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SUSTAINABLE DEVELOPMENT AND THE DIET OF LATHYRUS SATIVUS STRAIN RATS CAN IMPROVE CARDIOVASCULAR SAFETY BY CHANGING THE EXPRESSION OF PKC AND HIF1-α

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Abstract

The goal of this work is to investigate these relationships in the cardiovascular system and to explain their importance by citing appropriate sources. The cardiovascular system (CVS) plays a vital role in maintaining homeostasis by ensuring the efficient supply of oxygen and nutrients to various organs and tissues. Imbalances in this system can lead to the development of cardiovascular diseases, a significant cause of global mortality. Recent research has revealed intriguing connections between β-N-oxalyl L-α, β diamino propionic acid (β-ODAP), homoarginine (hArg), protein kinase C (PKC), and hypoxia-inducible factor 1-alpha (HIF-1α). We hypothesized that ODAP and homoarginine in *Lathyrus sativus*, as potential modulators of PKC and HIF-1α, might enhance the cardiovascular adaptive response to exercise. Swimming was employed as a physical exercise to promote the cardiovascular system's physiological responses in SD strain rats fed with *Lathyrus* diet or a normal diet for 30 days. Body weight gain and swimming ability time were recorded, and biochemical tests were performed on blood and tissue samples. Statistical analysis revealed a significantly lower body weight increase in *Lathyrus* diet-fed rats compared to normal diet-fed rats. Lipid profiles and marker enzymes were within normal ranges in both groups. Increased swimming ability time was observed in the *Lathyrus* diet group. These findings underscore the delicate balance between ODAP and hArg in cardiovascular health, suggesting *Lathyrus sativus* as a promising dietary supplement for performance enhancement, even at high altitudes.

Keywords: Cardiovascular system-*Lathyrus sativus*, diet, L-Homo Arginine-Hypoxia inducible factor-Weight loaded forced swimming test.

INTRODUCTION

Lathyrus sativus (i.e., grass pea), a non-protein amino acid, is found in plants seeds and is associated to neuro-lathyrism in humans. Over time, excessive use of β-N-Oxalyl-α, β-diamino propionic acid (β-ODAP) can lead to Lathyrism, a neurological illness [1]. Lathyrism causes paralysis and other motor disorders by degenerating neurons and muscles [2]. *Lathyrus sativus* contains two molecules essential for the circulatory system: neurotoxic amino acid (i.e., β-ODAP,), and homoarginine (hArg).

This paper will examine how these two chemicals affect cardiovascular health. *Lathyrus sativus* efficiently distributing oxygen and nutrients to organs and tissues, the cardiovascular system (CVS) maintains homeostasis [3]. Heart disease, a leading cause of death worldwide, can result from system imbalances. This essay examines these CVS linkages and discusses their importance using references [4]. β-ODAP has excitotoxic effects on the CVS by activating glutamate receptors. In the CVS, β-ODAP-induced excitotoxicity can cause endothelial cell

dysfunction, poor vaso-relaxation, and contribute to illnesses including hypertension and atherosclerosis [5-7]. Homoarginine (hArg) is an amino acid derivative with cardioprotective and vasoprotective properties. Homoarginine is structurally identical to arginine, another amino acid that produces nitric oxide. Endothelial nitric oxide synthase (eNOS) converts hArg into NO, causing vasodilation and controlling blood pressure [8-10].

This manuscript examines homoarginine's role in cardiovascular health. According to thorough studies, homoarginine affects the production of nitric oxide (NO), a strong vasodilator [11]. Endothelial function depends on NO, which regulates blood vessel tone and flexibility. Endothelial dysfunction, frequent in cardiovascular disease, impairs NO generation [12]. NO is made from L-arginine, which homoarginine is a precursor and it indirectly enhances NO production, increasing endothelial function and CVS integrity. Several studies show that homoarginine levels inversely affect major adverse cardiovascular events. Low homoarginine levels increase the risk of heart disease, stroke, and coronary artery disease [13]. Additionally, low hArg levels are linked to endothelial dysfunction, lower arterial elasticity, and impaired renal function, all of which indicate CVS impairment. Despite the expanding importance of homoarginine, more research is needed to completely understand its mechanisms of action and therapeutic potential. Additionally, studying the effects of homoarginine supplementation and nutrition on cardiovascular health may provide interventions [14].

