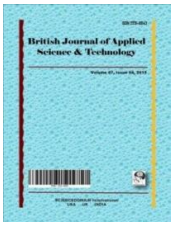

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The Effect of Chronic *Prosopis glandulosa* Treatment on Muscle Force Development and Fatigue Tolerance in Soleus Muscle

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Authors' contributions

This work was carried out in collaboration between all authors. All the authors contributed substantially to the design of the study. Author CG performed the statistical analysis and along with author DD wrote the protocol. The first draft of the manuscript and managed literature searches was conducted by author CG. Authors CG, DD and BH managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Muscle fatigue, which is the diminished ability of muscles to generate force, has been found to play a major role in limiting performance during physical activity. For centuries herbal remedies have been used in the plight to ameliorate fatigue and increase muscle strength, however many lacking scientific evidence.
Aims: This study was aimed to investigate the effects of *Prosopis glandulosa*, on skeletal muscle fatigue and muscle strength development.
Methodology: Adult, male, Wistar rats received daily oral administration of *Prosopis glandulosa*

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(100 mg/kg/day for 10 weeks). After 10 weeks the soleus muscles were excised from anaesthetized rats, weighed, measured and mounted for isometric force determination. Muscles were vertically suspended between two electrodes in Krebs Henseleit buffer solution in a water-jacketed organ bath. Isometric twitch- and tetanic force production, contraction time, half-relaxation time, force-frequency relationship and rate of fatigue were measured in response to electric field stimulation.

Results: *P. glandulosa* treatment had no significant effect on muscle fatigue tolerance, as both treated and untreated groups fatigued at the same rate. However, muscles from treated rats generated significantly increased force when the muscle was stimulated at different frequencies to generate a single twitch and tetanus and throughout a 2 minute fatigue protocol.

Conclusion: The use of an economical, natural and readily available substance such as the one we identified here, as treatment to increase muscle force generation could have far-reaching implications in not only the sporting arena, but also the health sector.

Keywords: Fabaceae; soleus muscle; slow-twitch muscle; electrical field stimulation.

1. INTRODUCTION

The use of herbal substances as performance-enhancers dates back to the early years of the Greek Olympians [1]. In recent years, it has been scientifically shown that certain herbal substances have anti-fatigue and ergogenic effects. An ergogenic aid can be broadly defined as a substance used for the purpose of enhancing physical performance, ranging from accepted practices (carbohydrate loading) to illegal and unsafe practices (anabolic-androgenic steroid use) [2,3].

Physical fatigue, also referred to as peripheral fatigue, is denoted as the deterioration of muscle performance during prolonged activity [4,5]. This decrease in force is reversible, as muscle performance can be recovered after sufficient rest and appropriate nutrition. The two main mechanisms thought to be responsible for this decrease in muscle performance are oxidative stress and exhaustion [6]. It has been found that intense exercise leads to the production and accumulation of excessive amounts of reactive oxygen species (ROS), including superoxide anions [7-10], hydrogen peroxide [8] and hydroxyl radicals [8,9,11], all of which result in oxidative stress injury to the body. Fatigue can therefore be delayed by pre-treating muscle with ROS-selective antioxidants [12-14]. Numerous studies have shown that antioxidant treatment, such as found in herbal preparations, can result in prolonged performance in endurance exercise by decreasing oxidative stress [12-14]. Korean ginseng, amongst others [15], has previously been found to enhance exercise and sport performance [13-14,16]. Conversely, the exhaustion theory proposes that muscle fatigue occurs as the result of energy source depletion and excess metabolite accumulation [6,17]. During anaerobic metabolism, adenosine triphosphate (ATP) is mainly produced through the degradation of phosphocreatine (PCr) and the breakdown of muscle glycogen, forming inorganic phosphates (P_i), lactate and hydrogen ions (H^+) as byproducts. Herbal substances have been found to exert its effects by increasing glycogen storage, thereby increasing the energy source and decreasing metabolite accumulation, thereby prolonging performance in exercise endurance.

