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Research Article

STUDY OF GROWTH PROMOTING EFFECTS OF AYU-707 - A POLYHERBAL POWDER IN IMMATURE RATS

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ABSTRACT

Objective: The growth promoting effects of AYU-707 was investigated in 1 and 2 months immature rats.

Methods: The AYU-707 was administered orally at a dose of 5 (1-month-old rats) and 6.5 g/kg body weight (BW) (2-month-old rats) for 45 days. During and end of the study, BW, tail length, body circumference, and feed efficiency were determined. After 24 hrs of the last treatment, the blood was withdrawn by retro-orbital puncture and blood, hemoglobin, serum alkaline phosphatase, serum glutamate-oxaloacetate transaminase, serum glutamic-pyruvic transaminase, calcium, total protein, and amylase were estimated. Then, the rats were sacrificed by overdose ketamine and the femur weight, tibial density, bone hydroxyproline content, and epiphyseal plate width were determined. Then, the spleen, lung, liver, kidney, heart, and uterus weights were determined.

Results: The preliminary phytochemical investigation confirms the presence of carbohydrate, amino acid, alkaloids, flavonoids, and tannins in AYU-707 powder. The rats treated with AYU-707 have shown significant differences in most of the growth enhancement parameters when compared to the normal rats.

Conclusion: From the results of the present study, it is concluded that AYU-707 formulated by M/s Ayurwin Pharma Pvt. Ltd., Bengaluru, possesses superior anabolic and growth promotion properties that may serve to promote the growth in children associated with growth-related disorders.

Keywords: Body circumference, Tibial density, Flavonoids, Hydroxyproline, Epiphyseal plate.

INTRODUCTION

The retardation of growth in children is caused by endocrine, metabolic, nutritional, and genetic factors. The important conditions that lead to decreased growth include growth hormone (GH) deficiency, metabolic acidosis, and malnutrition deficiencies. The human GH and insulin-like growth factor-1 (IGF-1) are important regulators of longitudinal growth. The synthesis and release of human GH from the anterior pituitary gland are promoted by GH-releasing hormone of the hypothalamus and inhibited by GH inhibiting hormone or somatostatin[4]. The current methods for increasing the growth in children include GH treatment alone or combination with gonadotropin-releasing hormone agonists. The excessive GH treatment may increase the rate of growth, rapid progression of puberty. The combination of GH and GnRH administration causes decrease in bone mineral density (BMD). The synthetic GH is expensive as it is obtained by DNA recombinant technology. Several studies reported that GH administration is beneficial for some children; it is non-therapeutically effective in all patients [1].

The minerals such as calcium, phosphorus, zinc, and magnesium; all are very essential for bone growth. These minerals are rich in vegetables, fruits, milk, and milk products [3].

The protein helps in the growth and repair of body tissue. The foods such as meat, milk, eggs, soy, and fish are rich in proteins. Vitamin D is essential in the absorption of calcium from food. It also regulates the body growth. It is found in egg yolk, fish, and liver. Early sunbath or sun salutes also helps in the synthesis of vitamin D [2].

An ideal natural growth promoting agent should promote the body growth and builds and tones the muscular mass by promoting new cell and tissue growth. It also strengthens the nervous system, maintains cholesterol level, helps insomnia, enhances memory, and should provide amino acid for the synthesis of human growth hormone in the anterior pituitary gland.

The use of herbal medicines and phytonutrients or nutraceuticals continues to expand rapidly across the world with many people now resorting to use these products for the treatment of various health challenges in different natural health-care settings. As the global use of herbal medicinal products continues to grow and many newer products are introduced into the market, public health issues and concerns surrounding their safety are also increasingly recognized. Although some herbal medicines have promising potential and are widely used, many of them remain untested and their use also not monitored [2].

METHODS

Materials

Drugs and chemicals were procured from VASA scientific suppliers Bengaluru. The biochemical kits used for the estimations were procured from Anjan distributors, authorized supplier of ERBA Diagnostics Mannheim. The sample AYU-707 was supplied by Ayurwin, Bengaluru - 560 010.

Instruments used

Oral gavage, syringe, Eppendorf tubes, capillary tubes, auto analyzer, microcentrifuge, micropipette, Sahli's hemoglobinometer, microscope.

Experimental design [6]

Preliminary phytochemical investigation

The preliminary phytochemical investigation was carried out in this phase as described by Khandelwal.