Recent research has found fascinating links between β-N-oxalyl L-α, β, ODAP, hArg, PKC, and HIF-1α [7, 13, 15]. However, new research suggests that *L. sativus* in the diet may improve cardiovascular health. HIF-1α and protein kinase C (PKC): Hypoxia-inducible factor 1 α (HIF-1α) is a fundamental regulator of cellular response, enabling adaptation and survival in low oxygen settings [16-19]. Stabilising HIF-1α during normoxia may be a promising treatment for disorders linked to low oxygen supply. Maintaining HIF-1α stability is critical during times of low oxygen availability. Biochemical substances and genetic factors are crucial for HIF1α stabilisation. Research indicates that ODAP can activate PKC and stabilise HIF-1α in normoxic circumstances. This study examines how β-ODAP and homoarginine in *Lathyrus sativus* stimulate PKC and stabilise HIF1-α in swimming rats under normoxic conditions, promoting cardiovascular protection [20-22].

MATERIALS AND METHODS

Procurement of animals and ethical clearance

Adult male Sprague Dawley (SD) strain rats weigh up to 120-150 g were acquired from National Centre for Animal Science, National Institute of Nutrition, Hyderabad, India. The animal experimentation protocol was approved by the institutional ethical committee (Reg. No.: CPCSEA/IAEC/JLS/09). All the animal experimentation was performed according the regulations laid by the animal ethics committee.

Animals

Rats were distributed into two sets for each containing twelve animals. One set of animals was served with the control diet (Table1) and the other with the *lathyrus diet* (Table 1). The study duration was 30 days. Encumbrance loaded enforced swimming test was with the SD strain rats

of both groups at alternate days for 30 days and at the termination of the study, blood was collected next euthanizing the rats by cervical dislocation and the biochemical analysis as explained in the later sections was carried out [23].

Table 1: Diet Composition

Dietary Component	***Lathyrus* diet**	**Control diet**
Protein (milk powder) (g)	15*	15
Fat (Butter) (g)	5	5
Carbohydrate (wheat flour) (g)	70	70
Cholesterol (g)	0.1	0.1
Fibre (cellulose) g	5	5
Coconut oil (ml)	1	1
Mg (%)	0.12	0.12
Ca (%)	0.9	0.9
P (%)	0.70	0.70
K (%)	1.2	1.2
S (%)	0.2	0.2
Na (%)	0.31	0.31
Cl (%)	0.64	0.64
Vitamin mix (g)	1	1

* Protein powder replaced by *Lathyrus* seed powder

Weight loaded forced swimming test

Weight loaded forced swimming test (FST) was performed according to a literature report with some modifications. In a small tank of 30 cm deep, at temperature 25 ± 2 °C comprising water FST was implemented [24]. Every alternate day, *Lathyrus* diet fed rats and control rats were exposed to FST supporting continuous load attached to their tails with an information block weighing just about 10 % of their body weight, which forces the animals to fast leg movements. Exhaustion was determined by observing their loss of coordinated movements and failure to swim [25].

Sample collection and preparation for analysis of marker enzymes and biochemical estimation

To minimize dietary impacts the animals remained fasted for 12 h. at the finishing point of experimental period and sacrificed around 8 am by cervical dislocation [26]. Cardiac puncture was made and blood was pinched into sterile syringes and then and there transferred into micro centrifuge tubes enclosing heparin, by centrifugation at 3000 rpm for 10 min. plasma was separated from cells [27]. The blood also been collected which was deprived of anticoagulant that was allowed to clot for 30 min., and then serum was separated by centrifugation [28]. For histological examinations, a small portion of tissues (heart, liver and arteries) were removed, splashed carefully by ice-cold saline and suspended in formalin buffer in polypropylene ampoules. Until the assays were carried out, the remaining tissue of each organ of every single

animal was put off in 0.15 M potassium chloride (KCl) contained poly propylene ampoules, sealed with para film, labelled carefully and frozen at -80 °C [29].

Analysis of marker enzymes

Activities of Marker enzyme were tested in plasma using the reported methods in the literature. The activity of lactate dehydrogenase (LDH) [30], creatinine kinase (CK) [31], aspartate transaminase (AST) [32], alkaline phosphatase (ALP) [33], alanine transaminase (ALT) [34], and γ-glutamyl transferase [35] were assayed using colorimetric methods by the reported methods

Biochemical estimations

Plasma total cholesterol (TC) levels and triglycerides (TRIGLY) levels were assessed with diagnostics kits from ERBA Diagnostics (Mumbai), following the methods defined by kit manufacturer. Serum HDL-C was determined by using a diagnostics kit from Siemens (Mumbai). The serum level of very low-density lipoprotein-cholesterol (VLDL-C) was calculated as VLDL-C = TG/5, and that of LDL-C was calculated as LDLC = TC – (HDL-C + VLDL-C).