The magnitude of muscle force generation is determined by mainly two factors, namely, (i) the size of the muscle recruited to generate the force and (ii) the muscle fiber type [18,19]. Force generation during contraction is related to the number of cross-links made between the actin and myosin chains [5]. Therefore, the more cross-links formed, the stronger the force of contraction. Hence, the maximal force of contraction depends upon the number of fibers a muscle contains. The type of fibers also plays an important role, as different types of muscle fibers possess different contractile properties [20]. It is known that a muscle composed of a high proportion of slow-twitch (Type I) fibers will be relatively weaker than a muscle of similar size with a high proportion of fast-twitch (Type II) fibers. Fiber composition is regulated in response to changes in physical activity, environment and pathological conditions [20], for example, endurance exercise training induces a fast-to-slow fiber type transition, transforming the myofibers to an increased oxidative metabolism [21-23]. Additional factors leading to fiber type transition include mechanical loading and unloading, hormones and aging [22].

The trend of utilizing herbal substances to improve performance has increased. One such herbal substance, under review for its potential performance-enhancing ability, is the dried and ground pods of the *Prosopis glandulosa* (Torr.) tree [Fabaceae] - commonly known as Honey mesquite. Mesquite, which is a common name for several species of the leguminous plants of the *Prosopis* genus, has been found to contain numerous phytochemicals eliciting various effects, such as anti-inflammatory effects [24].

To our knowledge, very few studies have been conducted on the *P. glandulosa* plant itself and no literature, except those from our laboratory [25,26], could be found regarding its potential clinical benefits. Therefore, we opted to scientifically examine the effects of *P. glandulosa* by electrically stimulating the isolated soleus muscle from rats to fatigue and determining the extent of recovery after the fatigue period. In addition, we also examined the magnitude of force development, with and without *P. glandulosa* treatment. If a cheap, natural and readily available substance is proven to augment muscle fatigue and increase muscle strength; it could have enormous implications in the sporting arena and health sector.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals used were purchased from Merck (Pty) Ltd – South Africa. The *P. glandulosa* preparation was supplied by Mr. P. Schoeman, who is the patent holder of the *P. glandulosa* supplement preparation method. A voucher herbarium specimen was prepared (ref. George 1, steu) and lodged at the Stellenbosch herbarium. The composition of the product was analyzed by the CSIR (SA), J. Muller Laboratories (Pty) Ltd. and the Central Analytical Facility of the Stellenbosch University and all three institutions concurred on the chemical content thereof. Appendix A contains an example of the chemical analysis of this proprietary drug, as analyzed by J. Muller Laboratories (Pty) Ltd.

2.2 Experimental Animals

Age- and weight-matched adult, male, Wistar rats (Charles River Laboratories International, Inc., Wilmington, MA) were used. All animals were housed at the Stellenbosch University Central Research Facility, Tygerberg, in temperature controlled rooms (22 – 24°C) and kept on a 12-hour light/dark cycle (lights on at 6:30 am). Rats were given *ad libitum* access to standard laboratory rat chow pellets (Nutroscience (Pty) Ltd, Malmesbury, SA) and tap water for the duration of the experimentation. The animals received humane care in accordance with the principles of the South African National Standard for the care and use of animals for scientific purposes (South African Bureau of Standards, SANS 10386, 2008). The project was approved by the Animal Research Ethics Committee of Sub-Committee B of Stellenbosch University (reference #10GK_HIL01).

2.3 P. glandulosa Treatment

The *P. glandulosa* powder used in the treatment protocol was from herbal origin and it consisted solely of the dry-milled pods of the *P. glandulosa* tree [25]. Rats were treated with *P. glandulosa* at a dose of 100 mg/kg/day for a total period of 10 weeks. *P. glandulosa* was weighed daily for each animal in the treatment group and set in a mixture of commercially available gelatin/ jelly cubes (Libstar Manufacturing solutions (Pty) Ltd, Johannesburg, SA, and Pioneer foods (Pty) Ltd, Paarl, SA respectively) of 1 ml volume. This jelly cubes were fed to each animal individually, to ensure absolute compliance and dose control. The dosage of 100 mg/kg/day *P. glandulosa* was calculated based on the daily dosage prescribed for human adults. We have previously shown this dose to elicit metabolic changes [25-26]. To accustom the animals to the researcher and the taste of the jelly cubes, all animals were fed placebo jelly cubes (jelly cubes without *P. glandulosa*) for 1 week prior to the start of the actual treatment program. During the 10 week experimental period, the control animals received placebo jelly cubes.

