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Catechin, an active constituent of green tea, preserves skeletal muscle activity in dexamethasone induced cachexia by increasing acetylcholine sensitivity in muscles of Wistar rats

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Chronic administration of glucocorticoids produces cachexia like symptoms such as muscular dystrophy, weight loss and skeletal muscle dysfunction. However, only limited options are available for treatment of this disease. One of the tea catechins, epigallocatechin-3-gallate attenuated skeletal muscle atrophy in cancer cachexia. In this context, we explored here (+)-catechin hydrate (catechin) for of its anticachectic activity in dexamethasone induced muscle dystrophy. Dosing of catechin at 100 mg/kg *p.o.* was continued for 5 days along with a daily dosing of dexamethasone at 0.6 mg/kg *i.p.* On the 6th day, animals were assessed for cachectic condition using changes in body weight, functional aspect of skeletal muscle such as muscle integrity, locomotor activity, handgrip strength, glucose uptake, responsiveness of skeletal muscle to acetylcholine, by estimating inflammatory parameters such as nitrite, myeloperoxidase in the gastrocnemius muscle and by evaluating plasma biochemical parameters such as triglycerides, total protein, albumin, creatinine, urea and IL-6 levels. Except for a few parameters, such as body weight, glucose uptake by hemi-diaphragm and triglyceride level, remaining parameters were significantly reversed by catechin treatment. The underlying mechanism of the myoprotective action of catechin has been postulated by the increased sensitivity of muscle to acetylcholine as demonstrated in this study, which might be responsible for prevention of muscle inflammation.

Keywords: Anticachectic activity, Cachexia, Glucocorticoids, Glucose uptake, Inflammation, Muscle dystrophy, Muscle wasting, Plasma IL-6 level, Skeletal muscle dysfunction, Weight loss

An increase in circulating glucocorticoids leads to anomaly to skeletal muscle characterized by muscle atrophy or complex metabolic syndrome (cachexia), which shows abnormalities in carbohydrate and fat metabolism, severe insulinopenia, metabolic acidosis, osteoporosis, weight loss, starvation, etc.[1-3]. Glucocorticoids are widely prescribed for the management of chronic inflammatory disorder, such as autoimmune, neuromuscular and lymphoproliferative Disorders[1]. However, long term administration of dexamethasone, a glucocorticoid, produces symptoms of cachexia-like syndrome resulting in muscle wasting and other complication, such as reduced food intake aberrant immune response, etc.[2]. This type of the complex metabolic syndrome was also observed in the different types of malignancies in cancer where the prevalence of cachexia was about 80% in upper gastrointestinal cancer patients and 60% in lung cancer patients[4]. The most prominent feature for diagnosis of cachexia is weight loss. The associated symptoms are anorexia, muscle protein breakdown, inflammation and insulin resistance[1]. It influences adversely the treatment options, quality of life and the patient`s survival. Cachexia in cancer patients accounts for 10-30% of the total cancer related deaths[4]. The exact cause of cachexia syndrome is not precisely known.

Despite the magnitude of the problem associated with the cachexia, progress in developing effective treatments to control cachexia is slow[4]. The anticachectic studies are generally focused on either reducing the anorexia or altered metabolism. The interventions have ranged from nutritional supplements to simple dietary intervention, e.g. offering small but energy dense meals[5]. Pharmacotherapy, on the other hand, includes progestagens[6], corticosteroids[7] and omega-3 fatty acid enriched with oral nutritional supplements[5]. Although corticosteroids and progestagens have temporarily improved appetite and body weight, their influence on weight gain often reflects unhelpful fluid retention and these drugs may exacerbate muscle weakness.

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Nonavailability of FDA approved drugs to treat cachexia and lack of evidence of reversing muscle loss with the agents used, demands further research of high methodological quality for finding a reliable drug. A group of flavonoids has been studied for the prevention of cancer induced cachexia. Curcumin and tea catechins are such few isolates, which had shown an ability to prevent cancer induced cachexia[8,9]. Epigallocatechin-3-gallate, an isolate obtained from green tea has been widely studied and shown potency to prevent cancer induced cachexia[9,10]. Besides, green tea catechins have been demonstrated to have antioxidant, anti-inflammatory and anticancer benefits. With this cue, we selected another important isolate of tea catechin, (+)–catechin hydrate for screening of their anticachectic activity against the dexamethasone induced model of muscle wasting.

Materials and Methods

Animals

Male Wistar rats, weighing 180-200 g were used for the study. The animals were obtained from Central animal research facility (CARF) of Manipal University. The animals were acclimatized for 7 days before the experiment under controlled conditions of temperature (23±2°C), humidity (50±5%) and 12 h light-dark cycles as prescribed by the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Animals were housed in sterile polypropylene cages with a bedding of sterile paddy husk and given free access to food and water. The studies conducted were approved by the Institutional Ethical Committee, Kasturba Medical College, Manipal [IAEC/KMC/53/2010-2011].

