein synthesis via upregulation of mammalian target of rapamycin (mTOR complex) (40). It was stated that mTORC1 is the main regulator of cellular growth as it promotes anabolic processes such as protein, lipid and nucleotide synthesis, whereas it inhibits catabolic pathways, such as autophagy (41) . Though, it is reported that the mTOR pathway controls important cellular processes including cell survival, mitochondrial biogenesis and function (42). Moreover, it was reported that hydrogen sulphide has an anti- inflammatory effect as it decreases the pro-inflammatory cytokines: tumor necrosis factor (TNF-a), IL-6, and IL-8 (43). As well, it inhibits the leukocyte adherence, leukocyte infiltration and edema formation mainly through activation of K_{ATP} channels (44).

Glibenclamide is the most widely used sulfonylurea drug for the treatment of type 2 diabetes mellitus. The main mechanism of glibenclamide is inhibition of ATP-sensitive potassium channel (K_{ATP}). Glibenclamide was used in this study to prove the significant role of K_{ATP} in the protective role of H_2S. Noteworthy, the present work stated that addition of glibenclamide to NAHS treatment to immobilized rats (IHG gp) led to similar changes in the relative muscle weight ratio of the tibialis anterior muscles as immobilized group (I gp). Also, we found that

contralateral tibialis (TC) in IHG group showed significant decrease in the relative weight ratio in the present study.

Furthermore, adding glibenclamide to the treated group (IHG gp) resulted in similar contractile properties as immobilized group (I gp). Moreover, addition of glibenclamide to treated group (IHG gp) showed significant increase in malondialdehyde together with significant reduction in the total antioxidant capacity in the tibialis anterior muscles same as the immobilized group (I gp). These stated results were confirmed by the histopathological examinations that demonstrated that adding glibenclamide to NAHS treatment in (IHG) group in the present thesis caused similar atrophic changes as (I) group. Also, (IHG) group showed diffuse marked positive staining and significant increase in percentage area stained with anti-Murf-1 in tibialis anterior muscles in the present work. So that, glibenclamide can be assumed to abolish the cytoprotective effect of hydrogen sulfide most probably through blocking K_{ATP} channels.

So, glibenclamide which is K_{ATP} channels blocker can reverse the protective effect of H_2S on the contractile properties of the skeletal muscle in the cast immobilization. Thus, we can suggest that H_2S mainly exerts its protective role in the skeletal muscle through activation of K_{ATP} channels.

Recommendations

Hydrogen sulfide donors can be used as additional dietary supplementations for the prevention of myopathy in prolonged cast immobilization.

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