Experimental rats were divided into 2 groups: a control placebo group (PLA), that received normal rat chow pellets and jelly cubes without *P. glandulosa* and a *P. glandulosa*-treated group (PG) that received normal rat chow and *P. glandulosa* mixed into jelly cubes (n = 10 in each group). A total of 20 isolated muscles were utilized, therefore 10 animals per experimental group (treatment vs. no treatment).

2.4 Sample Collection and Muscle Fatigue Stimulation Protocol

After 10-weeks of *P. glandulosa* treatment, the animals were weighed (to determine body mass)

and then received an overdose of sodium pentobarbital (200 mg/kg, intraperitoneal). The animals were continually monitored until loss of consciousness was reached, indicated by a total lack of response after a foot pinch, before any experimentation commenced.

Skeletal muscle fatigue was determined by methods previously described by Gordon et al. [27] and El-Khoury et al. [28]. Briefly, after the animals were euthanized with an overdose of sodium pentobarbital (200 mg/kg, intraperitoneal), one of the soleus muscles, with tendinous insertions intact, were removed and placed in ice-cold Krebs-Henseleit buffer (KHB). The KHB solution contained in mM: NaCl 119, KCl 4.74, $CaCl_2.2H_2O$ 1.25, $MgSO_4.7H_2O$ 0.6, KH_2PO_4 1.2, $NaHCO_3$ 24.9, Na_2SO_4 0.6 and glucose 10. The intact soleus muscle was then removed from the cold KHB buffer and vertically suspended between a pair of platinum electrodes in a 25 ml water-jacketed organ bath containing KHB solution. The KHB was continuously gassed (95% O2/5% CO_2) to maintain the pH at 7.4 and the temperature of the KHB was kept at 25°C. The physiological stability of rat skeletal muscle *in vitro* is temperature-dependent and stability for muscle strips of 1-2 mm diameter is better at 25°C compared to the *in vivo* temperature of 37°C [29]. The base of the muscle was fixed to an immobile hook and the other end tied to an isometric force transducer ((MLT 0202) ADInstruments, Inc., Colorado Springs, CO). The position of the force transducer could be adjusted by a micro-positioner, thus altering preload. The muscles were left to stabilize for 30 minutes before electrical stimulation commenced.

After an equilibration period of 30 min, the optimal length (*i.e.* muscle length producing maximal isometric twitch force) and supramaximal voltage was determined. Optimal muscle length and voltage were determined for each muscle by generating single twitch contractions at increasing muscle lengths and voltages respectively, until no increase in single-twitch force production was observed. The muscle length and voltage that generated the highest single twitch amplitude was then used throughout the entire stimulation protocol. The pulse duration was set to 1 msec for all twitch and tetanic contractions. The stimulation protocol consisted of the generation of a single twitch, force frequency curve to determine F_{max}, tetanus, a 2 minute stimulation period to determine fatigue resistance and ended off with two sets of tetanus stimulations at 5 and 20 minutes after fatigue. F_{max} was determined using brief, repeated stimulations at increasing pulse frequencies (1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Hz for 3 sec allowing a 2 min recovery interval between each stimulus). The greatest force achieved for each animal using this protocol, was considered the F_{max}. Following a 10 min resting period after F_{max} determination, muscle fatigue rate was determined over a 2 minute period of intermittent contractions, stimulating the muscle for 2 seconds on and 2 seconds off at a frequency of 40 Hz (predetermined to be F_{max}). Force was measured at 20 second intervals during fatigue. Muscle fatigue was established when the force generated after the fatigue protocol was 50% of the initial force generated. In the trial study, this was set at 2 minutes, as it took the soleus muscle 2 minutes to lose 50% of its initial force. Twitch amplitude (force), contraction time (time to peak tension) and half-relaxation time (time for peak force to decay by 50%) were determined before (BF) and after (AF) the fatigue protocol. Contraction time (time to peak tension) was defined as the time elapsed from the base to the peak of a single twitch. Half-relaxation time was defined as the time elapsed from the peak of a single twitch to the point of the twitch amplitude returning halfway to baseline. All muscle function data were collected through an AD Instruments Bridge Amp and Powerlab 4/30, and analyzed with Chart5 PowerLab software (ADInstruments, Inc., Colorado Springs, CO).