Chemicals

Catechin [(+) -catechin hydrate] and dexamethasone were procured from Sigma-Aldrich, USA. All other chemicals were procured from local reliable vendors.

Experimental design

Animals were grouped into three groups with six animals each. They were given vehicle/drug treatment once daily for a period of 5 days as follows. (a) Group I (control): 0.3% CMC alone in the volume of 10 mL/kg; (b) Group II: dexamethasone (0.6 mg/kg *i.p*.) treated control; and (c) Group III: dexamethasone (0.6 mg/kg *i.p*.) + catechin (100 mg/kg orally) treated group. In Gr. III, catechin was administered one hour prior to the administration dexamethasone . Body weight and food intake were measured daily before the treatment. The differences in the body weight between the first day, i.e., induction day and the 5th day of treatment were calculated and expressed in terms of percentage gain in body weight. On the 6th day, physical parameters namely muscle integrity, locomotor activity and hand grip strength/body weight ratio were evaluated. After that blood was withdrawn and plasma was separated from blood by centrifuging at 5000 rpm for 5 min. The separated plasma was used for estimation of biochemical parameters and evaluation of IL-6. The animals were then sacrificed by cervical dislocation, the diaphragm and the ileum were isolated for glucose uptake and muscle contractility, respectively.

Rota-Rod test

The muscle integrity and coordination were assessed using rota-rod apparatus. The speed of rota-rod was adjusted at 25 rpm, animals were placed on rota-rod. Time taken for the animal to fall from the rotating rod was noted and cut off time was 60 s.[11]

Locomotor activity

Locomotor activity was assessed using digital actophotometer (INCO, India). Before performing the locomotor task, the animals were placed individually in the activity meter for 3 min. Then, for the next 5 min, the ambulatory movements were recorded and expressed in terms of total photo beam counts for 5 min per animal[12].

Hand grip strength/body weight ratio

An animal cage with a metal grill on top was firmly fixed with sufficient weight in it. A rat was placed on the top of the metal grill of the cage and it was made to hold the grill firmly. The tail of the animal was tied to tourniquet which in turn was connected to a graduated water bottle with the help of a string running on a pulley. Water was gradually poured into the graduated bottle allowing it to apply force on the animal to pull it off from its grip on the cage. Water-flow into the bottle was stopped when the animal was just separated out of the metal grill. The grip strength of the animal was recorded as the weight of water in terms of cages that are required to loosen the grip of the animal from the cage. Subsequently, the ratio of hand-grip strength and body weight was calculated.

Parameters on the isolated tissues of the animals

Ex vivo glucose uptake by isolated rat hemidiaphragm

Glucose uptake by rat hemidiaphragm was estimated by the method described by Gowdra *et al*.[13].

In brief, overnight fasted Wistar rats were sacrificed by cervical dislocation and the diaphragm was dissected out. One half of the hemidiaphragm was transferred to an organ bath containing 5 mL of tyrode solution with 2% glucose maintained at 37°C and bubbled with atmospheric air. After 45 min of incubation, the glucose content of the solution was determined using glucose kits in an ELISA plate reader. The glucose uptake per gram of tissue was calculated as the difference in the initial and final glucose content in the medium.

Measurement of muscle contractility by isolated rat ileum preparation

Part of ileum was removed and immediately placed in a Petri dish containing the tyrode solution. One piece of ileum, 2-3 cm long was taken, tied with fine thread and mounted in an organ bath containing tyrode solution maintained at room temperature and aerated with 95% O_2 and 5% CO_2. Tissue was allowed to equilibrate for 30 min before adding the drugs to the organ bath. The concentration dependent response due to acetylcholine was recorded using a frontal writing lever. The concentration due to acetylcholine was recorded with increasing dose of acetylcholine (5-6 responses or until maximal response) Log dose *vs*. % response graphs were constructed and the EC50 values were determined[14].

Myeloperoxidase activity in gastrocnemius muscle

Fifty microliter sample/standard/50 mM phosphate buffers (pH 6) (blank) were added to the respective wells of a microplate. To the well, 250 µL O-dianisidine hydrochloride (ODA) 0.167 mg/mL in 50 mM phosphate buffer (pH 6) containing 0.0005 % hydrogen peroxide was added. The plate was then read at 490 nm after 5 and 15 min to monitor the reaction. After 15 min incubation, 50 µL 4M H_2SO_4 was added to stop the reaction and reading was taken. Percentage myeloperoxidase activity with respect to dexamethasone control was calculated. Results are expressed as mean percentage activity with respect to control ± SEM.[15,16]

Nitrite assay in gastrocnemius muscle homogenate

Muscle homogenate was centrifuged at 10000×g for 10 min at 4° Cand the supernatant was collected and used for nitrite estimation. About 100 µL of Griess reagent was added to 100 µL of supernatant and absorbance was measured spectrophotometrically at 542 nm[15,17]. A standard curve was prepared using sodium nitrite and the concentration was expressed in µg/mg of protein

Biochemical parameters of plasma samples

The separated plasma samples were analyzed for albumin, triglycerides, total protein, creatinine and urea using semi autoanalyzer as per the manufacturer's instruction provided with diagnostic kits from Aspen labs, Mumbai. Plasma IL-6 was estimated using ELISA kit of Invitrogen Corporation, USA.

