Original Article

Comprehensive Endurance Enhancing Effect of INDUS1710, a Composition of Standardised Fenugreek Seed Extracts, During Treadmill Running Exercise in Laboratory Rats

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Abstract

Background: Endurance, defined as the ability to sustain exercise, involves multiple organs and physiological processes. **Aim**: This study aimed to evaluate of INDUS1710, a composition of 4-hydroxyisoleucine, trigonelline, and select glycosides-based standardised fenugreek seed extracts, during treadmill running exercise (EXE) for endurance enhancement in laboratory rats. **Methods:** Male Wistar rats were randomly divided into six groups of 12 rats each and orally administered vehicle (distilled water, VC), INDUS1710 (20, 30, or 60 mg/kg), L-arginine (308 mg/kg), or vehicle for 28 days. All groups, except VC, underwent EXE without incline at a speed of 14 m/min for 6 days, followed by a speed of 20 m/min on the 7th day for 28 days until exhaustion. The physiological, functional, and metabolic parameters; relative organ weights; glycogen content of gastrocnemius muscle; and histological parameters of the heart were recorded. **Results:** Subacute supplementation with EXE of INDUS1710 resulted in a dose-dependent increase in time to exhaustion and prevented EXE-induced changes in organ function (heart, lungs, kidney, and liver), metabolic processes (carbohydrates, proteins, and lipids), and skeletal muscle glycogen content without causing pathological changes in skeletal or cardiac muscles. **Conclusion:** INDUS1710 supplementation with EXE showed comprehensive endurance enhancement efficacy and safety in laboratory rats.

Keywords: Cardiovascular, endurance, fenugreek seed, respiratory, skeletal muscle

Introduction

The ability to maintain the physical or mental effort for an extended period, known as endurance, is crucial for both physical and mental health and overall well-being.[1] Several endurance types, namely cardiovascular, skeletal, muscular, and mental endurance, involve different mechanisms, such as the ability of the heart to pump blood efficiently,[2] lungs to oxygenate blood,[3] and muscles to use oxygen to produce energy[4] along with central fatigue and homeostatic principles.[5]

Several food ingredients,[6] nutraceutical supplements,[7] and health drinks[8] have been recommended to improve endurance performance. Plant-based natural compounds, such as extracts, have been reported to improve endurance.[9] Caffeine and other stimulants are used to enhance endurance,[7] but excessive consumption can harm sleep, health, and cognitive function, causing memory problems, dependence, and withdrawal symptoms.[10] However, the use of many plant-based sports nutrition options lacks standardisation, quality control, or insufficient clinical evidence[11] and the risk of side effects.[12] In addition, the endurance process involves multiple physiological systems including cardiovascular, respiratory, and musculoskeletal systems. Therefore, a plant-based supplement with benefits to multiple organs

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while maintaining a robust safety profile is required for a comprehensive endurance enhancement.[13]

Fenugreek (*Trigonella foenum-graecum*, L.), a spice ingredient, is known for its diverse beneficial effects on endurance-related organs such as the heart, lungs, and skeletal muscle[14] Fenugreek seeds supplementation to endurance exercise to obese women improves body composition, lipid profile and insulin resistance.[15] The existing body of evidence on fenugreek seed extracts standardised to bioactive markers like 4-hydroxyisolecuine (4-HI), trigonelline,[16,17] and glycosides [18,19] have suggested the potential for endurance enhancement. The major branched-chain amino acid (BCAA) component of fenugreek seed is 4-HI.[20] An oral supplement containing 4-HI is reported to enhance glycogen synthesis,[21] glucose supply,[22] insulin secretion,[20] and glucose uptake [22] in skeletal muscles. Fenugreek seed extract rich in 4-HI in mice during forced swimming exercise improves endurance capacity.[23] Trigonelline, a major alkaloid in fenugreek seeds, enhances dopaminergic transmission.[24,25] As brain dopamine-associated circuits are involved in reward processing and motivation,[26] Trigonelline-containing extracts can be used to reduce central fatigue and enhance endurance, through possible modulation of brain dopamine [25,27] and associated reward processing [28]and motivation process [29] In addition, the glycosides-based standardised fenugreek seed extract was reported endurance-related physiological benefits such as senolytic action[30] with improvement of lung function in animals,[31] muscle strength, power, and lean mass in resistance-trained males, [32,33] and muscle recovery in non-resistance-trained subjects.[19] In addition, the robust safety profile of 4-HI +trigonelline-[34] and glycoside- [19,35-37] based standardised extracts has also been confirmed.

Taken together, the composition of fenugreek seed extract standardised to 4-HI, trigonelline, and glycosides can provide comprehensive endurance enhancement with benefits to multiple organs.[13] Therefore, this study evaluated the 4-HI, trigonelline, and glycosides-based standardised composition of fenugreek seed extract (INDUS1710) on endurance-related systems (cardiovascular, respiratory, skeletal muscles) and processes (metabolism) during treadmill running exercise in laboratory rats.

Materials and Methods

The test extract

The test extract, INDUS1710, was obtained as powder and was the composition of standardised fenugreek seed extracts, available as a dietary ingredient, Enducor.[38] INDUS1710 was the composition of IDM01 (standardised to 4-HI and trigonelline)[34] and SFSE-G (standardised to glycosides),[36] with a final composition of 15.84% 4-HI, 21.88% trigonelline, and 15.94% select glycosides (vicenin 1, vicenin 2, vicenin 3, schaftoside, isoschaftoside, orientin, isoorientin, vitexin, isovitexin, vitexin-2-o-rhamnoside, and steroidal saponins). L-arginine (98% purity, Sigma-Aldrich, Bangalore, India) was used as a positive control. Biochemical estimation kits and other chemicals were bought from the Coral Clinical Systems (Goa, India).

Animals

Wistar rats (male, 3–4 months old, weighing 190–230 g) were acquired from the National Institute of Biological Sciences (Pune, India) and used in the study. The rats were maintained in animal houses using recommended conditions as per the Indian ethical standards and regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)[39,40] The approval of the study protocol was taken from Institutional Animal Ethics Committee (IAEC) of the institution where the study was conducted.

Groupings and treatments

The study included 72 rats, which were randomised into six groups (G1–G6) of 12 rats each. Groups G1 and G2 were the vehicle control (VC) and treadmill running exercise (EXE control groups, respectively, which received daily gavage treatment with 10 mL/kg distilled water (vehicle). The G3 group, L-Arginine (308), was a positive control and received daily oral gavage treatment of l-arginine (308 mg/kg) The other groups (G4–G6) received daily oral gavage of INDUS1710 (20, 30, or 60 mg/kg). All treatments were orally gavaged once daily 30 min before EXE from D1 to D28.

EXE schedule

The schedule of EXE with the measurements performed during the study is presented in Figure 1. All groups of rats, except G1, underwent acclimatisation followed by the EXE schedule to measure the time to exhaustion as an indicator of endurance exercise using a rodent treadmill (VJ Instruments, Karanja Lad, India), as previously reported.[41] During acclimatisation, each rat was placed on a rodent treadmill (VJ Instruments, Karanja Lad, India) with speed of 14 meters/min (0.8 km/h) with no incline, for 5 min for six days (D1 to D6). On the 7th day of each week for the next 28 days (D7, D14, D21, and D28), each rat was placed on a treadmill running at 20 meters/min (1.2 km/h) with no incline until exhaustion was achieved. Exhaustion was considered when the rat received an electric shock three consecutive times to prevent running on a treadmill. Body weight (g), food intake (g), and water consumption (mL) were recorded before dosing on D1 and D28.