Specific force was calculated in N/cm^2 of muscle cross-sectional area. The latter was approximated by dividing the dry-weight of the muscle by the product of optimal length and muscle density (assumed to be 1.056 g/cm^3). The force transducer was calibrated using known weights. The contraction time and half-relaxation time were measured as indices of isometric twitch kinetics. For the fatigue protocol, values were normalized by expressing the force generated at each 20 second time point, as a percentage of the initial force at the beginning of the fatigue trial.

2.5 Statistical Analysis

All data are presented as mean±standard error of the mean (SEM), unless otherwise stated. Statistical significance between two groups was assessed via a Student t-test and between two or more groups; a two-way ANOVA was used, followed by a Bonferroni post-hoc test. $P \leq 0.05$ was considered as statistically significant.

Statistical analysis of data was performed using GraphPad Prism, version 5.

3. RESULTS

3.1 Effect of *P. glandulosa* Treatment on Body Mass and Muscle Biometrics

Rats were matched for body mass at the onset of the 10 week *P. glandulosa* treatment and treatment was found to have no effect on weight gain. Skeletal muscle biometrics (mass, optimal length and width), which is a key determinant of the force output, displayed no significant differences between the treated and untreated groups (Table 1). In essence, the soleus muscles of the PLA and PG where biometrically similar.

3.2 Effect of *P. glandulosa* Treatment on the Force-Frequency Relationship

The force-frequency relationship, which is the sigmoid relationship between a muscle's activation frequency and the consequent isometric force output, displayed a similar trend for both muscles in the treated and untreated groups. This trend is displayed in Fig. 1B, representing the force generated at each frequency, expressed as a % of the maximum force generated. In contrast, the absolute values of the force generated at different frequencies (Fig. 1A) displays that the soleus muscles of the treated rats generated significantly more force, during electrical stimulation, compared to the untreated rats, at all the different frequencies ($P<$ .001). As illustrated in Fig. 1A, the force generated by the soleus muscle of the untreated rats incrementally increased from 15.34±2.92 N/cm^2 at a frequency of 5 Hz to a maximum force of 47.77±5.73 N/cm^2 at a frequency of 40 Hz, where after the generated force slowly decreased to a force equal to 38.55±6.27 N/cm^2 at a frequency of 100 Hz. A similar trend is followed by the treated rats, however, at significantly higher levels. The force generated by the soleus muscle of the *P. glandulosa* treated rats incrementally increased from 24.37±3.18 N/cm^2 at a frequency of 5 Hz to a maximum force of 61.65±5.05 N/cm^2 at a frequency of 40 Hz, before slowly declining to a force of 53.48±6.41 N/cm^2 at a frequency of 100 Hz. The level of force generated peaked at a frequency of 40 Hz in both groups.

3.3 Effect of *P. glandulosa* Treatment on Fatigue Tolerance

The 2 minute intermitted stimulation (fatigue protocol) was sufficient to significantly decrease the force generated by both the treated and untreated group by at least 50%. In other words, the force measured after the 2 minute fatigue protocol was 50% lower than the force measured before the induction of fatigue (18.03±3.36 vs. 42.62±5.00 N/cm^2; $P<0.0001$) (Fig. 2B). *P. glandulosa* treatment was unable to reduce fatigue tolerance, as fatigue development was not significantly different between the treated versus untreated group at any point during the 2 minute fatigue protocol. However, the initial force generated, was significantly higher in the treated group, when compared to the untreated group (56.39±4.21 vs. 42.62±5.00 N/cm^2; $P< 0.001$).

3.4 Effect of *P. glandulosa* Treatment on Contractile Properties, Before and After Fatigue

The induction of muscle fatigue resulted in the significant reduction in both twitch- and peak tetanic force generated by the soleus muscle, when comparing PLA (BF) to PLA (AF) and PG (BF) to PG (AF). Therefore as a consequence the twitch/tetanus ratio was significantly reduced after fatigue compared to before fatigue. Despite fatigue ensuing, the contraction time was unaffected by *P. glandulosa* treatment, remaining constant throughout. Ten weeks of *P. glandulosa* treatment sufficiently increased force generated by the soleus muscle, as depicted by the significantly elevated twitch- and peak tetanic

Table 1. Biometric characteristics of the experimental animals before and after *P. glandulosa* treatment

