Original Article

Sugarcane Bagasse Extract Improves Muscle Endurance and Increases Both Muscle Density and Muscle Size of Skeletal Muscle in Rats

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Background: The physical fatigue can disturb the quality of work outcome and quality of life. However, the pharmacological therapy against this condition has not yet satisfied the requirement of people. Therefore, the anti-fatigue regimen which is cheap, safe, less toxic and easy to approach is required. Based on the role of oxidative stress on the contractile dysfunction of muscle, the antifatigue effect of sugarcane pomace which possesses antioxidant effect has gained attention. However, no evidence concerning this issue is available.

Objective: To determine the anti-fatigue effect and the possible mechanism of the extract of sugarcane bagasse.

Materials and Methods: Male Wistar rats were randomly divided into 5 groups: control, vehicle, sugarcane bagasse extract 50, 150, 450 $mg.kg^{-1}$BW. Rats in all groups except those in control group were treated with the assigned substance once daily via oral route for 28 days. Exhaustive swimming time was assessed every 7 days. At the end of study, gastrocnemius, soleus and extensor digitorum longus (EDL) were isolated and determined oxidative stress markers and the muscle fiber density and diameter.

Results: All doses of extract used in this study showed anti-fatigue effect. The possible mechanism might depend on the dose of extract. Low and medium doses exerted the effect via the alteration of muscle morphology in gastrocnemius and soleus respectively whereas high dose of extract showed the effect mainly via the decreased oxidative stress in soleus muscle.

Conclusion: Sugarcane bagasse extract has the potential to be developed as an anti-fatigue active ingredient in health products. However, further studies are required to elucidate the precise underlying mechanism and the possible active ingredients.

Keywords: Sugarcane bagasse, Anti-fatigue, Oxidative stress, Muscle morphology

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Physical fatigue is characterized by the deterioration in physical performance resulting from excessive exertion[1]. It is commonly found during sport and exercise activities. However, this condition is also observed as a secondary outcome in many diseases and health conditions during performance of daily activities[2]. Recent findings have demonstrated that physical fatigue disturbs the quality of life and performance output but long-term accumulated fatigue can induce death due to overwork or "Karoshi"[1]. Numerous factors such as energy exhaustion and the increased oxidative stress have been reported to play pivotal roles on fatigue. A growth of evidence has pointed out that contracting muscle during exercise produces both reactive oxygen and nitrogen species and these oxidative stress species in turn induces oxidative damage to both protein and lipid in the contracting myocyte of muscle. Both the oxidative damage and the disturbance of signal transduction induced by oxidative stress lead to contractile dysfunction and finally resulting in muscle weakness and fatigue[3].

Currently, pharmacological therapy against this condition has not yet satisfied the requirement of people. Great attention has been paid to seek an anti-fatigue regimen which is cheap, safe, less toxic and easy to approach in order to improve physical performance and postpone fatigue conditions especially in athletes. Recent researches have revealed that substances possessing antioxidant activity also show anti-fatigue effect[4-6]. Therefore, the development of anti-fatigue regimen from substance possessing antioxidant activity has been focused.

Sugarcane or *Saccharum offinarum*, one of the important crops in the Northeast of Thailand, is belonging to a family of Poaceae. Both juice and bagasse of sugarcane

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contain abundant phenolic compounds[7]. In addition, they also exhibit antioxidant activity. Based on the role of oxidative stress in the pathophysiology of fatigue and the anti-fatigue effect of substances possessing antioxidant activity, the anti-fatigue and the protective effects against muscle damage of sugarcane in exercise-induced fatigue rats has gained attention. To the best of our knowledge, no scientific data were available until now. Therefore, the authors aimed to determine the anti-fatigue effect and the protective effect of sugarcane in skeletal muscle of a rat model of load-induced endurance swimming.

Materials and Methods

Preparation of sugarcane bagasse extract

Bagasse of sugar cane (var.K 92) was dried in oven at temperature less than 60°C. Then the dried sugar cane bagasse was ground into small pieces using kitchen grinder and prepared as 50% hydroalcohol by maceration method. The extract was filtrated and concentrated to dryness under reduced pressure in a rotatory evaporator. The percent yield of extract was 10.48 and the extract contained phenolic compounds at concentration of 158.33±0.001 mg of Gallic acid equivalent 100 g^{-1} of plant). The extract was kept in a dark bottle at -20°C until it was used.