Measurements

On D28 of the experiment, after 1 h of endurance capacity evaluation, the parameters related to respiratory function,[42] cardiovascular (electrocardiographic, arterial, and ventricular) function,[43] biochemical measurements for metabolic (carbohydrate, protein, lipid) endurance,[44] liver function, and kidney function. In addition, on D28 after functional measurements, the weights of organs (heart, liver, spleen, kidneys, and skeletal muscles, namely, the

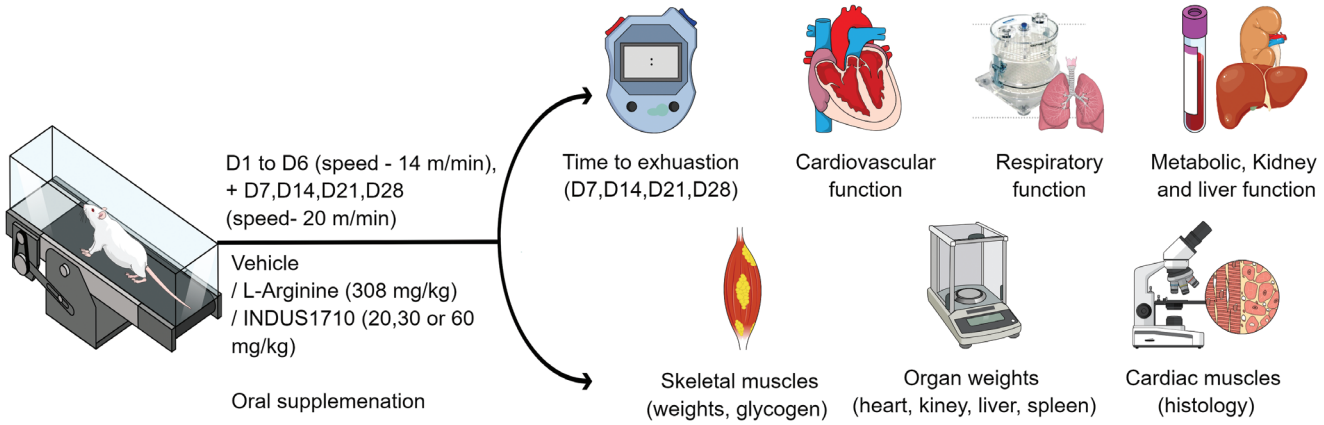

Figure 1: Schedule of EXE and measurements. D = day of study, m/min = meters per minutes

soleus, gastrocnemius, and levator ani), glycogen content measurement in the gastrocnemius muscle.[45] and histology (heart and skeletal muscles) were performed on euthanised (cervical dislocation) rats. Details of the measurements, equipment, methods, and list of parameters are presented in Table 1.

Statistical Analysis

Data are represented as mean ± standard error of the mean (SEM) and were analyzed using GraphPad Prism 5.0 for Windows (GraphPad, San Diego, CA). Time-to-exhaustion data were analyzed using a two-way repeated analysis of variance (ANOVA) and Bonferroni's test. Other data were analyzed for each parameter on each day using separate one-way ANOVA and Dunnett's test. Differences were considered statistically significant at $P < 0.05$.

Results

The results obtained from data on time spent by rats on the treadmill until exhaustion are shown in Table 2. On D1, no groups showed significant differences (v/s VC or EXE control groups) during the endurance test (treadmill speed 20 m/min at 0% grade until exhaustion was achieved). However, the EXE control group rats had a significant ($P < 0.05$ or $P < 0.001$) increase in time to exhaustion (v/s VC) from D7 onwards up to D28. L-arginine (308 mg/kg), and INDUS1710 (20, 30, or 60 mg/kg)-treated rats showed significant ($P < 0.001$) increases in the time to exhaustion from D14 and D21 onwards, respectively (Table 2). On D28, the time to exhaustion was prolonged by 6.3% in the L-arginine-treated group and by 5.85%, 8.95%, and 12.06% in the INDUS1710 (20, 30, and 60 mg/kg)-treated groups.

Body weights and dietary intake data are presented as the mean change from the baseline (Table 3). The increase in body weight in the EXE was shown significantly ($P < 0.001$) higher (16%) than that in the VC group. In addition, body weight increases after 28-day treatment with L-arginine (5%) or INDUS1710 (up to 7%) has no significant difference from EXE. The food and water intake in the EXE group on D28 was significantly ($P < 0.001$) more than VC group, whereas L-arginine or INDUS1710 (all doses) showed a statistically significant ($P < 0.001$) decrease.

The results of analysis from respiratory (Table 4) and cardiovascular function (Table 5) parameters and percent changes with statistical significance (Table 6) were presented in tabular form. Similarly, the results of the analysis of metabolic and biochemical measurements, including liver and kidney functions (Table 7) and percent changes (Table 8), were presented in tabular forms. These results showed the efficacy of 28-day supplementation of INDUS1710 in the prevention of EXE-induced changes in endurance-related parameters, such as respiratory, cardiovascular, metabolic, liver and kidney functions.

Effects of INDUS1710 on organ weights and muscle glycogen content

Rats in the EXE control group showed a significant decrease ($P < 0.001$) in gastrocnemius muscle glycogen and a significant increase ($P < 0.001$) in the relative weights of heart, liver, kidneys, gastrocnemius muscle and levator ani muscle (Table 9). Daily subacute treatment with l-arginine significantly prevented the EXE-induced increase in relative organ weights of heart, liver, kidneys and soleus muscle, and gastrocnemius muscles and decrease in muscle glycogen, without effects on the relative wight of spleen and levator ani. INDUS1710 supplementation significantly ($P < 0.001$ or $P < 0.001$) and dose-dependently prevented the EXE-induced increase in relative weights of heart, liver, and gastrocnemius muscle and decrease in muscle glycogen without significant changes in relative weights of kidneys, spleen, soleus muscle and levator ani muscles.

Effect of INDUS1710 on the gross morphology and histology of heart

The gross morphology of representative heart samples from rats in the EXE control groups showed a marked increase in ventricle thickness, indicating hypertrophy, compared to the hearts of rats without exercise (VC). Representative heart

Table 1: Details of measurements, equipment, methods, and list of parameters

Sr. No.	Measurement	Equipment	Method and parameters
1.	**Lung function measurements**	Whole-body flow-through plethysmograph: EMKA Technologies, France	**Method**: On D28, after one h of endurance capacity evaluation, the respiratory parameters of each rat in the unrestrained state for 10 min were recorded:
			Pulse oxygen (PO), Peak inspiratory flow (PIF), Peak expiratory flow (PEF), Tidal volume (TV), Expired volume (EV), Frequency (breaths per minute), Enhanced pause (Penh) for 10 minutes
2.	**Cardiovascular function measurements**	Eight-channel recorder PowerLab instrument	**Method** On D28, rats anesthetised with urethane (1.25 g/kg, i.p.), then, Millar mikro-tip transducer catheter was inserted into the left ventricle via the right carotid artery and connected to a bio-amplifier to measure following parameters:
		Data acquisition system- LabChart 7.3 Windows, AD Instrument Pvt. Ltd., Bella Vista, Australia	**Electrocardiography** (PR interval, RR interval, QRS complex, Corrected QT interval (QTc)
		Millar mikro-tip transducer catheter: Model SRP-320, Millar Instruments, Houston, TX)	**Arterial** (Heart rate, mean arterial blood pressure)(MABP), Left ventricular end systolic (LVESP) and diastolic pressure (LVEDP),
			Ventricular (Steepest slope during the upstroke [dP/dt(max)], Steepest slope during the downstroke [dP/dt(min)], Contractility index, Stroke volume, End diastolic volume, Pressure time index, Tau)
3.	**Biochemical measurements**	Microcentrifuge: (Model no. 5810, Eppendorf, Hamburg, Germany	**Method:** On D1 and D28, blood collected by retro-orbital puncture under anesthesia. Serum separated by centrifugation (4°C, 7000 rpm, 15 min) to measure following parameters:
			Carbohydrate metabolism (Random serum glucose)
		UV-visible spectrophotometer: Jasco V-530, Tokyo, Japan	**Protein metabolism** (Total protein (TP), Lactate dehydrogenase (LDH), Creatine kinase (CK),
			Lipid metabolism (Total cholesterol (TC)
		Biochemical reagent kits: Coral Clinical Systems, Goa, India	Triglycerides (TG), High-density, lipoprotein (HDL), Low-density lipoprotein (LDL)
			Liver function (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT)
			Kidney function (Urea, Creatinine, Uric acid, Blood urea nitrogen (BUN)
4.	**Organ weights**	Electronic weighing Balance: Scaletec Mechatronics Private Limited, Vadodara, India	**Method**: Rats was euthanised at the end of 28-day treatment and after functional measurements.
			Skeletal muscles (soleus, gastrocnemius, and levator ani) of the left and right sides of the rats separated and washed immediately with saline to remove blood and fluids.
			Organ weights (six rats per group) was measured
5.	**Gastrocnemius muscle glycogen**	Tissue homogeniser: Remi Motors, Mumbai, India	**Method**: Six samples of gastrocnemius tissues homogenate with 0.1 M Tris-HCl buffer (pH 7.4) to concentration of 10% w/v.
			Glycogen content measured as mg of glycogen/100 g of tissue
6.	**Histology**	Light microscope: Magnus, MLX Plus, New Delhi, India	**Method:** Two samples from each organ preserved in formaldehyde solution (10%) were sectioned, stained with hematoxylin and eosin (H&E).
			Sections of heart stained with H&E (Left Ventricular wall thickness) and Sirius Red (Collagen deposition and infiltration) on scale: : 0 (absent), + (slight), ++ (mild), +++ (moderate) and ++++ (Severe)