Statistical analysis

All the values represent mean and SEM of six animals. Data were analyzed by One-way ANOVA followed by Dunnett's post hoc analysis using GraphPad Prism 5 (demo version), GraphPad Software, La Jolla California USA, www.graphpad.com.

Results

Effect of drug treatment on the body weight and food intake of the animals in the dexamethasone induced cachexia

Five-day administration of dexamethasone produced a significant (P <0.001) reduction in body weight and food intake in the animals. However, the treatment of catechin could not reverse the effect of dexamethasone (Fig. 1).

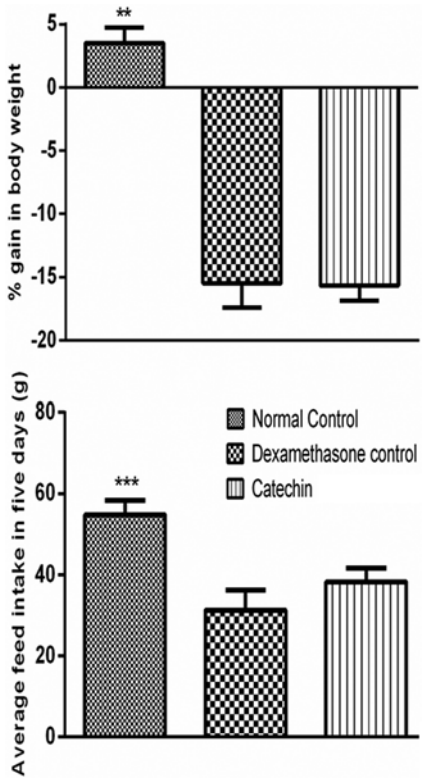

Fig. 1 — Effect of catechin treatment on the body weight and food intake. [All values are mean±SEM of six animals, where **P <0.01 compared to dexamethasone control, ***P <0.001 compared to dexamethasone control]

Effect of drug treatment on muscle integrity and locomotor activity in the dexamethasone-induced cachexia

Dexamethasone caused a significant (P <0.001) reduction in the muscle integrity compared to normal control. On the other hand, the treatment with catechin significantly (P <0.01) reversed the influence of dexamethasone on muscle integrity (Fig. 2).

The locomotor activity of animals treated with dexamethasone significantly (P <0.001) reduced compared to normal control. Catechin treatment significantly (P <0.01) antagonized the debilitating effect of dexamethasone on locomotor activity. Handgrip strength to the body weight ratio was significantly (P <0.001) decreased in animals treated with dexamethasone. The effect of catechin was found to be significant (P <0.05) on handgrip strength to body weight ratio compared to the dexamethasone-treated group (Fig. 2).

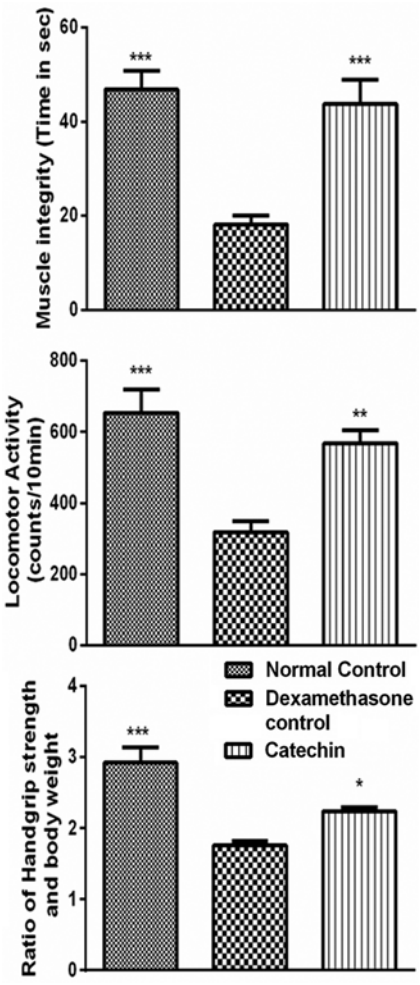

Fig. 2 — Effect of catechin treatment on functional parameters in the dexamethasone-induced cachexia. [All the values are mean±SEM of six animals, where *P <0.05 compared to dexamethasone control, **P <0.01 compared to dexamethasone control, ***P <0.001 compared to dexamethasone control]

Effect of drug treatment on muscle contractility by isolated rat ileum preparation

The EC_{50} of acetylcholine to produce contraction on the isolated rat ileum of a control group of animals was 629.65±2.25 ng/mL. Dexamethasone significantly (P <0.01) increased the EC50 of acetylcholine (770±26.83 ng/mL) compared to normal control, while catechin significantly (P <0.001) reversed the effect of dexamethasone (EC50 120.55±46.72 ng/mL) (Fig. 3).