Animals

Healthy male Wistar rats (200 to 250 g) were purchased from National Laboratory Animal Center, Salaya, Nakorn Pathom and they were housed in group of 6 per cage in standard metal cages at 24±2°C on 10:14 h light-dark cycle. All animals were given access to food and water ad labium. The experiments were performed to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC). The experimental protocols were approved by the Institutional Animal Care and Use Committee.

General procedure

After the acclimatization for 2 weeks, rats were randomly divided into 5 groups consisting of 8 per each group. The first group was designated as control group which received no treatment. The second group was vehicle treated group which was treated with vehicle or propylene glycol. The third to fifth groups were designated as experimental groups which received various doses of sugarcane bagasse extract in doses of 50, 150 and 450 $mg.kg^{-1}$ BW once daily via oral route. The treatments were performed at a period of 28 days. The animals were assessed anti-fatigue effect by using exhaustive swimming test every 7 days throughout the experimental period. At the end of study period, gastrocnemius, soleus and extensor digitorum longus (EDL) were isolated and determined muscle density and muscle size. The oxidative stress markers including malondialdehyde (MDA) level and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in all muscles mentioned earlier were also evaluated.

Exhaustive swimming test

After the treatment, all rats rested for 30 minutes before subjected to exhaustive swimming test. In this test, each animal was placed in the swimming tank (50x50x40 cm) 30 cm deep with water maintained at 25±2°C. Constant load weighing 10% of body weight was loaded at the tail of each animal. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 5 sec[8]. The assessment was performed every 7 days throughout the study period.

Histological examination of skeletal muscles

At the end of experiment, gastrocnemius, soleus and extensor digittorum longus (EDL) were isolated and fixed with 10% formaldehyde. Then, they were dehydrated, cleared and embedded in paraffin blocks. Transverse section of muscle was prepared at 5 µm and stained with haematoxylin and eosin (H&E). Muscle density and muscle size were evaluated under light microscope.

Determination of oxidative stress markers

The oxidative stress markers including malondialdehyde (MDA) level and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes were also determined. MDA level was evaluated by using thiobarbituric acid reacting substances (TBARS) assay[9]. The activity of SOD was determined via the xanthine/xanthine oxidase reaction whereas the activity of catalase was measured as disappearance of hydrogen peroxide at 240 nm[10] and the activity of GPx was determined based on the glutathione recycling method by using 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) and glutathione reductase[11].

Statistical analysis

All parameters were compared using one-way analysis of variance (ANOVA). LSD post hoc test was used to identify specific mean differences. They were represented as mean ± standard error mean (mean ± SEM). Statistical analysis was carried out using SPSS version 15. Differences were considered significant at p-value <0.05.

Results

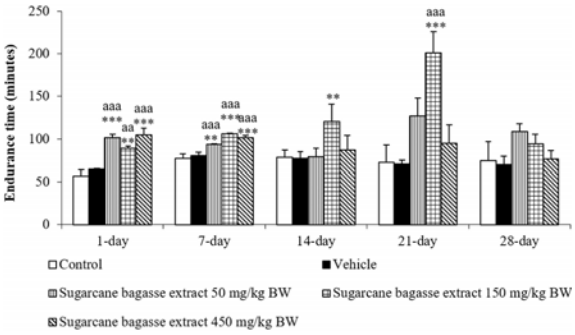
Effect of sugarcane bagasse extract on swimming time

Figure 1 showed that after the single administration, rats which received vehicle showed no significant difference in swimming time when compared with control rats. However, rats which received single treatment of sugarcane bagasse extract at doses of 50, 150 and 450 $mg.kg^{-1}$ showed the enhanced swimming time (p-value <0.001, 0.01 and 0.001, respectively; compared with rats which received vehicle). When the treatment was prolonged to 7 days, the significant increase of swimming time in rats which received the sugarcane bagasse extract at all doses mentioned earlier were still observed (p-value <0.01, 0.001 and 0.001, respectively; compared with rats which received vehicle). However, at 14 and 21 days of treatment, only rats which received the extract

at dose of 150 mg.kg^{-1} significantly enhanced the swimming time (p-value <0.01 and 0.001 respectively; compared with rats which received vehicle). No significant changes of this parameter were observed in any groups at 28-day period.