	PLA	PG	p-values
Body mass (g)	438.00±14.97	426.43±16.26	Not significant
Muscle mass (g)	0.20±0.01	0.19±0.01	Not significant
Muscle dry-mass (g)	0.03±0.00	0.03±0.00	Not significant
Optimal muscle length (mm)	31.20±0.66	31.14±0.99	Not significant
Muscle width (mm)	4.60±0.24	4.43±0.20	Not significant
Muscle/ body mass ratio	0.04±0.00	0.05±0.00	Not significant

The data are expressed as mean±SEM; Analysis by Student t-test; n = 10, Per group

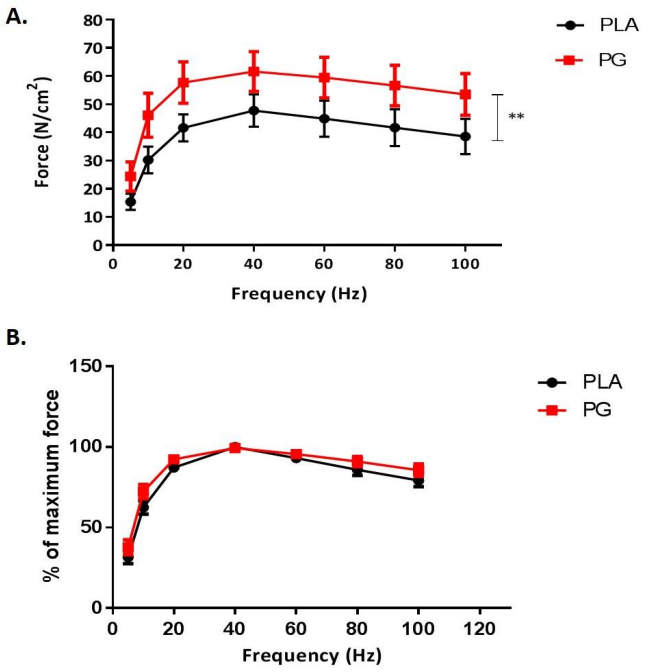

Fig. 1. Force-frequency relationship characteristics of rat soleus muscle in control and *P. glandulosa*-treated rats

*(A) Represents the specific force generated by the soleus muscles at the different frequencies and (B) represents the force generated at each frequency when expressed relative to the maximum force generated. The data are expressed as mean ± SEM. Analysis were by two-way ANOVA. n = 10, per group; ** P< 0.001 PLA vs. PG (10 Hz to 100 Hz)*

force production at baseline (PG (AF) vs. PLA (AF)). *P. glandulosa* treatment also resulted in a significantly increased half-relaxation time post-fatigue, compared to the untreated controls (Table 2).

3.5 Fatigue Results in Decreased Force Production

Force produced by the soleus muscle, directly after fatigue (5 minutes), was significantly reduced in both untreated (26.39±5.98 vs. 47.91±2.60 N/cm^2; P < 0.0001) and treated (28.45±5.07 vs. 62.20±2.68 N/cm^2; P < 0.0001) groups, compared to their respective baseline values. Conversely, a 20 minute resting period resulted in the muscles recovering most of its initial force in untreated (42.12±7.16 vs. 26.39±5.97 N/cm^2; *P*< 0.001) and treated groups (52.37±7.48 vs. 28.45±5.07 N/cm^2; P < 0.001). *P. glandulosa* treatment only had a significant effect on the force generated before the fatigue protocol (62.20±2.68 vs. 47.91±2.60 N/cm^2; *P* = 0.05), with no significant differences observed between the treated and untreated groups 5 minutes and 20 minutes after fatigue (Fig. 3).

4. DISCUSSION

In the current study, we examined the possible strength-increasing and anti-fatigue effects of *P. glandulosa* on soleus muscle, obtained from healthy rats. In this *ex vivo* study, intense exercise was mimicked by electrically stimulating soleus muscles to fatigue and determining the recovery of the muscles after fatigue induction.

Indeed, we present compelling evidence for an effect of *P. glandulosa*. Despite *P. glandulosa* not increasing the endurance capacity of isolated skeletal muscles above (Fig. 2), treatment with these pulverized pods resulted in a significantly increased force generation, throughout force-frequency determination (Fig. 1). The force generated by the soleus muscle of the untreated rats, incrementally increased reaching its maximum force at 40 Hz, where after the generated force slowly decreased again.