Effect of drug treatment on glucose uptake by isolated rat hemidiaphragm

In the rat hemidiaphragm preparation, glucose uptake was significantly (P <0.01) declined in the dexamethasone-treated group. catechin failed to reverse the effect of dexamethasone, in fact, it significantly (P <0.01) decreased the glucose uptake compared to dexamethasone group (Fig. 3).

Effect of drug treatment on myeloperoxidase (MPO) activity and nitrite level in gastrocnemius muscle

In the gastrocnemius muscle, MPO and nitrite level were significantly (P <0.01) elevated after the treatment of dexamethasone. Treatment with catechin (P <0.05) prevented the rise in the level of the MPO. Similar effects were observed on the nitrite levels by the treatment of catechin (P <0.01) (Fig. 4).

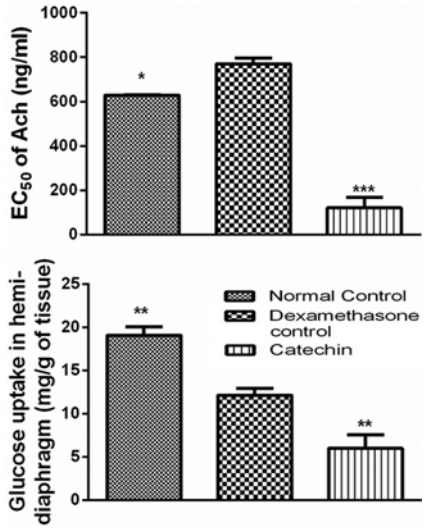

Fig. 3 — Effect on acetylcholine sensitivity and glucose uptake. [All the values are mean±SEM of six animals, where *P <0.05 compared to dexamethasone control, **P <0.01 compared to dexamethasone control, ***P <0.001 compared to dexamethasone control]

Effect of drug treatment on biochemical parameters of the plasma samples

Dexamethasone treatment showed a significant (P <0.01) reduction in plasma albumin, total protein levels, and creatinine level. Catechin treatment significantly reversed the decrease in albumin, plasma protein and creatinine level by dexamethasone. Urea levels were significantly (P <0.01) raised under the influence of dexamethasone, while significant reversal was observed in the catechin treatment group. Besides, we observed a significant (P <0.001) increase in plasma triglyceride levels in dexamethasone-treated rats while no significant effects were observed with catechin group (Fig. 5).

IL-6 levels in the plasma of dexamethasone-treated group significantly (P <0.001) increased compared to normal control group, while treatment with catechin significantly (P <0.001) prevented this rise (Fig. 6).

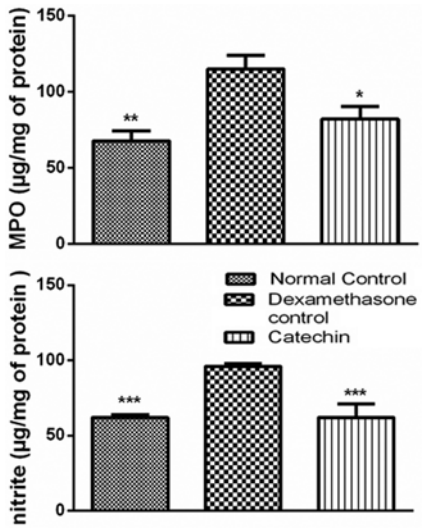

Fig. 4 — Effect on myeloperoxidase and nitrite levels in gastrocnemius muscle homogenate. [All the values are mean±SEM of six animals, where *P <0.05 compared to dexamethasone control, **P <0.01 compared to dexamethasone control, ***P <0.001 compared to dexamethasone control]

Discussion

A number of anticlastogenic agents are used along with glucocorticoid therapy and cancer chemotherapy for preventing their side effects. Among these notable agents, tea catechins are reported to possess anti-cancer effects besides anti-clastogenic property[18,19]. However, these catechins are not evaluated for their anticachectic activity against dexamethasone in spite of their known anti-oxidant and immunomodulatory

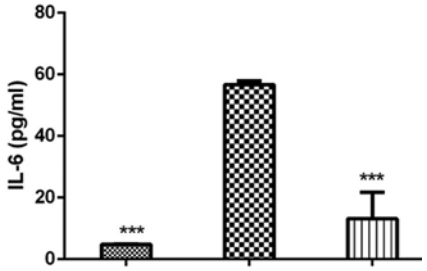

Fig. 6 — Effect on Plasma IL-6 level. [All the values are mean±SEM of six animals, where ***P <0.001 compared to dexamethasone control]