samples from rats with l-arginine and INDUS1710 (30 and 60 mg/kg) but not INDUS1710 (20 mg/kg) on subacute oral administration showed a noticeable reduction. These results were confirmed by the cell architecture showing the thickness of heart ventricles Figure 2. The histology of heart sections (Sirius red staining) from representative rats in the EXE showed marked deposition of collagen (Figure 3). In contrast, such deposition was not visible in the heart sections of the rats from VC. Oral treatment with INDUS1710 (20 mg/kg) did not protect rats from marked deposition of collagen in heart, as shown in the representative sections. However, heart sections of INDUS1710 (30 and 60 mg/kg) or l-arginine supplemented rats showed noticeably less amount of collagen deposition.

Effect of INDUS1710 on histology of skeletal muscles

The histology of skeletal muscle samples, namely gastrocnemius, levator ani, and soleus muscles, obtained from a representative group of rats subjected to endurance exercise without treatment (EXE control) or treated with

Table 2: Effect of INDUS1710 on Time to exhaustion (min)

Day	VC	EXE Control	Treatment (mg/kg, po) + EXE			
			L-arginine (308)	INDUS1710 (20)	INDUS1710 (30)	INDUS1710 (60)
D1	41.33 ± 0.48	42.83 ± 0.30	41.50 ± 0.42	41.16 ± 0.30	43.16 ± 0.30	42.33 ± 0.48
D7	42.16 ± 0.60	44.66 ± 0.33###	45.50 ± 0.42	44.83 ± 0.60	45.00 ± 0.36	44.66 ± 0.42
D14	46.00 ± 0.36	46.00 ± 0.44#	48.16 ± 0.83***	48.00 ± 0.25	47.50 ± 0.42	47.33 ± 0.49
D21	46.50 ± 0.22	47.66 ± 0.21###	50.33 ± 0.49***	50.33 ± 0.49***	51.66 ± 0.21***	52.83 ± 0.16***
D28	46.66 ± 0.21	48.33 ± 0.16###	51.50 ± 0.22***	51.16 ± 0.30***	52.66 ± 0.21***	54.16 ± 0.16***

Values are expressed as mean ± standard error of mean. The numbers in parentheses indicate the doses in mg/kg. n = 12; data were analyzed using Two-way ANOVA followed by Bonferroni's post-test. # $P < 0.05$, ### $P < 0.001$ (v/s VC), *** $P < 0.001$ (v/s EXE control). VC- Vehicle control. D – Day of study, EXE – Treadmill running exercise.

Table 3: Effect of INDUS1710 on changes from baseline in body weight, food intake, and water intake on D28

Parameter	VC	EXE Control	Treatment (mg/kg, po) + EXE			
			L-arginine (308)	INDUS1710 (20)	INDUS1710 (30)	INDUS1710 (60)
Body weight (g)	57.83 ± 4.73	27.92 ± 2.49###	27.00 ± 3.13	33.92 ±2.67	38.50 ±3.02	36.33 ±2.25
Food Intake (g)	0.35 ± 0.14	4.90 ± 0.18###	2.60 ± 0.06***	3.10 ± 0.12***	2.00 ± 0.09***	1.10 ± 0.07***
Water intake (ml)	0.00 ± 0.15	16.0 ± 0.60###	6.00 ± 0.45***	13.0 ± 0.45***	12.00 ± 0.45***	8.00 ± 0.45***

Values are expressed as mean ± standard error of mean. The numbers in parentheses indicate the doses in mg/kg. n = 12; Data were analyzed using One-way ANOVA followed by Dunnett's test for each parameter. ### P <0.001 (v/s VC), *** $P < 0.001$ (v/s EXE control). VC- Vehicle control, EXE – Treadmill running exercise

Table 4: Effect on respiratory function parameters on D28

Parameter	VC	EXE Control	Treatment (mg/kg, po) + EXE			
			L-arginine (308)	INDUS1710 (20)	INDUS1710 (30)	INDUS1710 (60)
Pulse Oxygen (%)	95.92 ± 0.39	90.50 ± 0.28###	94.33 ± 0.14***	90.92 ± 0.22	91.75 ± 0.28**	92.75 ± 0.18***
Peak Inspiratory Flow (m/s)	14.00 ± 0.34	18.00 ± 0.26###	15.08 ± 0.22***	17.75 ± 0.45	16.42 ± 0.14***	15.75 ± 0.13***
Peak Expiratory Flow (m/s)	23.25 ± 0.22	19.25 ± 0.18###	20.83 ± 0.40***	19.00 ± 0.21	20.33 ± 0.14***	20.83 ± 0.24***
Tidal volume (ml)	1.30 ± 0.019	1.06 ± 0.018###	1.20 ± 0.008***	1.04 ± 0.014	1.12 ± 0.016	1.15 ± 0.015**
Expired Volume (ml)	1.068 ± 0.005	0.848 ± 0.013###	1.032 ± 0.006***	0.863 ± 0.014	0.922 ± 0.008***	0.965 ± 0.005***
Frequency lof breathings (breaths per min)	236.10 ± 1.63	311.80 ± 1.59###	279.80 ± 1.46***	308.30 ± 1.78	293.60 ± 0.96***	284.10 ± 1.20***
Penh (sec)	0.82 ± 0.006	1.64 ± 0.042###	0.92 ± 0.007***	1.52 ± 0.032**	1.24 ± 0.022***	1.10 ± 0.021***

Values are expressed as mean ± S.E.M, Numbers in parentheses indicate dose in mg/kg. n = 12; data were analyzed using One-way ANOVA followed by Dunnett's test for each parameter. ### $P < 0.001$ (v/s VC), *** P <0.001 (v/s EXE control), VC- Vehicle control, EXE – Treadmill running exercise

INDUS1710 or L-arginine, showed normal tissue architecture and increased cell size (hypertrophy) compared to sections of respective organs without exercise (VC), which indicates their repeated use during exercise.

Discussion

This study evaluated the effects of INDUS1710 (20, 30, and 60 mg/kg) supplementation with EXE on different types of endurance in rats. A treadmill fatigue test allowing rats to run until exhaustion. This simple, high-throughput, involuntary, and offers many advantages over wheel-running exercises for measuring fatigue-like behavior for endurance performance.[46] Involuntary physical activity to avoid electrical shock is an indicator of endurance exercise capacity in animals.[47] Subacute oral treatment with INDUS1710 extended the time to exhaustion, indicating improved endurance, as previously reported.[48] This was accompanied by reduced food and water intake, suggesting better energy utilisation by INDUS1710.