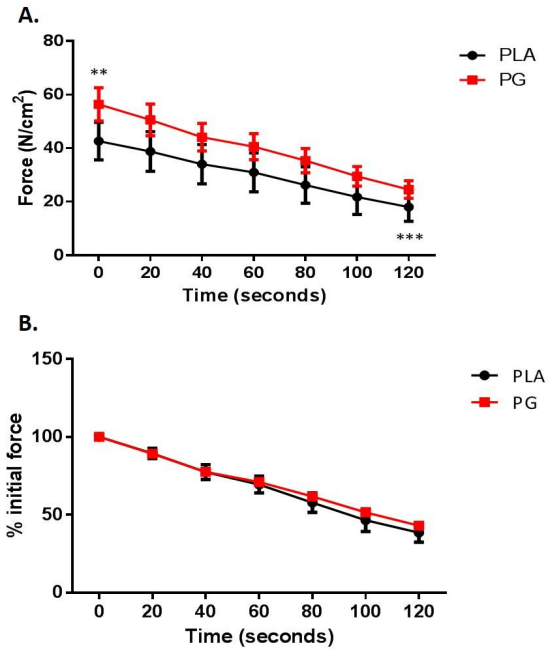

Fig. 2. Fatigue characteristics for soleus muscle in control and *P. glandulosa*-treated rats

*(A) Represents the force generated by the soleus muscles and (B) represents the force, expressed as a % of the initial force generated. The data are expressed as mean ± SEM. Analysis was by two-way ANOVA. n = 10, per group; ** P< 0.001 t = 0 min, PG vs. PLA; *** P< 0.0001 t = 0 min vs. t = 120 min, PLA; *** P< 0.0001 t = 0 min vs. t = 120 min, PG*

A similar trend was observed in the treated group; however, the force generated by the soleus muscles of the treated rats was significantly higher at all measured frequencies (Fig. 1A). This same phenomenon of increased force production was observed when the muscles were stimulated to generate a single twitch or tetanus (Table 2), prior to the induction of fatigue. This augmented effect disappeared after fatigue, as no significant differences were observed during either a single twitch or tetanic stimulation (Table 2) when measured directly after (5 minutes) the fatigue protocol. A 20 minute resting period allowed the muscle to recover most of its initial force in both the untreated and treated groups, when compared to the force generated directly after fatigue (5 minutes) (Fig. 3). Therefore, one may assume that this augmented effect on force will persist if the muscle is left to completely recover.

It is well known that the magnitude of muscle force generation is determined mainly by the size (biometrics) of the muscle and the muscle fiber type [18-19]. The soleus muscles used in this study seemed phenotypically similar, across the treated and untreated groups, as there were no significant biometrical differences found, i.e. the mass, length and width of the muscles in the different experimental groups did not statistically differ from one another (Table 1). Therefore, a likely explanation for the increase in muscle strength lies in the muscle fiber composition. Muscle fiber composition is regulated in response to various factors, such as changes in physical activity. Numerous studies have shown that endurance exercise training induces a fast-to-slow fiber type transition [21-23]. Pathological conditions, mechanical loading and unloading, hormones and aging have also been found to elicit transition in fiber type phenotype [20,22]. From the results obtained in this study we theorized that *P. glandulosa* treatment triggers the transition of muscle fiber type from an oxidative slow-twitch to an oxidative fast-twitch phenotype. Although scant research is available, in which an herbal substance *per se* was responsible for muscle fiber type transition, there are studies alluding to this phenomenon [30]. Wang et al. [30], for example, found the ratio of slow-twitch fibers in soleus muscles decreasing significantly in fructose-fed rats compared to controls and that treatment with Jiang-Tang-Ke-Li (Chinese medicine) recovered the composite ratio of these fibers.