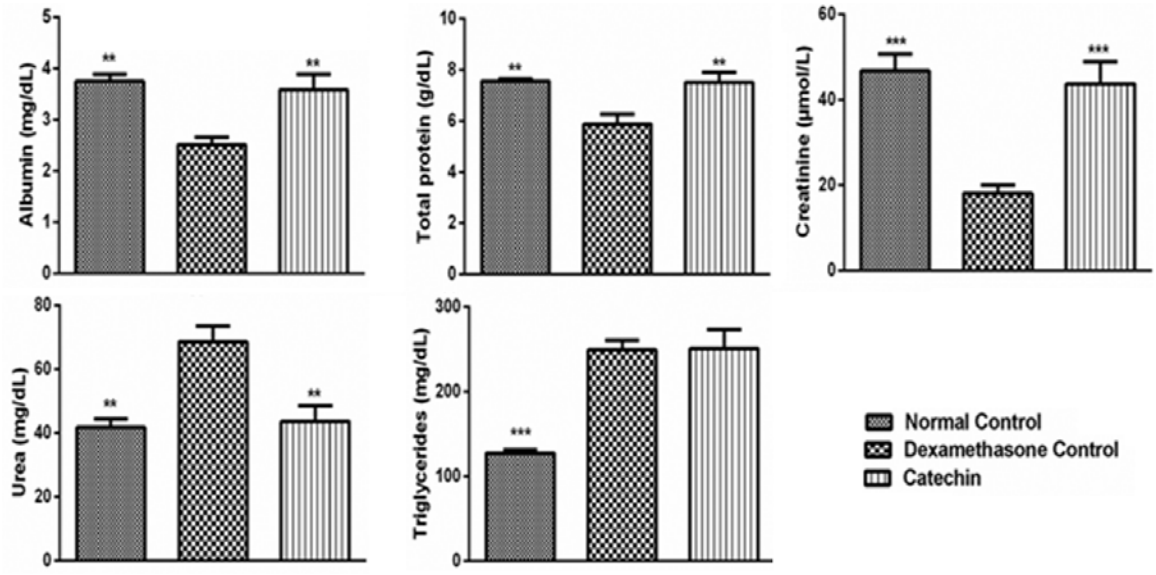

Fig. 5 — Effect on biochemical parameters. [All the values are mean±SEM of six animals, where **P <0.01 compared to dexamethasone control, ***P <0.001 compared to dexamethasone control]

properties[20,21]. In the view of this, the present study was undertaken to assess the anticachectic activity of (+)-catechin hydrate in the dexamethasone-induced cachectic model in Wistar rats. The dose of dexamethasone was selected based on the reports of Ma *et al*.[22], while test drug dose was selected based on the earlier reports on hepatoprotection for (+)-catechin hydrate by Raj *et al*.[23] and obesity modulation effect for a green tea catechin by Yan *et al*.[24].

Loss of body weight along with anorexia are the major symptoms of dexamethasone induced cachexia/anorexia syndrome[25]. In the present study, similar findings were obtained after dexamethasone administration, a significant weight reduction was observed in dexamethasone administered control rats compared to normal control rats. Catechin was unable to prevent the weight loss caused by dexamethasone. This action of catechin can be justified by the fact that catechin leads to weight loss along with an increase in oxidation of fat[26]. However, compared to dexamethasone, there was no extra weight loss observed after catechin treatment. Thus, it can be concluded that it has no additive effect on dexamethasone induced weight reduction and also, it cannot prevent the weight loss by dexamethasone. The dexamethasone treatment also showed a decrease in food intake in the animals, which might have contributed to the overall decrease in the body weight. The treatment with catechin improved food intake compared to dexamethasone alone treatment, however, it was not significant.

The decrease in skeletal muscle activity in dexamethasone induced cachexia-anorexia syndrome was characterized by evaluating functional aspects of muscle, directly by muscle integrity, locomotor activity, handgrip strength and indirectly by glucose uptake and the responsiveness of skeletal muscle to acetylcholine[27]. Dexamethasone, which significantly decreased muscle integrity, and hand grip strength compared to control, were effectively prevented by catechin treatment. Similar ameliorative effect of catechin on correcting locomotor incoordination caused by dexamethasone is interlinked with its effectiveness in maintaining skeletal muscle integrity and reflected their beneficial role in dexamethasone induced cachectic conditions. Skeletal muscle activity largely depends on the nicotinic receptor stimulation caused by cholinergic transmission. The cholinergic transmission also regulates the inflammatory activities of macrophages/monocytes and T-lymphocytes. The activation of the nicotinic receptor (nAChRα7) on these cells prevents skeletal muscle inflammation by reducing metalloprotease MMP-9 activity, TNFα and NFkB contents and by increasing muscular regeneration and preventing apoptosis[28]. In the present study, the sensitivity of muscle to acetylcholine was decreased significantly by dexamethasone, which was improved significantly by catechin. This effect of catechin might be due to anticholinesterase activity of catechin as reported by Cheruku *et al*.[20].