Prolonged or intense physical activity leads to decreased muscle performance, resulting in fatigue.[49] Factors like energy metabolism muscle contractile properties, cardiovascular and pulmonary performance, and mental function contribute to fatigue during endurance exercise.[50] Muscle fatigue is largely influenced by Adenosine triphosphate (ATP) availability which is a

Table 5: Effects on cardiovascular parameters on D28

Parameter	VC	EXE Control	Treatment (mg/kg, po) + EXE			
			L-arginine (308)	INDUS1710 (20)	INDUS1710 (30)	INDUS1710 (60)
Electrocardiographic parameters						
PR Interval (ms)	30.65 ± 0.18	29.41 ± 0.18$^{###}$	30.84 ± 0.14***	29.51 ± 0.22	29.75 ± 0.13	31.05 ± 0.19***
RR Interval (ms)	130.70 ± 0.14	125.40 ± 0.34$^{###}$	130.90 ± 0.20***	126.00 ± 0.40	127.50 ± 0.60**	130.00 ± 0.29***
QRS Complex (ms)	18.36 ± 0.05	16.58 ± 0.16$^{###}$	18.36 ± 0.08***	17.40 ± 0.04***	17.69 ± 0.04***	18.25 ± 0.04***
QTc Interval (ms)	107.90 ± 0.24	114.30 ± 0.50$^{###}$	108.50 ± 0.29***	111.00 ± 2.10	109.80 ± 0.47**	108.40 ± 0.21***
Arterial parameters						
Heart rate (BPM)	341.80 ± 0.60	345.70 ± 0.66$^{#}$	340.70 ± 0.61***	344.20 ± 1.04	345.2 ± 1.30	339.7 ± 0.42***
Systolic BP (mmHg)	108.50 ± 1.68	120.80 ± 1.99$^{###}$	113.5 ± 1.68*	121.30 ± 1.66	113.70 ± 2.58	113.70 ± 1.72
Diastolic BP mmHg)	75.83 ± 0.94	89.67 ± 1.22$^{###}$	82.50 ± 1.02***	84.67 ± 1.05**	83.67 ± 0.92**	84.00 ± 1.06**
MABP (mmHg)	86.67 ± 0.92	100.00 ± 1.09$^{###}$	92.83 ± 0.87***	96.83 ± 0.83	93.50 ± 1.12***	94.00 ± 0.82***
LVESP (mmHg)	126.40 ± 0.82	138.00 ± 0.74 $^{###}$	136.70 ± 1.21	137.10 ± 0.36	136.30 ± 0.66	135.20 ± 0.54
LVEDP (mmHg)	6.59 ± 0.08	17.17 ± 0.33$^{###}$	17.36 ± 0.06	17.54 ± 0.09	17.55 ± 0.07	17.43 ± 0.08
Ventricular parameters						
dP/dt(max) (mmHg/s)	3267 ± 8.01	2436 ± 13.33$^{###}$	2993 ± 15.31***	2540 ± 14.31***	2745 ± 10.43***	2917 ± 18.98***
dp/dt(min) (mmHg/s)	-1062 ± 11.51	-1425 ± 9.88$^{###}$	- 1054 ± 19.05***	- 1349 ± 13.74**	- 1260 ± 15.89***	- 1172 ± 13.11***
Contractility Index (1/s)	56.28 ± 0.42	32.58 ± 0.61$^{###}$	50.87 ± 0.73***	32.98 ± 0.60	35.91 ± 0.18**	45.72 ± 0.95***
Stroke Volume (μl)	125.30 ± 1.30	86.83 ± 0.60$^{###}$	107.20 ± 0.600***	89.00 ± 0.58	91.67 ± 0.66***	97.33 ± 0.56***
End Diastolic Volume (μl)	199.50 ± 1.56	258.50 ± 1.60$^{###}$	213.20 ± 1.64***	246.20 ± 2.68**	226.20 ± 3.30***	219.30 ± 2.23***
Pressure Time Index (mmHg.sec)	15.33 ± 0.56	22.17 ± 0.48$^{###}$	17.33 ± 0.49***	19.67 ± 0.42**	17.83 ± 0.48***	16.67 ± 0.49***
Tau (ms)	5.62 ± 0.14	8.26 ± 0.06$^{###}$	6.12 ± 0.09***	7.76 ± 0.08**	7.22 ± 0.04***	6.56 ± 0.08***
Ejection Fraction (%)	62.86 ± 1.06	33.60 ± 0.30$^{###}$	50.29 ± 0.42***	36.18 ± 0.56*	40.58 ± 0.72***	44.40 ± 0.52***

Values are expressed as mean ± S.E.M, Numbers in parentheses indicate dose in mg/kg. n = 12; data were analyzed using One-way ANOVA followed by Dunnett's test for each parameter. $^{#}P < 0.05$, $^{###}P < 0.001$ (v/s VC), *** $P < 0.001$ (v/s EXE control), VC- Vehicle control, EXE – Treadmill running exercise.

required energy source for muscle contraction.[50] Regular exercise uses skeletal muscle's ability to oxidise substrates and generate ATP.[49] The key factors in reduced ATP production and muscle endurance decline are depleted glycogen stores, [51] pH alterations, [52] and build-up of metabolic by-products, such as the lactate and hydrogen ions in muscle fibers.[53] Training-induced mitochondrial biogenesis intensification enhances muscle's metabolic status throughout exercise and increases resistance to fatigue.[54]

Subacute oral treatment with INDUS1710 caused an increase in body weight (although not significant) and gastrocnemius muscle, despite reduced food intake. Exercise increases calorie use and short-term weight loss,[55] but weight can be regained owing to metabolic changes[56] energy re-balance,[57] or muscle mass gain,[58] and could have contributed to INDUS1710's endurance-boosting effects.

Regular exercise and good physical fitness have been found to improve pulmonary function.[59] The capacity of the respiratory system supports maximal oxygen transport in normally exercising healthy humans,[60] but limits heavy and sustained endurance exercises.[60] Spirometric respiratory function tests help to better understand lung size and physiology.[61] Endurance exercise and aerobic training have been reported to stimulate lung function in healthy adults[59] and elderly women.[62] The time to exhaustion during exercise largely depends on the tissue oxygen saturation.[63] Intense exercise decreases arterial blood oxygen[64] enhances respiratory airflow and expiratory volume,[65] increases frequency with lower tidal volume[66] and increases the size of respiratory muscles.[67,68] In addition, increased muscle strength, with improved thoracic mobility and balance between lung and chest elasticity gained from regular exercise, are important factors for improved pulmonary function in healthy adults.[59] The results of this study on enhanced pulmonary function, including increased pulse oxygen, pulmonary airflow (PIF), lung capacity (tidal volume), airway reactivity to oxygen (measured by Penh), and strength of respiratory muscles (due to hypertrophy of skeletal muscle), contributed significantly to endurance capacity enhancement shown by subacute INDUS1710 supplementation to EXE.