Effect of sugarcane bagasse extract on oxidative stress markers

Since accumulative lines of evidence pointed out that oxidative stresses play an important role on fatigue mechanism, we also determined the oxidative stress status in all types of skeletal muscle by using gastrocnemius as the representative of mixed type muscle whereas soleus and EDL were used as representatives of slow twitch and fast twitch muscles respectively. Our results showed that the only significant change of oxidative markers was the reduction of MDA in soleus muscle (p-value <0.01; compared with rats which received vehicle). No significant changes of SOD, CAT and GPx were observed in any groups. In addition, rats which received other treatments also failed to show the significant reduction of MDA level in skeletal muscle as shown in Table 1.



aa,aaa p-value <0.01 and 0.001 respectively; compared with control group; **,*** p-value <0.01 and 0.001 respectively, compared with rats which received vehicle

Figure 1. Effect of sugarcane bagasse extract on swimming time (n = 8/group). Data were presented as mean ± SEM.

Effect of sugarcane bagasse extract on diameter and density of muscle fiber

Figure 2 showed the effect of sugarcane bagasse extract on the size of various muscle types. It was found that vehicle produced no significant changes on muscle fiber size in gastrocnemius, soleus and EDL. Both rats which received glucose and rats which received low dose of extract showed the increased in muscle diameter in gastrocnemius (p-value <0.05; compared with vehicle treated group) whereas no significant changes in soleus and EDL were observed. In addition, rats which treated with medium dose of extract showed the enhanced muscle diameter in EDL (p-value <0.001; compared with vehicle treated group) while those which received high dose of extract showed the increased muscle diameter in soleus (p-value <0.05; compared with vehicle treated group).

The effect of sugarcane bagasse extract on the density of muscle fibers of gastrocnemius, soleus and EDL was also evaluated and results were shown in Figure 3. It was found that vehicle also did not produce significant change of this parameter in all muscle mentioned above. Rats which received medium dose of extract showed the increased muscle density in soleus (p-value <0.001; compared with vehicle treated group) whereas rats which treated with high dose of extract showed the increased in muscle density in EDL (p-value <0.001; compared with vehicle treated group).

Discussion

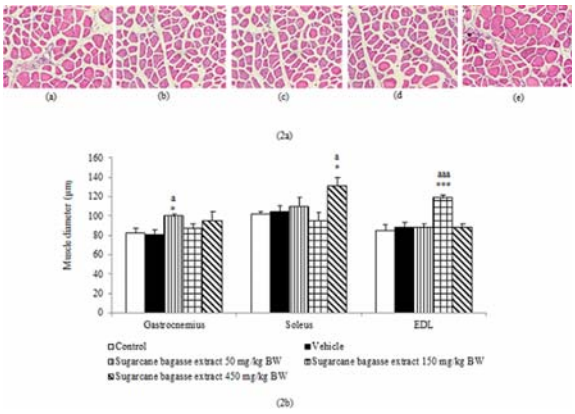
Exhaustive swimming test has been widely used for testing the effect of interventions such as supplement and training[12]. In this test, weight is attached to the tail of rodent animals and the animals must swim with the attached weight until they are unable to remain on the surface. According to this method, the weights are used to reduce the time to exhaustion because unloaded rats can swim for many hours in the normal condition[12].

Although oxidative stresses are reported to contribute an important role on muscle fatigue, our data showed that only significant reduction of MDA level was observed in soleus, a representative of slow twitch muscle. These data suggested that sugarcane bagasse extract at dose

Table 1. Effect of various concentrations of sugarcane bagasse extract on malondialdehydel level and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes in extensor digitorum longus (EDL) muscle

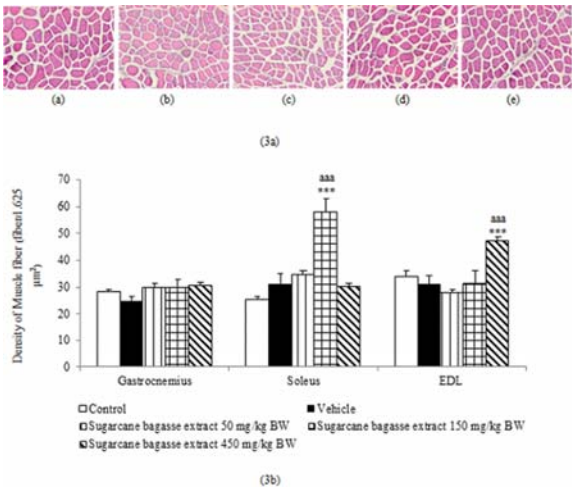
Treatment groups	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
Control	4.09±0.76	8.34±1.54	0.08±0.02	0.89±0.15
Vehicle	4.37±0.86	8.64±0.96	0.08±0.01	0.72±0.07
Sugarcane bagasse extract 50 mg/kg BW	3.13±1.17	10.26±2.88	0.09±0.02	0.97±0.05
Sugarcane bagasse extract 150 mg/kg BW	2.33±0.43	12.17±2.22	0.13±0.06	1.18±0.20
Sugarcane bagasse extract 450 mg/kg BW	0.146±0.27[a,**]	11.33±0.73	0.08±0.01	1.09±0.07