Enzymatic markers of inflammation play an important role in characterizing cachexia associated muscle wasting. Since the sensitivity of skeletal muscle to acetylcholine was increased by catechin, it might have a protective role against inflammation and related inflammatory myopathy. To confirm this, MPO and nitrite levels in gastrocnemius muscle homogenate were assessed. The earlier studies suggest that inflammatory markers increase significantly in the cachexia syndrome, which shows the recruitment of immune cells, such as neutrophil, monocytes and macrophages to muscle, leading to muscular dystrophy. In the present study, dexamethasone treatment significantly elevated MPO and nitrite levels in the gastrocnemius muscle. Catechin significantly prevented the rise in MPO and nitrite level in the muscle, which might be due to the poor infiltration of polymorphonuclear cells/neutrophil. These polymorphonuclear cells and some other tissues like myocytes and endothelial cells secrete IL-6, which is a key regulatory cytokine for inflammation. The circulatory plasma IL-6 is short-lived and concentration may increase for a short period after acute strenuous exercise. However, the increased level of IL-6 for a longer time is observed in various pathological conditions of cachexia, associated with cancer and other metabolic syndromes namely diabetes and inflammation[29]. The level of IL-6 is also reported to increase in long term administration of dexamethasone due to the combined effect of insulin resistance and increased the formation of advanced glycation end product leading to the generation of proinflammatory signals such as IL-6[30]. In the present study, chronic administration of dexamethasone showed a significant rise in IL-6 level which was significantly prevented by catechin administration. Thus, it confirmed the myoprotective effect of catechin by inhibition of inflammatory signal. Earlier studies also supported catechins role in inhibition of IL-6 levels[31] and suppression of muscle inflammation[32].

Glucose is the primary source of energy for normal skeletal muscle functions. The muscle contractile

activity during exercise largely depends on skeletal muscle glucose transport and glucose homeostasis. Dexamethasone is known to cause insulin resistance in rat skeletal myocytes without altering GLUT4 expression[33]. In our study, insulin-mediated glucose uptake was significantly decreased in dexamethasone group, which was consistent with the previous reports[34]. Interestingly, glucose uptake was further decreased in catechin treated animals which might be due to inhibition of translocation of GLUT4 transporters as was observed earlier in 3T3-L1 adipocytes[34,35]. Insulin resistance is one of the important factors leading to dyslipidaemia[2,36]. Dexamethasone increases not only insulin resistance but also splanchnic triglyceride production along with low lipoprotein lipase activity in adipose tissue. We found similar results in dexamethasone treated control animals, the triglyceride levels were significantly elevated compared to normal animals. However, treatment with catechin could not reverse the condition.

Decreased levels of total protein and albumin is associated with cachexia in the murine model due to decreased expression of albumin gene by the raised level of TNFα[37,38]. We got similar finding in the present study, levels of total protein and albumin were decreased significantly by the chronic administration of dexamethasone. The levels of total protein and albumin were significantly reversed by the co-treatment with catechin, which further supported the anticachectic activity of catechin. Creatinine production depends on muscle mass of the individual. A lower plasma creatinine and high level of urea represent muscle-wasting conditions associated with renal dysfunction[39]. In the present study, the significantly lower value of serum creatinine and high value of urea were observed in dexamethasone alone treated group, which represented a muscle wasting condition. Catechin significantly prevented these changes induced by dexamethasone, which might represent protection from the loss of muscle mass and improvement in renal function.

Conclusion

Catechin showed improvement in the impaired functional parameters of muscles by increasing acetylcholine sensitivity and preventing inflammatory condition induced by dexamethasone. However, it could not reverse the body weight loss caused by the dexamethasone treatment.

References

1 Fry CS, Nayeem SZ, Dillon EL, Sarkar PS, Tumurbaatar B, Urban RJ, Wright TJ, Sheffield-Moore M & Tilton RG, Choudhary S, Glucocorticoids increase skeletal muscle NF-κB inducing kinase (NIK): links to muscle atrophy. *Physiol Rep*, 4 (2016). pii: e13014.

2 Goncalves-Neto LM, Ferreira FB, Souza L, dos Santos C, Boschero AC, Facundo VA, Santos AR, Nunes EA & Rafacho A, Disruption of glucose tolerance caused by glucocorticoid excess in rats is partially prevented, but not attenuated, by arjunolic acid. *Indian J Exp Biol*, 52 (2014) 972.