The cardiorespiratory system's ability to provide oxygen to muscles is the limiting factor for maximal oxygen uptake.[69,70] Changes in lung performance increase maximal cardiac output, and high oxygen consumption capacity, especially during endurance exercise.[70]

Table 6: Effects on percent (%) changes in respiratory and cardiovascular function parameters

Parameter	EXE control	% change with Treatment (mg/kg, po) + EXE	
		L-arginine (308)	INDUS 1710 (60) maximum % change
Respiratory			
Pulse Oxygen (%)	5.67 ↓	4.23 ↓	2.48 ↓
Peak Inspiratory Flow (m/s)	28.57 ↑	16.22 ↓	12.50 ↓
Peak Expiratory Flow (m/s)	17.20 ↓	8.20 ↑	8.20 ↑
Tidal volume (ml)	18.46 ↓	13.20 ↑	8.49 ↑
Expired Volume (ml)	20.5 ↓	21.17 ↑	12.94 ↑
Frequency of breathings (breaths per min)	32.06 ↑	10.26 ↓	8.89 ↓
Penh (sec)	100 ↑	43.90 ↓	32.92 ↓
Cardiovascular – Electrocardiographic parameters			
PR Interval (ms)	4.04 ↓	4.86 ↑	5.57 ↑
RR Interval (ms)	4.05 ↓	4.38 ↑	3.66 ↑
QRS Complex (ms)	9.69 ↓	10.73 ↑	10.07 ↑
QTc Interval (ms)	1346.83 ↑	5.07 ↓	5.16 ↑
Cardiovascular – Arterial parameters			
Heart rate (BPM)	1.14 ↑	1.44 ↓	1.73 ↓
Systolic BP (mmHg)	11.33 ↓	6.04 ↓	5.87 ↓
Diastolic BP (mmHg)	18.25 ↓	7.99 ↓	6.32 ↓
MABP (mmHg)	15.38 ↓	7.17 ↓	6.00 ↓
LVESP (mmHg)	9.17 ↓	0.94 ↓	2.02 ↓
LVEDP (mmHg)	160.54 ↓	1.10 ↑	1.51 ↑
Cardiovascular – Ventricular parameters			
dP/dt(max) (mmHg/s)	25.43 ↓	22.86 ↑	19.74 ↑
dp/dt(min) (mmHg/s)	34.18 ↓	26.03 ↓	17.75 ↓
Contractility Index (1/s)	42.11 ↓	56.13 ↑	40.33 ↑
Stroke Volume (μl)	30.70 ↓	23.45 ↑	12.09 ↑
End Diastolic Volume (μl)	29.57 ↑	17.52 ↓	15.16 ↓
Pressure Time Index (mmHg.sec)	44.61 ↑	21.83 ↓	24.80 ↓
Tau (ms)	46.97 ↑	25.90 ↓	20.58 ↓
Ejection Fraction (%)	46.54 ↑	49.67 ↑	32.14 ↑

Comparisons (EXE Control v/s Vehicle control; Arginine (308) and INDUS1710 (60) v/s EXE control. ↓ indicate decrease and ↑ indicate increase. EXE – Treadmill running exercise. Values are expressed as mean ± S.E.M, Numbers in parentheses indicate dose in mg/kg. n = 12; data were analyzed by one-way ANOVA followed by Bonferroni post-test for each parameter on each day. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ (v/s VC), *** $P < 0.001$ (v/s EXE control). VC- Vehicle control, EXE – Treadmill running exercise.

Endurance exercise training has been linked to heart rate variability (HRV) owing to autonomic dysfunction.[71] Abnormalities in ECG parameters indicate cardiac autonomic dysfunction.[72] Endurance training has been shown to result in hemodynamic adaptations, such as baroreflex-mediated tachycardia, which is a result of an imbalance in increased vagal and decreased sympathetic outflow, as observed in mouse studies.[73] Rats that undergo endurance exercise are known to increase in heart rate variability, heart rate, QTc, and other ECG parameters.[74] Subacute INDUS1710 treatment prevents EXE-induced ECG and diastolic blood pressure changes, suggesting improved cardiovascular endurance.[73] Reduced HRV in INDUS1710 treated rats suggested stabilisation of the autonomic nervous system and improved baroreflex control of blood pressure, leading to endurance benefits. The absence of any effects on systolic blood pressure, LVESP, and LVEDP during EXE showed the cardiovascular safety of INDUS1710 in rats.

Acute exhaustive exercise increases end-systolic volume, reduces ejection fraction, and weakens preload-adjustable heart contractility and mechanical efficiency.[75] The prevention of EXE-induced reduction of dp/dt contractility, stroke volume, ejection fraction, and increased end-diastolic volume, pressure time index, tau, thinner walls left ventricles, and reduced collagen deposition in cardiac tissues by INDUS1710 supplementation, indicates better cardiac function and contractility, perhaps through the amelioration of heart hypertrophy or inflammation.

Endurance exercise places significant strain on the cardiovascular system, resulting in significant adaptations

Table 7: Effect on metabolic and biochemical parameters

Parameter	Day	VC	EXE Control	Treatment (mg/kg, po) + EXE			
				L-arginine (308)	INDUS1710 (20)	INDUS1710 (30)	INDUS1710 (60)
Carbohydrate Metabolism							
Glucose (mg/dL)	D1	108.60 ± 4.42	112.70 ± 2.76	98.61 ± 5.12	101.30 ± 3.84	103.40 ± 3.84	104.60 ± 3.00
	D28	108.20 ± 4.29	99.47 ± 2.17	93.69 ± 4.11	91.80 ± 3.11	93.38 ± 3.10	90.81 ± 1.54
Protein Metabolism							
TP (g/dL)	D1	6.50 ± 0.19	6.24 ± 0.13	6.89 ± 0.20	6.40 ± 0.16	6.43 ± 0.24	6.62 ± 0.16
	D28	6.64 ± 0.20	9.42 ± 0.21###	7.17 ± 0.21***	7.36 ± 0.18***	7.08 ± 0.26 ***	7.09 ± 0.17***
LDH (IU/L)	D1	332.6 ± 18.27	287.30 ± 16.45	310.60 ± 15.25	250.50 ± 4.66	285.10 ± 6.42	330.50 ± 11.03
	D28	368.0 ± 18.73	1334.00 ± 27.67###	651.00 ± 10.70***	841.60 ± 16.08***	673.50 ± 13.38***	641.90 ± 10.07***
CK (IU/L)	D1	79.20 ±1.06	133.60 ± 58.56	77.51 ± 1.53	79.20 ± 0.76	73.57 ± 3.09	74.83 ± 2.10
	D28	91.01 ± 2.68	386.80 ± 13.65###	167.80 ± 7.51***	255.10 ± 1.16***	215.50 ± 9.20***	208.30 ± 8.70***
Lipid metabolism							
TC (mg/dL)	D1	67.41 ±1.04	66.03 ± 1.09	66.64 ± 0.90	66.52 ± 0.78	66.46 ± 0.80	67.63 ± 0.76
	D28	102.9 ± 4.08	73.38 ± 2.65###	66.97 ± 2.27	84.00 ± 2.85	101.6 ± 4.46***	97.70 ± 3.32***
TG (mg/dL)	D1	59.76 ± 1.15	58.26 ± 1.02	58.94 ± 1.16	58.34 ± 1.60	59.10 ± 1.06	57.04 ± 1.09
	D28	80.03 ± 1.43	59.43 ± 1.04###	62.84 ± 1.35	67.01 ± 1.63***	65.56 ± 1.26**	62.40 ± 1.32
HDL (mg/dL)	D1	43.18 ± 1.18	48.60 ± 2.04	48.61 ± 1.40	50.09 ± 1.25	53.54 ± 2.78	53.73 ± 1.52
	D28	43.72 ± 1.22	39.73 ± 1.69	46.91 ± 1.34	42.00 ± 1.07	47.19 ± 2.37	47.10 ± 1.28
LDL (mg/dL)	D1	39.94 ± 1.14	40.80 ± 0.66	40.26 ± 1.06	39.96 ± 1.18	38.99 ± 1.26	38.91 ± 1.12
	D28	66.11 ± 3.64	42.40 ± 0.70###	52.74 ± 3.54*	51.27 ± 2.21*	49.34 ± 1.70	45.56 ± 1.20
Liver function							
AST (IU/mL)	D1	13.00 ± 0.87	16.33 ± 0.92	16.50 ± 1.58	16.17 ± 1.50	13.83 ± 1.36	16.83 ± 1.22
	D28	13.75 ± 0.93	36.33 ± 2.69#	27.00 ± 2.08	32.92 ± 3.40*	27.75 ± 2.02	25.83 ± 2.57*
ALT (IU/mL)	D1	19.42 ± 2.36	47.92 ± 4.26#	35.00 ± 5.24	44.67 ± 4.58	34.83 ± 5.14	36.50 ± 3.74
	D28	18.25 ± 2.29	16.83 ± 2.34	20.08 ± 3.48	24.25 ± 2.86	24.92 ± 2.28	23.92 ± 2.56
Kidney function							
Urea (mg/dL)	D1	24.92 ± 1.28	25.42 ± 1.30	28.33 ± 1.12	27.50 ± 2.10	26.92 ± 1.32	26.67 ± 1.04
	D28	17.80 ± 0.31	20.41 ± 0.36###	18.20 ± 0.06***	19.89 ± 0.08	19.29 ± 0.20**	18.98 ± 0.21***
Creatinine (mg/dL)	D1	0.54 ± 0.02	0.55 ± 0.02	1.32 ± 0.55	0.61 ± 0.08	0.56 ± 0.03	0.58 ± 0.03
	D28	0.54 ± 0.02	0.72 ± 0.02###	0.54 ± 0.03***	0.74 ± 0.02	0.64 ± 0.03	0.58 ± 0.02**
Uric acid (mg/dL)	D1	3.54 ± 0.06	3.57 ± 0.18	3.56 ± 0.08	3.71 ± 0.08	3.70 ± 0.06	3.56 ± 0.09
	D28	3.58 ± 0.07	4.00 ± 0.07##	3.60 ± 0.08**	3.90 ± 0.08	3.80 ± 0.08	3.59 ± 0.09**
BUN (mg/dL)	D1	8.28 ± 0.14	8.36 ± 0.13	8.30 ± 0.02	8.08 ± 0.16	8.52 ± 0.08	8.53 ± 0.08
	D28	8.32 ± 0.14	9.54 ± 0.17###	8.50 ± 0.03***	9.29 ± 0.04	9.02 ± 0.09**	8.86 ± 0.09***