Data were presented as mean ± SEM.
[a] p-value <0.05 compared with control group, ** p-value <0.01 compared with rats which received vehicle (n = 8/group)



[a,aaa] *p*-value <0.05 and 0.001 respectively; compared with control group. [*,***] *p*-value <0.05 and 0.001 respectively; compared with rats which received vehicle

Figure 2. Effect of sugarcane bagasse extract on diameter of muscle fiber. 2a) Photographs showing histomorphology of soleus muscle in various groups; (a) Control (b) Vehicle treated group (c) Low dose of sugarcane bagasse extract (50 mg.kg^{-1} BW) (d)Medium dose of sugarcane bagasse extract (150 mg.kg^{-1} BW) (e) High dose of sugarcane bagasse extract (150 mg.kg^{-1} BW) 2b) Effect of the sugarcane bagasse extract on diameter of muscle fiber in various types of skeletal muscle fiber (n = 8/group). Data were presented as mean ± SEM.



[aaa] *p*-value <0.001 compared with control group, [***] *p*-value <0.001 compared with rats which received vehicle

Figure 3. Effect of sugarcane bagasse extract on density of muscle fiber. 3a) Photographs showing histomorphology of EDL in various groups; (a) Control (b) Vehicle treated group (c) Low dose of sugarcane bagasse extract (50 mg.kg^{-1} BW) (d) Medium dose of sugarcane bagasse extract (150 mg.kg^{-1} BW) (e) High dose of sugarcane bagasse extract (150 mg.kg^{-1} BW) 3b) Effect of the sugarcane bagasse extract on density of muscle fiber in various types of skeletal muscle fiber (n = 8/group). Data were presented as mean ± SEM.

of 450 mg.kg^{-1} decreased excess oxidative stress in slow twitch muscle which used main energy from oxidative phosphorylation process. Since no tight relationship of the longer swimming time and the decreased oxidative stress was observed especially at low and medium doses of sugarcane bagasse extract, the main mechanism to increase swimming time induced by low and medium doses of sugarcane bagasse of extract should be related to other factors rather than oxidative stress.

Interestingly, our data have demonstrated that rats which received medium dose of sugarcane bagasse extract showed the increased muscle fiber density in soleus whereas those which received the high dose extract showed the elevation of muscle density in EDL. In addition, the increased muscle diameter was also observed in gastrocnemius and EDL of rats which received low and medium doses of extract. It has been reported that the increased slow twitch muscle fiber is responsible for the increased muscle endurance[13]. Therefore, the enhanced muscle fiber density especially in slow twitch and mixed type muscle might be responsible in part for the increased swimming time induced by medium dose of sugarcane bagasse extract. Although the elevation of muscle density in EDL was also observed in rats treated with high dose of sugarcane bagasse extract, this change might play minor role on the increased swimming time because fast twitch muscle did not play a great role on endurance and no significant increase in swimming time in rats which received high dose of extract was observed during 14 days of treatment until the end of experiment. Since the fast twitch muscle plays an important role on force production, the increased muscle size observed in EDL of rats which treated with medium dose of extract might contribute some role on the increased force and speed of swimming. However, this required further investigation.

The current data showed the differential vulnerability to sugarcane bagasse extract. Gastrocnemius was less sensitive to the sugarcane bagasse extract whereas soleus muscle showed the highest sensitive to this extract. The different vulnerability might be attributed partly to the difference in distribution of growth factor[14] and non-enzymatic antioxidant and mitochondria density[15]. Since anti-fatigue effect was previously observed in rats treated with polyphenol compounds[16], the anti-fatigue effect of sugarcane bagasse extract observed in this study might be related with the phenolic compounds in the extract. Since numerous factors exert the great influence on physical fatigue,

no simple linear relationship between the doses of extract and this parameter was observed.

Conclusion

The present study has demonstrated that sugarcane bagasse extract possesses anti-fatigue effect which extends the exhaust swimming time. The possible mechanism may depend on the dose of extract. Low and medium doses exert the effect via the alteration of muscle morphology in gastrocnemius and soleus, respectively: whereas the high dose of extract shows the effect mainly via the decreased oxidative stress in soleus muscle. Further studies are required to elucidate the precise underlying mechanism and the possible active ingredients.

What is already known on this topic?