3 Banji D, Banji OJ, Chiluka VL & Abbagoni S, Role of Triticum aestivum aqueous extract in glucocorticoid induced osteoporosis in rats. *Indian J Exp Biol*, 52 (2014) 153.

4 Pettersen K, Andersen S, Degen S, Tadini V, Grosjean J, Hatakeyama S, Tesfahun AN, Moestue S, Kim J, Nonstad U, Romundstad PR, Skorpen F, Sørhaug S, Amundsen T, Grønberg BH, Strasser F, Stephens N, Hoem D, Molven A, Kaasa S, Fearon K, Jacobi C & Bjørkøy G. Cancer cachexia associates with a systemic autophagy-inducing activity mimicked by cancer cell-derived IL-6 trans-signaling. *Sci Rep*, 7 (2017):2046.

5 Harvie M, Nutritional supplements and cancer: potential benefits and proven harms. *Am Soc Clin Oncol Educ Book* (2014) e478.

6 Berenstein EG & Ortiz Z, Megestrol acetate for the treatment of anorexia-cachexia syndrome. *Cochrane Database Syst Rev*, (2005) Cd004310.

7 Willox JC, Corr J, Shaw J, Richardson M, Calman KC & Drennan M, Prednisolone as an appetite stimulant in patients with cancer. *Br Med J* (*Clin Res Spl Edn*), 288 (1984) 27.

8 Siddiqui RA, Hassan S, Harvey KA, Rasool T, Das T, Mukerji P & DeMichele S, Attenuation of proteolysis and muscle wasting by curcumin c3 complex in MAC16 colon tumour-bearing mice. *Br J Nutr*, 102 (2009) 967.

9 Wang H, Lai YJ, Chan YL, Li TL & Wu CJ, Epigallocatechin-3-gallate effectively attenuates skeletal muscle atrophy caused by cancer cachexia, *Cancer lett*, 305 (2011) 40.

10 Mirza KA, Pereira SL, Edens NK & Tisdale MJ, Attenuation of muscle wasting in murine CC myotubes by epigallocatechin-3-gallate. *J Cachexia Sarcopenia Muscle*, 5 (2014) 339

11 Verma MK, Goel R, Nandakumar K & Nemmani KVS, Effect of D-Ala2GIP, a stable GIP receptor agonist on MPTP-induced neuronal impairments in mice. *Eur J Pharmacol*, 804 (2017):38.

12 Nampoothiri M, John J, Kumar N, Mudgal J, Nampurath GK & Chamallamudi MR, Modulatory role of simvastatin against aluminium chloride-induced behavioural and biochemical changes in rats. *Behav Neurol,* 2015 (2015) 210169.

13 Gowdra VS, Mudgal J, Bansal P, Nayak PG, Manohara Reddy SA, Shenoy GG, Valiathan M, Chamallamudi MR & Nampurath GK, Synthesis, characterization, and preclinical evaluation of new thiazolidin-4-ones substituted with *p*-chlorophenoxy acetic acid and clofibric acid against insulin resistance and metabolic disorder. *Biomed Res Int*, 2014 (2014) 620434.

14 Morales MA, Ahumada F, Castillo E, Burgos R, Christen P, Bustos V & Muñoz O, Inhibition of cholinergic contractions of rat ileum by tkopane-type alkaloids present in Schizanthus hookeri. *Z Naturforsch C*, 68 (2013) 203.

15 Kumar N, Rai A, Reddy ND, Raj PV, Jain P, Deshpande P, Mathew G, Kutty NG, Udupa N & Rao CM, Silymarin liposomes improves oral bioavailability of silybin besides targeting hepatocytes, and immune cells. *Pharmacol Rep*, 66 (2014) 788.

16 Manohara Reddy SA, Mudgal J, Bansal P, Vasanthraju SG, Srinivasan KK, Rao CM & Gopalan Kutty N, Antioxidant, anti-inflammatory and anti-hyperglycaemic activities of heterocyclic homoprostanoid derivatives. *Bioorg Med Chem*, 19 (2011) 384.

17 Ratajczak-Wrona W, Jablonska E, Garley M, Jablonski J & Radziwon P, Role of ERK1/2 kinase in the expression of iNOS by NDMA in human neutrophils. *Indian J Exp Biol*, 51 (2013) 73.

18 Gupta S, Saha B & Giri AK, Comparative antimutagenic and anticlastogenic effects of green tea and black tea: a review. *Mutat Res*, 512 (2002) 37.

19 Jain P, Kumar N, Josyula VR, Jagani HV, Udupa N, Rao CM & Raj PV, A study on the role of (+)-catechin in suppression of HepG2 proliferation via caspase dependent pathway and enhancement of itsin vitro and in vivo cytotoxic potential through liposomal formulation. *Eur J Pharm Sci*, 50 (2013) 353.