Treatment protocol

Animals

Wistar rats (24) were used in the study. The animals were kept under standard conditions of light and dark cycle with food and water. Animals acclimatized to laboratory condition before the test. 24 Wistar rats (age = 1 and 2-month-old) were divided into 4 groups (n=6) and treated for 45 days as described in Table 1. Animals were housed in cages with paddy husk as bedding, at temperature of 27±2°C, and 12:12 hrs light and dark cycle. The study protocol was presented in the IAEC meeting held on November 13, 2015. The IAEC approval No: PESCP/IAEC/23/2015, PES College of Pharmacy, and registration No: 600/PO/Ere/S/02/CPCSEA.

Group I - Control (1-month-old) received vehicle (milk), Group II - Control (2-month-old) received vehicle (milk). Group III (1-month-old) received 5 g/kg body weight (BW) p.o. AYU-707, and Group IV (2-month-old) received 6.5 g/kg BW p.o. AYU-707. All treatment groups received treatment for 45 days orally.

On 46th day, the animals were separated; and BW, body length, tail length, and body circumference of each rat were measured. Then, blood was withdrawn from individual animals of all the groups by retro-orbital plexus puncture under anesthesia. The blood was drawn into Eppendorf tubes without any anticoagulant agent. The tubes were allowed for blood clotting by incubating in an upright position at room temperature for 30-45 minutes. These tubes were then centrifuged at 2000 rpm for 10 minutes. Then, the supernatant serums were transferred to different Eppendorf tubes at room temperature using a micropipette. The obtained serum was used for the estimation of alkaline phosphatase (ALP), aspartate transaminase (AST) (serum glutamate-oxaloacetate transaminase [SGOT]), alanine transaminase (ALT) (serum glutamic-pyruvic transaminase [SGPT]), calcium, total protein, and amylase using Erba diagnostic kits and autoanalyzer.

RESULTS

Preliminary phytochemical investigation

As per the observations of the phytochemical investigation, it is confirmed that AYU-707 powder consists of carbohydrate, amino acid, alkaloids, flavonoids, and tannins.

Effect on BW, tail length, body length, body circumference, and feed efficiency in normal and AYU-707 treated rats[7]

As per the obtained data, it is observed that the normal control rats (Group I) gained 97.83 g within the 45-day study period, whereas the Group III rats (treated with AYU-707) gained 113.33 g. Hence, there is an additional weight gain of 15.5 g in rats treated with AYU-707. This gives the experimental evidence for the growth promoting the effect of AYU-707 in rats.

As per the study data, it is observed that the normal control rats (Group II) gained 50.16 g within the 45-day study period, whereas the Group IV rats (treated with AYU-707) gained 91.83 g. Hence, there is additional weight gain of 41.67 g in rats treated with AYU-707. This gives the experimental evidence for the growth promoting the effect of AYU-707 in rats (Fig. 1 and Table 2).

Table 1: Treatment schedule

Groups (n=6)	Treatment	Dose and route	Treatment schedule
I	Normal control (1-month-old) Vehicle - Milk	Oral	Day 1-45
II	Normal control (2-month-old) Vehicle - Milk	Oral	Day 1-45
III	AYU-707 in milk(1-month-old)	5 g/kg BW p.o.	Day 1-45
IV	AYU-707 in milk (2-month-old)	6.5 g/kg BW p.o.	Day 1-45

Tail length

As per the study data, the control rats Group I have shown 4.63 cm (13.63-9 = 4.63 cm) of tail length increment during the study period, whereas the rats treated with AYU-707 (Group III) have shown tail 6.03 cm (14.96-8.93 = 6.03 cm) of tail length increment after the study. Hence, AYU-707 treated rats have shown average 1.40 cm additional tail length growth as compared to normal rats. This also confirms the growth promotion effect of AYU-707 (Fig. 2).

As per the study data, the control rats Group II have shown 5.26 cm of tail length increment during the study period, whereas the rats treated with AYU-707 (Group IV) have shown 6.1 cm of tail length increment after the study. Hence, AYU-707 treated rats have shown average 0.85 cm additional tail length growth as compared to normal rats. This also confirms the growth promotion effect of AYU-707.