Values are expressed as mean ± S.E.M, Numbers in parentheses indicate dose in mg/kg. n = 12; data were analyzed by one-way ANOVA followed by Bonferroni post-test for each parameter on each day. # P <0.05, ### P < 0.001 (V/s VC), *** P <0.001 (v/s EXE control). VC- Vehicle control, EXE – Treadmill running exercise.

in endurance athletes.[76] In some cases, these adaptations can cause long-term harm, such as myocardial fibrosis, in elite or veteran athletes.[76] Myocardial fibrosis occurs when cardiac fibroblasts remodel the myocardium by depositing excessive extracellular matrix, which reduces heart tissue compliance and contractility.[77] INDUS1710 treatment prevented EXE-induced reduction in dP/dt(max) and dP/dt(min), a sensitive index of myocardial contractility and ventricular stiffness.[78] to improve myocardial oxygen balance,[79] and resulted in increased exercise time to exhaustion.

Ventricular stiffness and collagen deposition-induced fibrosis in heart muscles were detected using Sirius Red staining.[80] Sirius red stained of heart sections from INDUS1710-supplemented rats showed protection from EXE-induced fibrotic adaptations (ventricular stiffness and collagen deposition) in cardiac muscles. These effects can be assigned to the antifibrotic efficacy of glycoside content of INDUS1710 as reported earlier animal studies.[31,81]

High-intensity training and other endurance exercises enhance cardiorespiratory fitness, longevity, and slow ageing.[82] Muscular endurance is the capacity of skeletal muscles to sustain contractions, resist fatigue, and recover after exercise.[83] The strength and endurance performance of athletes is affected by the type of skeletal muscle.[84] For

Table 8: Effects on percent changes in metabolism and biochemical parameters

Parameter	Day	EXE control	Treatment (mg/kg, po) + EXE	
			L-arginine (308)	INDUS 1710 (60 maximum % change
Carbohydrate Metabolism				
Glucose (mg/dL)	0	3.63 ↑	12.50 ↓	7.19 ↓
	28	8.77 ↓	5.81 ↓	8.71 ↓
Protein Metabolism				
TP (g/dL)	0	4.16 ↓	10.42 ↑	6.09 ↑
	28	29.51 ↑	23.89 ↓	24.73 ↓
LDH (IU/L)	0	15.76 ↓	8.11 ↑	15.04 ↑
	28	176.69 ↑	389.47 ↓	382.63 ↓
CK (IU/L)	0	40.71 ↑	41.98 ↓	43.99 ↓
	28	76.47 ↑	56.62 ↓	46.15 ↓
Lipid metabolism				
TC (mg/dL)	0	2.09 ↓	0.92 ↑	2.42 ↑
	28	40.23 ↓	8.74 ↓	33.14 ↑
TG (mg/dL)	0	2.57 ↓	1.17 ↑	2.09 ↓
	28	34.66 ↓	5.74 ↑	5.00 ↑
HDL (mg/dL)	0	11.15 ↑	0.02 ↑	10.56 ↓
	28	10.04 ↓	18.07 ↑	18.55 ↑
LDL (mg/dL)	0	0.35 ↑	0.45 ↓	2.9 ↓
	28	56.66 ↓	24.98 ↑	7.96 ↑
Liver function				
AST (IU/mL)	0	20.39 ↑	1.04 ↑	3.06 ↑
	28	62.15 ↑	25.68 ↓	83.95 ↓
ALT (IU/mL)	0	59.47 ↑	26.96 ↓	23.83 ↓
	28	8.44 ↓	19.31 ↑	42.13 ↑
Kidney function				
Urea (mg/dL)	0	1.97 ↓	11.45 ↑	4.92 ↑
	28	12.79 ↑	10.83 ↓	7.01 ↓
Creatinine (mg/dL)	0	1.82 ↑	136.36 ↑	5.45 ↓
	28	25.00 ↑	25.00 ↓	19.44 ↓
Uric acid (mg/dL)	0	0.84 ↑	0.28 ↓	0.28 ↓
	28	10.50 ↑	10.00 ↓	10.25 ↓
BUN (mg/dL)	0	0.96 ↑	0.72 ↓	2.03 ↑
	28	12.79 ↑	10.90 ↓	10.27 ↓

Comparisons (EXE Control v/s Vehicle control; Arginine (308) and INDUS1710 (60) v/s EXE control. ↓ indicate decrease and ↑ indicate increase, EXE – Treadmill running exercise.

example, sprinters have faster fiber-type dominance, whereas endurance runners have slower fiber-type dominance.[85] Therefore, endurance enhancement efficacy of INDUS1710 can be mediated through skeletal muscle hypertrophy and subsequent increase in aerobic capacity.

A strong correlation between endurance and carbohydrate metabolism. Endurance athletes require more carbohydrates than others to sustain energy during training and competition.[86] Glucose with stored skeletal muscle glycogen are used for ATP the breakdown for energy source for sudden needs during exercise.[49] Carbohydrate-derived glycogen in skeletal muscles is the main substrate during high-intensity exercise, but it is depleted quickly.[51] Furthermore, a strong relationship between muscle glycogen and fatigue and decreased ATP synthesis rates.[51] and endurance capacity during cycling.[87] During endurance exercise, glycogen levels decrease and lactate increases.[88] EXE with or without INDUS1710 had no effect on random plasma glucose levels during the study. INDUS1710 supplementation reduced gastrocnemius muscle glycogen content, suggesting a glycogen-sparing action. These results are in agreement with the previously reported glycogen-sparing action of fenugreek extract,[89] acceleration of the rate of glycogen resynthesis by 4-hydroxyisoleucine rich,[21] and improved repetitions to failure (muscle endurance) by glycoside[32] rich fenugreek extract. The storage of muscle glycogen leads to myofibril growth and hypertrophy.[90] The increased cell size of gastrocnemius muscles with INDUS1710 supplementation may have led to the enhanced glycogen storage and endurance capacity observed in our study.