Table 2. Contractile properties of soleus muscle from control vs. *P. glandulosa*-treated rats before and after fatigue

	PLA (BF)	PG (BF)	PLA (AF)	PG (AF)	p-value
Twitch force (N/cm^2)	7.80±0.65	11.95±0.72*	3.09±0.41***	4.22±0.17***	*p<0.05 PG (BF) vs. PLA (BF) ***p<0.0001 PLA (AF) vs. PLA (BF); PG (AF) vs. PG (BF)
Contraction time (ms)	150.00±10.00	114.29±3.49	120.00±5.48	128.57±5.62	Not significant
Half-relaxation time (ms)	447.50±10.37	442.86±11.88	387.50±16.01	453.21±9.83*	*p<0.05 PG (AF) vs. PLA (AF)
Tetanic force (N/cm^2)	47.91±2.60	62.20±2.68*	26.39±5.98***	28.45±1.92***	*p<0.05 PG (BF) vs. PLA (BF) ***p<0.0001 PLA (AF) vs. PLA (BF); PG (AF) vs. PG (BF)
Twitch/Tetanus ratio	15.83±1.49	19.16±1.50	11.90±2.40*	14.75±1.04*	*p<0.05 PLA (AF) vs. PLA (BF); PG (AF) vs. PG (BF)

The data are expressed as mean±SEM; Analysis by two-way ANOVA; n = 10

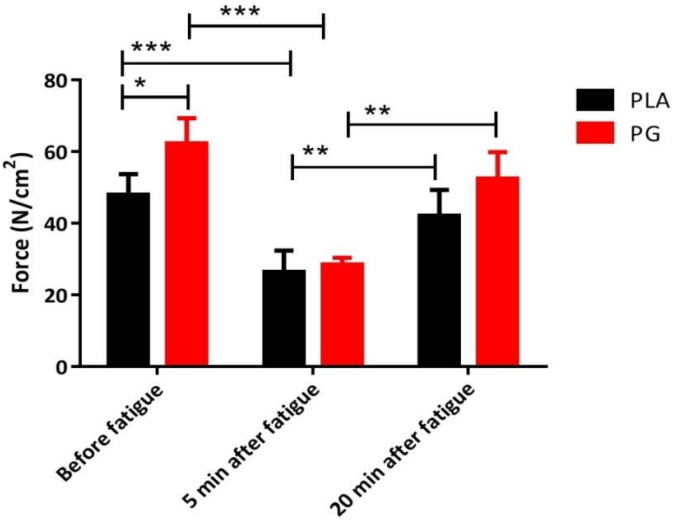

Fig. 3. Tetanic force production before, 5- and 20 min after fatigue

*The data are expressed as mean ± SEM; Analysis by two-way ANOVA; n = 10, per group; * P= 0.05 PLA vs. PG (Before fatigue); ** P< 0.001 PLA vs. PLA (5 min vs. 20 min); PG vs. PG (5 min vs. 20 min); *** P< 0.0001 PLA vs. PLA (Before fatigue vs. 5 min); PG vs. PG (Before fatigue vs. 5 min)*

Motor units containing fast-twitch fibers are typically larger than motor units containing slow-twitch fibers. This difference inevitably means that when a single fast-twitch fiber motor unit is stimulated, more muscle fibers contract than when a slow-twitch fiber motor unit is stimulated. Therefore, since more fibers are stimulated to contract in fast-twitch fiber motor units, more force is produced. Thus muscle composed of a high proportion of fast-twitch fibers will be relatively stronger than a muscle of similar size with a high proportion of slow-twitch fibers.

According to literature, the oxidative slow-twitch fibers and the oxidative fast-twitch fibers react similarly with regards to fatigue development, i.e. they have both been found to be "fatigue-resistant" [31]. This similarity with regards to fatigue development can also be seen in our data, as there were no significant differences observed between the treated and untreated groups (Fig. 2), depicted by the rate of fatigue development. In addition, the time it takes for oxidative fast-twitch fibers to develop maximum tension are faster than that of the oxidative slow-twitch fibers [31]. This phenomenon is also evident in our results, as the contraction time of the muscles in the untreated group is relatively slower than that of the treated group (Table 2). I.e. the muscles of the treated animals contracted at a faster rate and therefore reached its maximum tension faster.

5. CONCLUSION

In conclusion, the data obtained from this study is novel as there is no known literature on the effect of *P. glandulosa* on force generation or fatigue tolerance during muscle stimulation. In addition, since *P. glandulosa* is categorized as an invader tree in South Africa – and probably other countries of export too – it illustrates the ethnopharmacological significance of our novel results. This tree seems to be an ideal candidate for harvesting for natural medicine, as there is no imminent risk of depleting natural resources of the plant. The use of an economical, natural and readily available substance such as the one we identified here, as treatment to increase muscle force generation could have far-reaching implications in not only the sporting arena, but also the health sector.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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