Body circumference

As per the study data, the control rats Group I have shown 4.37 cm (13.08-8.71=4.37 cm) of body circumference increment during the study period, whereas the rats treated with AYU-707 (Group III) have shown 5.04 cm (14.25-9.21=5.04 cm) of body circumference enhancement after the study. Hence, AYU-707 treated rats have shown average 0.37 cm additional body circumference growth as compared to normal rats. This also confirms the growth promotion effect of AYU-707 (Fig. 3).

As per the study data, the control rats Group II have shown 3.78 cm of body circumference increment during the study period, whereas the rats treated with AYU-707 (Group IV) have shown 5.27 cm of body circumference increment after the study. Hence, AYU-707 treated rats

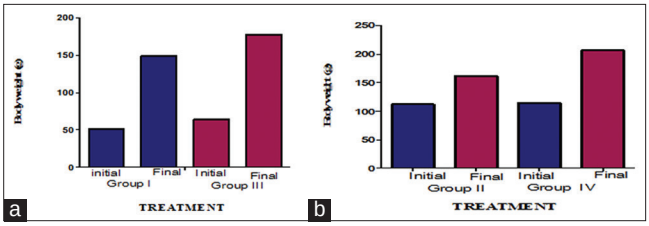

Fig. 1: (a and b) Body weight

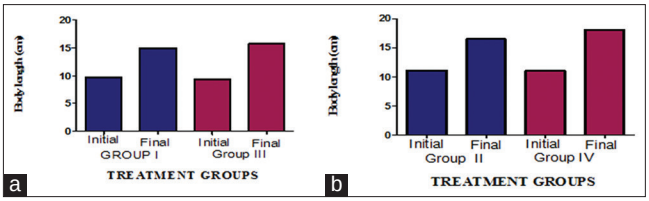

Fig. 2: (a and b) Body length

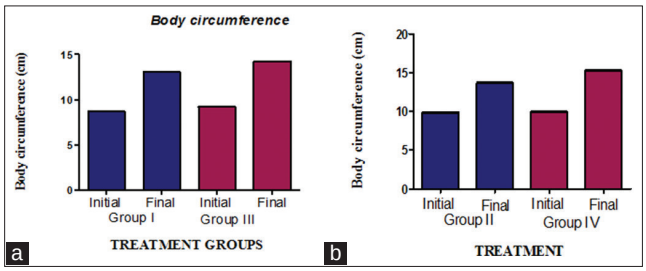

Fig. 3: (a and b) Body circumference

Table 2: Effect on BW, tail length, body length, and body circumference in normal and AYU-707 treated rats

Group n=6	BW in g		Tail length in cm		Body length in cm		Body circumference in cm		Feed efficiency in g
	I	F	I	F	I	F	I	F	
I	51.33	149.16	9.18	13.63	9.66	14.96	8.71	13.08	26.86
II	112	162.16	9.65	14.91	11.13	16.50	9.88	13.66	13.99
III	64.33	177.66	8.93	14.96	9.43	15.76	9.21	14.25	27.20
IV	114.83	206.66	9.73	15.83	11.10	18.08	9.98	15.25	21.22

BW: Body weight

have shown average 1.49 cm additional body circumference growth as compared to normal rats. This also confirms the growth promotion effect of AYU-707.

Feed efficiency

The rats of Group III administered with AYU-707 have not shown any significance difference when the results are compared to control rats (Group I). However, the rats of Group IV have shown a significant increase in the percentage of feed efficiency when the results are compared to normal rats (Group II).

Effect on serum ALP, SGPT, and SGOT in normal and AYU-707 treated rats (Table 3)

Effect of AYU-707 on serum ALP, SGPT, and SGOT

ALP

Serum ALP level was measured using Erba diagnostic kit and semi autoanalyzer. Under standard conditions, the absorbance was measured at 405 nm and the ALP activity was calculated using the formula ALP activity (IU/L) = ΔA/min × Factor (2764). The normal serum ALP level in rats 56.80-120 U/L.

SGOT

SGOT or AST level was measured using Erba diagnostic kit and semi autoanalyzer. Under standard conditions, the absorbance was measured at 340 nm and the serum SGPT activity was measured using the formula (IU/L) = ΔA/min × Factor (1768). The normal serum SGOT levels in rats 45.7-80.8U/L.