Physical fatigue disturbs the quality of life and performance output, energy exhaustion and the increased oxidative stress have been reported to play important roles on fatigue. Antioxidant activity shows anti-fatigue effect. Sugarcane or *Saccharum offinarum*, one of the important crops in the Northeast of Thailand, both juice and bagasse of sugarcane contain abundant phenolic compounds, which exhibit antioxidant activity.

What this study adds?

Sugarcane bagasse extract possesses anti-fatigue effect which extends the exhaust swimming time. The possible mechanism via the alteration of muscle morphology in gastrocnemius and soleus and also the decreased oxidative stress in soleus muscle.

Acknowledgements

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Potential conflicts of interest

The authors declare on conflicts of interest.

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สารสกัดจากชานอ้อยช่วยเพิ่มความทนทานของกล้ามเนื้อและเพิ่มทั้งความหนาแน่นและขนาดของกล้ามเนื้อในกล้ามเนื้อโครงร่างในหนู

สุภาพร มัชฉิมะปุระ, จินตนาภรณ์ วัฒนธร, วิภาวี ทูคำมี

ภูมิหลัง: การล้าทางกายจะรบกวนผลลัพธ์ของงานและคุณภาพชีวิต อย่างไรก็ตามยังไม่มีการรักษาทางยาที่ต้านภาวะนี้ให้ผลพึงพอใจตามความต้องการของประชาชน ดังนั้นสารที่มีฤทธิ์ต้านล้าที่ราคาถูก ปลอดภัย มีความเป็นพิษน้อย และง่ายต่อการเข้าถึงจึงเป็นที่ต้องการ สืบเนื่องจากบทบาทของความเครียดออกซิเดชันต่อหารทำให้การหดตัวของกล้ามเนื้อผิดปกติทำให้ฤทธิ์ต้านล้าของสารสกัดชานอ้อยซึ่งมีฤทธิ์ต้านอนุมูลอิสระได้รับความสนใจ อย่างไรก็ตามไม่พบหลักฐานเกี่ยวกับประเด็นนี้

วัตถุประสงค์: เพื่อตรวจสอบฤทธิ์ต้านการล้าและกลไกการออกฤทธิ์ของสารสกัดจากชานอ้อย

วัสดุและวิธีการ: หนูขาววิสตาร์แบ่งออกเป็น 5 กลุ่มคือกลุ่มควบคุม กลุ่มที่ได้รับสารนำส่ง กลุ่มที่ได้รับสารสกัดชานอ้อยขนาด 50, 150 และ 450 mg.kg-1BW หนูทุกกลุ่มยกเว้นที่อยู่ในกลุ่มควบคุมได้รับการป้อนสารตามที่กำหนดวันละครั้งเป็นเวลา 28 วัน ทำการประเมินการว่ายน้ำจนเหนื่อยล้าทุก ๆ 7 วัน ในตอนท้ายของการศึกษาได้ทำการแยกกล้ามเนื้อแกสตรอซนีเมียส กล้ามเนื้อโซเลียส และกล้ามเนื้อเอกเทนเซอร์ดิจิตอรุ่มลองกัส ออกมาจากร่างกายและนำไปตรวจสอบหาตัวบ่งชี้ความเครียดออกซิเดชันและความหนาแน่นของเส้นใยกล้ามเนื้อและขนาดเส้นผ่าศูนย์กลางของกล้ามเนื้อ

ผลการศึกษา: สารสกัดทุกขนาดที่ใช้ในการศึกษาครั้งนี้แสดงฤทธิ์ต้านความเหนื่อยล้า โดยกลไกที่เป็นไปได้อาจขึ้นอยู่กับขนาดปริมาณของสารสกัดขนาดต่ำและขนาดกลางออกฤทธิ์ผ่านทางการเปลี่ยนแปลงของสัณฐานวิทยาของกล้ามเนื้อในกล้ามเนื้อแกสตรอคนีเมียสและกล้ามเนื้อโซเลียสตามลำดับ ในขณะที่สารสกัดปริมาณที่สูงออกฤทธิ์หลักผ่านการลดความเครียดออกซิเดชันในกล้ามเนื้อโซเลียส

สรุป: สารสกัดจากชานอ้อยมีศักยภาพที่จะนำมาพัฒนาเป็นสารออกฤทธิ์ในการต้านล้าในผลิตภัณฑ์เพื่อสุขภาพ อย่างไรก็ตามมีความจำเป็นต้องศึกษาเพิ่มเติมเพื่อหากลไกพื้นฐานของการออกฤทธิ์ที่แม่นยำและสารที่น่าจะเป็นสารออกฤทธิ์