The treatment INDUS1710 with EXE decreased blood protein levels, suggesting enhanced utilisation and greater retention of dietary proteins for better endurance, as

Table 9: Effect on relative organ weights and skeletal muscle glycogen content

Relative weight or glycogen content (Units)	VC	EXE Control	Treatment (m/kg, po) + EXE			
			L-arginine (308)	INDUS1710 (20)	INDUS1710 (30)	INDUS1710 (60)
Heart (mg/g)	483.10 ± 13.16	608.20 ± 13.65###	561.20 ± 8.09***	591.00 ± 8.68	569.40 ± 12.18**	568.30 ± 11.28***
Liver (mg/g)	39.78 ± 1.15	49.17 ± 0.57###	45.77 ± 0.63*	47.33 ± 0.11	44.89 ± 0.86**	43.75 ± 0.79***
Spleen (mg/g)	02.75 ± 0.06	03.00 ± 0.09	02.93 ± 0.08	02.93 ± 0.08	02.90 ± 0.07	02.88 ± 0.09
Left Kidney (mg/g)	10.40 ± 0.38	12.82 ± 0.27###	11.79 ± 0.22*	12.43 ± 0.07	11.97 ± 0.30	11.72 ± 0.22*
Right Kidney (mg/g)	10.50 ± 0.3	12.95 ± 0.27###	11.91 ± 0.23*	12.55 ± 0.07	12.09 ± 0.30	11.84 ± 0.22*
Soleus muscle (mg/g)	127.30 ± 1.15	140.80 ± 2.09###	131.00 ± 0.37*	139.80 ± 1.70	136.80 ± 1.64	137.50 ± 0.85
Levator ani muscle (mg/g)	01.70 ± 0.04	01.92 ± 0.04###	01.93 ± 0.03	01.87 ± 0.02	01.90 ± 0.04	01.85 ± 0.04
Gastrocnemius Muscle (mg/g)	07.03 ± 0.21	12.66 ± 0.18###	10.26 ± 0.19***	07.89 ± 0.09***	08.69 ± 0.25***	09.11 ± 0.18***
Gastrocnemius muscle glycogen (mg/100 g of tissue)	1.51 ± 0.06	0.93 ± 0.02###	1.28 ± 0.04***	1.04 ± 0.04	1.21 ± 0.004***	1.25 ± 0.02***

Values are expressed as mean ± S.E.M, Numbers in parentheses indicate dose in mg/kg. n = 6; data were analyzed using One-way ANOVA followed by Dunnett's test for each parameter. # $P < 0.05$, ### $P < 0.001$ (v/s VC), *** $P < 0.001$ (v/s EXE control). VC – Vehicle control, EXE – Treadmill running exercise.

suggested previously.[91] The results of INDUS1710 supplemented group showing dose-dependent decreases in EXE-induced LDH and CK elevation, as expected from endurance exercise.[88,92] BCAA supplementation reduces the LDH and CK accumulation from prolonged exercise[93] and may stimulate muscle protein synthesis.[94] As a result, it is highly likely that 4-HI, a branched-chain amino acid (BCAA) found in INDUS1710, was responsible for the decreased LDH and CK. Physically active and fit individuals show metabolic adaptations to endurance exercise by lowering TC, TG, and LDL and increasing HDL cholesterol levels[95,96] and fat utilisation,[97] especially in adipose and skeletal muscle,[98,99] fatty acid oxidation,[70,100] and HDL cholesterol levels.[101] The retention of HDL and LDL levels while preventing decrease of TC and TG with enhanced endurance in INDUS1710 supplementation to EXE suggests a lower requirement of fuel (fatty acid oxidation). These results are in agreement with reports that carbohydrates are mainly used instead of fats during aerobic exercise.[102]

During endurance exercise, with glycogen breakdown, LDH converts lactate to pyruvate and further to acetyl-CoA, carbon dioxide, and Nicotinamide adenine dinucleotide owing to energy demand.[103,104] In addition, glucose gets converted to pyruvate in the cytoplasm, then reduced to lactate LDH, bypassing mitochondrial oxidation.[105] Therefore, decreased blood lactate levels by INDUS1710 supplementation contribute to recovery improvement and endurance enhancement.[103] In mitochondria, pyruvate drives ATP production via oxidative phosphorylation and multiple biosynthetic pathways,[104] whereas pyruvate dehydrogenase (PDH) catalyzes the decarboxylation of pyruvate to maintain oxidative ATP synthesis.[106] Fenugreek extract rich in 4-HI and trigonelline, INDUS1710 markers, reported to protect PDH in mitochondria isolated from many goat vital organs.[107,108] Therefore, the protective endurance exercise-induced increase in LDH and decrease in PDH induced by INDUS1710 may have maintained oxidative ATP synthesis to enhance endurance.

Intense exercise is known to increase liver enzymes, AST and ALT,[109] indicating muscle inflammation[110,111] not liver damage.[112] Glycoside-rich standardised fenugreek seed extract was reported to reduce pro-inflammatory cytokines IL1 and IL6 in young athletes.[19] Hence, the reduction in EXE-induced liver enzymes by INDUS1710 supplementation is contributed by anti-inflammatory glycosides content.

In this study, INDUS1710 supplementation prevented the EXE-induced increases in urea, uric acid, BUN and creatinine levels. During intensive exercise, increased protein degradation and turnover increase the free amino acid pool,[113] leads to increased levels of creatinine, uric acid, urea, CK, LDH, AST, and ALP during acute exercise[114] and resistance training.[115,116] The efficacy of BCAA supplementation with exercise to enhance endurance has been proposed to occur through the prevention of acute kidney failure-induced muscle wasting.[117] Thus, the BCAA content (4-HI) in INDUS1710 may help maintain normal kidney parameters during EXE.

Mental endurance, a capacity to focus, attention and stamina, is critical for enhanced exercise performance[118] The mental endurance involves reducing mental fatigue, perceived exertion, and boredom.[119] In addition, changes in brain neurotransmission,[120] particularly noradrenaline and dopamine, play important roles in mental endurance.[118]