20 Cheruku SP, Ramalingayya GV, Chamallamudi MR, Biswas S, Nandakumar K, Nampoothiri M, Gourishetti K & Kumar N, Catechin ameliorates doxorubicin-induced neuronal cytotoxicity in *in vitro* and episodic memory deficit in *in vivo* in Wistar rats. *Cytotechnology*. 70 (2018):245.

21 Ranjith-Kumar CT, Lai Y, Sarisky RT & Cheng Kao C, Green tea catechin, epigallocatechin gallate, suppresses signaling by the dsRNA innate immune receptor RIG-I. *PLoS One*, 5 (2010) e12878.

22 Ma K, Mallidis C, Bhasin S, Mahabadi V, Artaza J, Gonzalez-Cadavid N, Arias J & Salehian B, Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression. *Am J Physiol Endocrinol Metab,* 285 (2003) E363

23 Vasanth Raj P, Nitesh K, Sagar Gang S, Hitesh Jagani V, Raghu Chandrashekhar H, Venkata Rao J, Mallikarjuna Rao C & Udupa N, Protective Role of Catechin on d-Galactosamine Induced Hepatotoxicity Through a p53 Dependent Pathway. *Indian J Clin Biochem*, 25 (2010) 349

24 Yan J, Zhao Y & Zhao B. Green tea catechins prevent obesity through modulation of peroxisome proliferator-activated receptors. *Sci China Life Sci*, 56 (2013):804.

25 Moinard C, Minet-Quinard R, Gachon F, Cynober L & Vasson M-P, Dexamethasone treatment induces long-lasting hyperleptinemia and anorexia in old rats. *Metabolism*, 50 (2001) 1054.

26 Türközü D &Tek NA, A minireview of effects of green tea on energy expenditure. *Crit Rev Food Sci Nutr,* 57 (2017):254

27 Murphy KT, Chee A, Trieu J, Naim T & Lynch GS, Importance of functional and metabolic impairments in the characterization of the C-26 murine model of cancer cachexia. *Dis Model Mech*, 5 (2012) 533.

28 Leite PEC, Lagrota-Candido J, Moraes L, D'Elia L, Pinheiro DF, da Silva RF, Yamasaki EN & Quirico-Santos T, Nicotinic acetylcholine receptor activation reduces skeletal muscle inflammation of mdx mice. *J Neuroimmunol*, 227 (2010) 44.

29 Carson JA & Baltgalvis KA, Interleukin-6 as a Key Regulator of Muscle Mass during Cachexia. *Exerc Sport Sci Rev*, 38 (2010) 168

30 Bighetti BB, Assis GF, Vieira DC, Violato NM, Cestari TM, Taga R, Bosqueiro Jé R & Rafacho A, Long-term dexamethasone treatment alters the histomorphology of acinar cells in rat parotid and submandibular glands. *Int J Exp Pathol*, 95 (2014) 351.

31 Hosokawa Y, Hosokawa I, Ozaki K, Nakanishi T, Nakae H & Matsuo T, Tea polyphenols inhibit IL-6 production in tumor necrosis factor superfamily 14-stimulated human gingival fibroblasts. *Mol Nutr Food Res*, 54 (Suppl. 2) (2010) S151.

32 Haramizu S, Ota N, Hase T & Murase T, Catechins suppress muscle inflammation and hasten performance recovery after exercise. *Med Sci Sports Exerc*, 45 (2013) 1694.

33 Brown P, Badal S, Morrison S & Ragoobirsingh D, Acute impairment of insulin signalling by dexamethasone in primary cultured rat skeletal myocytes. *Mol Cell Biochem*, 297 (2007) 171.

34 Ueda M, Furuyashiki T, Yamada K, Aoki Y, Sakane I, Fukuda I, Yoshida K & Ashida H, Tea catechins modulate the glucose transport system in 3T3-L1 adipocytes. *Food Funct*, 1 (2010) 167.

35 Strobel P, Allard C, Perez-Acle T, Calderon R, Aldunate R & Leighton F, Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. *Biochem J*, 386 (2005) 471.

36 Ginsberg HN, Zhang YL & Hernandez-Ono A, Regulation of plasma triglycerides in insulin resistance and diabetes. *Arch Med Res*, 36 (2005) 232.

37 Brenner DA, Buck M, Feitelberg SP & Chojkier M, Tumor necrosis factor-alpha inhibits albumin gene expression in a murine model of cachexia. *J Clin Invest*, 85 (1990) 248.

38 Visser M, Kritchevsky SB, Newman AB, Goodpaster BH, Tylavsky FA, Nevitt MC & Harris TB, Lower serum albumin concentration and change in muscle mass: the Health, Aging and Body Composition Study. *Am J Clin Nutr*, 82 (2005) 531.

39 Zaman T, Filipowicz R & Beddhu S, Implications and importance of skeletal muscle mass in estimating GFR at dialysis initiation. *J Ren Nutr*, 23 (2013) 233.