SGPT or ALT

Serum SGPT level was measured using Erba diagnostic kit and semi autoanalyzer. Under standard conditions, the absorbance was measured at 340 nm and the SGPT activity was calculated using the formula SGPT activity (IU/L) = ΔA/min × Factor (1746). The normal serum SGPT levels in rats 17.5-30.2 U/L.

The serum ALP level in Group I normal control rats was found to be 76.46±5.96 U/L. The rats (Group III) treated with AYU-707 have shown a significant increase (107.33±17.51 U/L) when compared to normal control rats. Similarly, Group I normal control rats were found to be 82.91±4.28 U/L. The rats treated with AYU (Group IV) have also shown a significant increase in the serum ALP level (136.81±16.83 IU/L) when compared to normal (Group II) rats. The increased ALP level is may be due to increased osteoblast activity in developing due to accelerated bone growth due to AYU-707. The insignificant activity of serum SGOT and SGPT in AYU-707 treated rats, when compared to the normal rats, suggests that AYU-707 is safer to vital organs such as liver.

Effect on serum Ca^{2+}, amylase, and total protein in normal and AYU-707 treated rats (Table 4)

Serum calcium, amylase, and total protein measured using Erba diagnostic kits (Fig. 4).

Serum calcium

Under standard conditions, the absorbance was measured using semi autoanalyzer, and serum calcium was calculated using formula:

Table 3: Different serum parameters: ALP, SGPT, and SGOT

Groups n=6	ALP (U/L)	SGPT (U/L)	SGOT (U/L)
I	76.46±5.96	34.47±10.17	83.38±12.91
II	82.91±4.28	33.88±10.57	77.49±15.30
III	107.33±17.51	32.11±10.39	59.22±9.27
IV	136.81±16.83	33.59±15.69	62.17±7.19

ALP: Alkaline phosphatase, SGOT: Serum glutamate-oxaloacetate transaminase, SGPT: Serum glutamic-pyruvic transaminase

Table 4: Effect of AYU-707 on serum calcium, amylase, and total protein

Groups	Calcium (g/dL)	Amylase (IU/dL)	Total protein (g/dL)	Hb content g/dL
I	10.24±0.133	157.44±50.81	8.11±0.858	15.63
II	9.99±0.230	140.23±46.74	8.57±1.220	14.50
III	10.69±0.251	189.76±31.32	10.06±1.100	17.36
IV	10.26±0.127	203.31±40.92	9.07±1.542	17.68

Hb: Hemoglobin

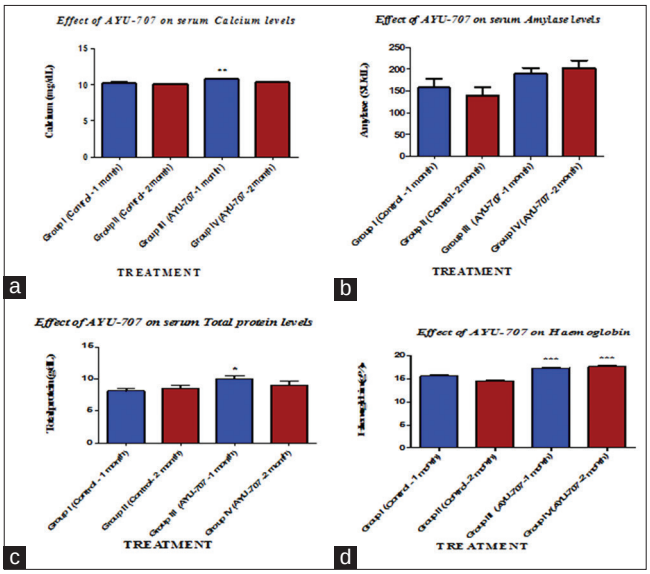

Fig. 4: (a) Effect of AYU-707 on serum calcium levels, (b) Effect of AYU-707 on serum amylase levels, (c) effect of AYU-707 on serum total protein levels, (d) effect of AYU-707 on hemoglobin

Calcium (mg/dl) = Absorbance of test/absorbance of standard × Concentration of standard (mg/dl). The normal serum calcium levels in rats - 3.11-11 mg/dL.

Serum amylase

The absorbance was measured using semi autoanalyzer at 405 nm and serum amylase was calculated using formula. Amylase activity (U/L) = ΔA/min × F (3128). The normal serum amylase activity in rats - 138-313 IU/dL.