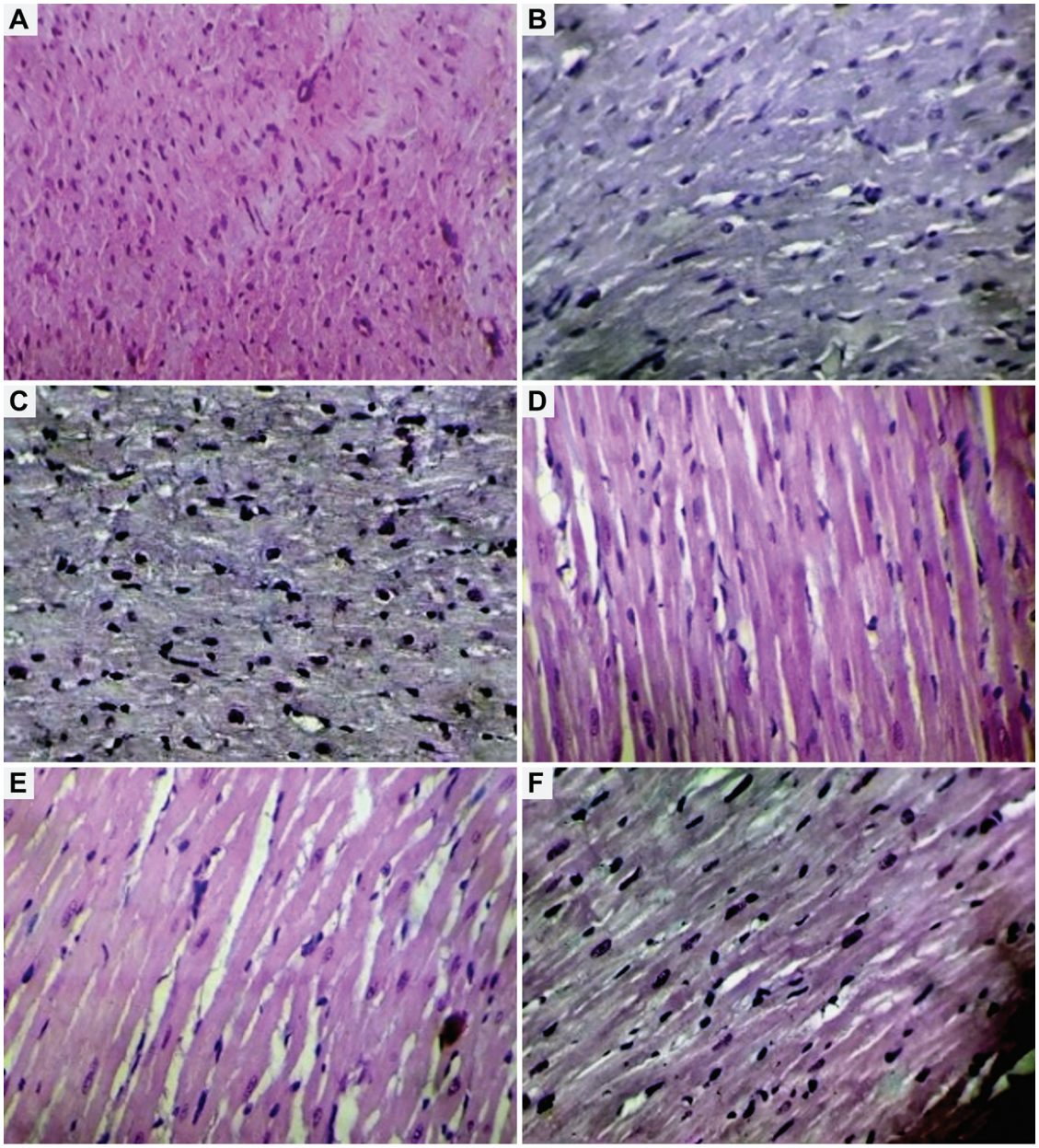

Figure 2: Photomicrograph showing representative sections of hearts showed thickness of left ventricular walls of rats stained with Haematoxylin and eosin (A) vehicle control, VC with normal thickness (0) (B) EXE control with severe thickness (++++) (C) L-arginine (308 mg/kg) with mild thickness (++) (D) INDUS1710 (20 mg/kg, oral) with moderate (+++) (E) INDUS1710 (30 mg/kg) with mild thickness (++) and (F) INDUS1710 (60 mg/kg) with mild thickness (++), magnification 10×

The dprevious reports on the markers of INDUS1710 (i.e. 4-HI and trigonelline) indicated that the compound may maintain and enhance brain noradrenaline and serotonin levels in response to physiological stress.[121,122] Furthermore, fenugreek seed extract, standardised to trigonelline (a marker of INDUS1710), possess dopaminergic potential in animal[25] and clinical studies.[123]

This study provides preclinical evidence of endurance enhancement for INDUS1710, a standardised extract of fenugreek seed, with specified concentrations of marker compounds on multiple organs, like the heart, lungs, skeletal muscles, and metabolic processes. Therefore, INDU1710 can be developed as a safe and effective food supplement to improve overall endurance after suitable clinical studies.

Conclusion

This study showed the comprehensive endurance-enhancing potential of INDUS1710 supplementation in exercising rats via multiple mechanisms.

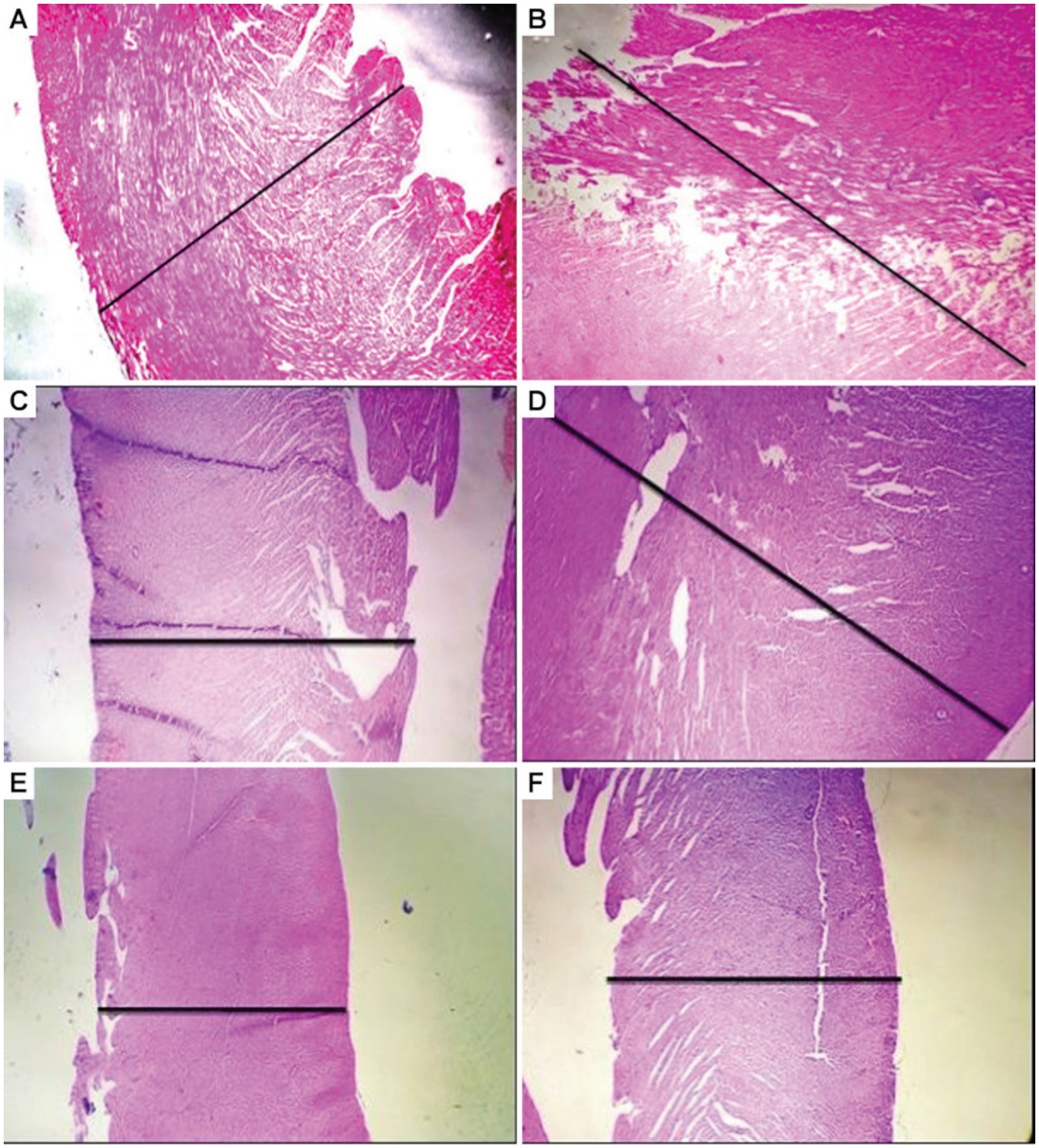

Figure 3: Photomicrograph showing representative sections of hearts of rats stained with Sirius red (A) vehicle control, VC with no collagen deposition (B), EXE control with severe collagen deposition (C), L-arginine (308 mg/kg) with mild collagen deposition, (D) INDUS1710 (20 mg/kg, oral) with moderate collagen deposition (E) INDUS1710 (30 mg/kg with mild collagen deposition, and (F) INDUS1710 (60 mg/kg) with mild collagen deposition, magnification 40×

Ethical approval

The approval of the study protocol (approval number: CPCSEA/PCL02/2016-17) was taken from the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University (Pune, India) where the study was conducted.

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Conflict of interest

The authors declare no conflicts of interest.

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