Serum total protein

The absorbance was measured using semi autoanalyzer at 546 nm and serum total protein was calculated using formula. Total protein (mg/dl) = Absorbance of test/Absorbance of standard × Concentration of standard. The normal serum total protein level in rats - 5.6-7.6 g/dL

The insignificant difference in the serum calcium level in both Group III and IV rats when compared the normal Groups (I and II) was observed. The serum calcium may be utilized for the growth of developing bone in AYU-treated rats. Hence, no elevation of serum calcium level in AYU-treated rats.

The rats treated with AYU-707 (Group III and IV) have shown an insignificant increase in the serum amylase activity when compared to the normal rats (Group I and II). This result indicates that AYU-707 administered in 1 and 2 months rats may stimulate proliferation of pancreatic cells and increases the content of amylase enzyme in the blood.

The rats treated with AYU-707 (Group III) have shown a significant increase in the serum total protein level when compared to the normal rats (Group I). The rats treated with AYU-707 (Group IV) have shown an insignificant increase in the serum total protein level when compared to the normal rats (Group I). This result indicates that AYU-707 administered in immature rats may stimulate the expression of various growth factors (enzymes/proteins) and plasma proteins.

The rats treated with AYU-707 have shown significant increase (17.36 and 17.68 g/dL) in hemoglobin (Hb) content when compared to normal rats (15.63 and 14.5 g/dL). These results are in accord with the earlier animal studies report and suggest that the GH-IGF axis is involved in the physiological elevation of Hb level during childhood.

Effect on rat femur weight, tibial density, epiphyseal plate width, and Bone hydroxyproline in normal and AYU-707 treated rats (Table 5)[5,8,9]

The rats treated with AYU-707 have not shown a significant change in the weight of femur weight (0.62±0.0242, 0.67±0.0163 g) when compared to normal rats (0.53±0.0178, 0.62±0.0183).

The rats treated with AYU-707 have shown a significant increase in the tibial density (1.48±0.0147 and 1.50±0.0348 g/L) when compared to the tibial densities of normal rats (1.36±0.0265 and 1.38±0.0466 g/L). This result indicates the bone formation and bone strengthening effects of AYU-707 in rats.

The rats treated with AYU-707 have shown a significant increase in the bone hydroxyproline content 13.40±0.390 and 15.76±0.550 mg/g bone when compared to the bone hydroxyproline content of the normal rats (8.63±0.401, 9.72±0.257 mg/g bone). The result indicates the collagen stabilizing effect of AYU-707 on rat bone (Figs. 5 and 6).

Effect on spleen, uterus, kidney, lungs, liver, and heart weight in normal and AYU-707 treated rats (Table 6)

The rats treated with AYU-707 (Group III and IV) not shown a significant change in the spleen when the results are compared to the normal rats (Group I and II). The rats treated with AYU-707 (Group III) have shown a significant increase in the weight of the uterus (2.6 g) when compared to the uterine weight (2.04 g) of the normal control rats (Group I). However, rats treated with AYU-707 (Group III) have not shown a significant increase in the weight of the uterus (2.65 g) when compared to the uterine weight (2.44 g) of the normal control rats (Group I). The rats treated with AYU-707 have not shown a significant change in the kidney, lung, and liver weights when the results are compared to the normal rats.

Table 5: Effect on femur weight, tibial density, epiphyseal plate width, and bone hydroxyproline in normal and AYU-707 treated rats

Groups n=6	Femur weight (g)	Tibial density (g/ml)	Epiphyseal plate width (µm)	Bone hydroxyproline (mg/g)
I	0.53±0.0178	1.36±0.0265	9.33±1.211	8.63±0.401
II	0.62±0.0183	1.38±0.0466	8.66±0.816	9.72±0.257
III	0.62±0.0242	1.48±0.0147	12.66±0.816	13.40±0.390
IV	0.67±0.0163	1.50±0.0348	14.83±1.940	15.76±0.550

DISCUSSION

The insignificant activity of serum SGOT and SGPT in AYU-707 treated rats, when compared to the normal rats, suggests that AYU-707 is safer to vital organs such as liver. The significant increase in the serum amylase activity in AYU-707 administered rats indicates that AYU-707 administered in 1 and 2 months rats may stimulate proliferation of pancreatic cells and increases the content of amylase in the blood. The increased total protein levels in AYU-707 administered in immature rats may stimulate the expression of various growth factors (enzymes/proteins) and plasma proteins. The increase in serum Hb level is in accord with earlier animal studies report and suggests that the GH-IGF axis

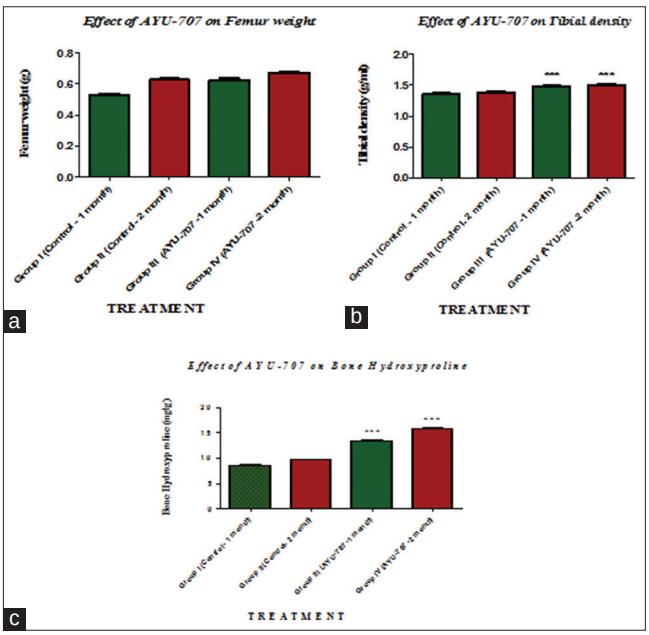

Fig. 5: (a) Effect of AYU-707 on femur weight, (b) effect of AYU-707 on tibial density, (c) effect of AYU-707 on bone hydroxyproline

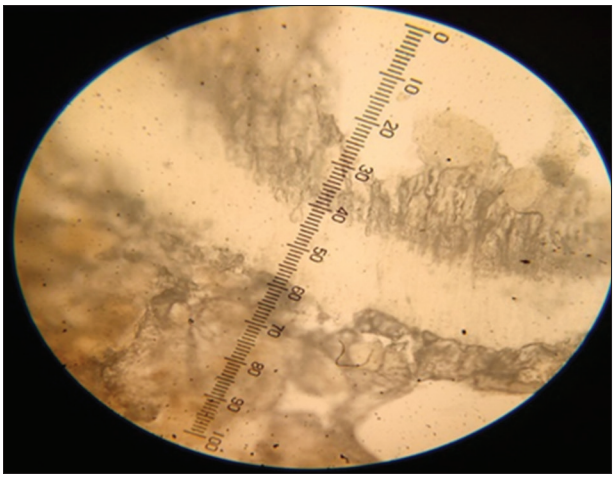

Fig. 6: Width of epiphysis plate

Table 6: Effects on spleen, uterus, kidney, lungs, liver, and heart weight in normal and AYU-707 treated rats

Group n=6	Spleen weight in g	Uterus weight in g	Kidney weight in g	Lungs weight in g	Liver weight in g	Heart weight in g
I	4.48	2.04	9.16	9.09	34.43	3.60
II	4.67	2.44	9.65	9.16	37.33	3.79
III	4.59	2.60	9.09	9.20	35.40	4.27
IV	4.91	2.65	9.56	9.32	40.65	4.58

is involved in the physiological elevation of Hb level during childhood. The significant increase in femur weight and tibial density in AYU-707 treated rats indicates the bone formation and bone strengthening effects of AYU-707 in rats. The elevated level of hydroxyproline level in AYU-707 treated rats indicates the collagen stabilizing the effect of AYU-707 on rat bone. Hydroxyproline is a non-proteinogenic amino acid formed by the post-translational hydroxylation of proline. Hydroxyproline is a major component of collagen, where it serves to stabilize the helical structure. The AYU-treated rats shown significant increase in the length of epiphyseal thickness when compared to the thickness of the epiphyseal plate of the normal control rats. This confirms the growth promotion effect of AYU-707.

CONCLUSION

From the results of the present study, it is concluded that AYU-707 formulated by M/s Ayurwin Pharma Pvt. Ltd., Bengaluru, possesses superior anabolic and growth promotion properties that may serve to promote the growth in children associated with growth-related disorders.

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