Statistical analysis: It was implemented by means of t-test unpaired. For twelve rats, for each set and results were articulated as mean ± standard error of mean (SEM). A value of $P<0.05$ was consider statistically significant.

Unpaired t -test with Welch's correction: Welch's t- test is a two-sample position test which is used to test premise that the two equal populations have mean or almost equal mean. t-value means a critical t value of 2.101 where if the absolute value of t-static is larger than t critical value it is said to be the population is significantly different.

RESULTS

Body weight: Average body weight gain conducted for 12 animals in each group.

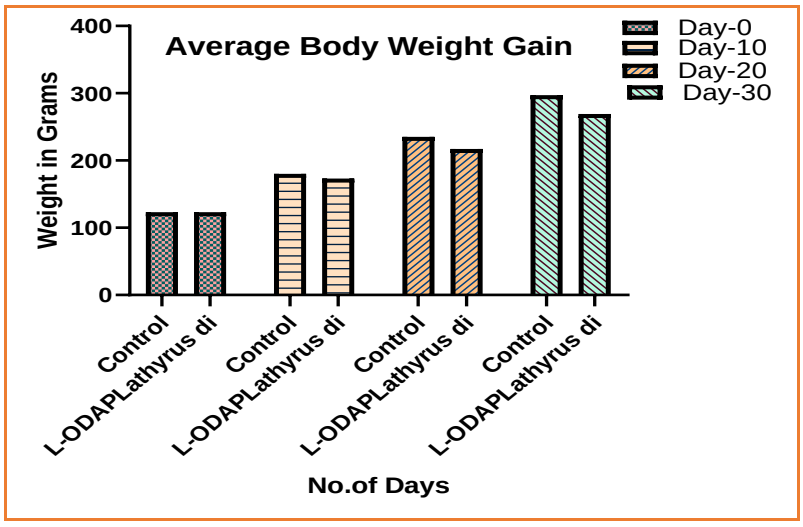

Figure 1: Body weight gain recorded for control and *Lathyrus* diet groups recorded for on X-axis and weight in grams on Y-axis

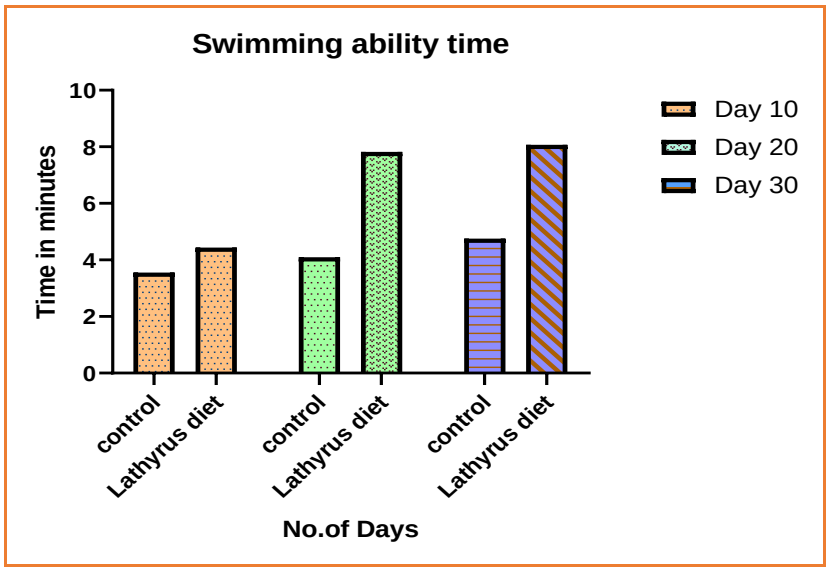

Figure 2: Swimming ability time recorded for control and L-ODAP fed groups on X-axis and number of days on Y-axis

Lipid Profile

We tested blood samples for toxicology and found no difference in lipid profile and marker enzymes in control and Lathyrus diet-fed rats, which average body weight.

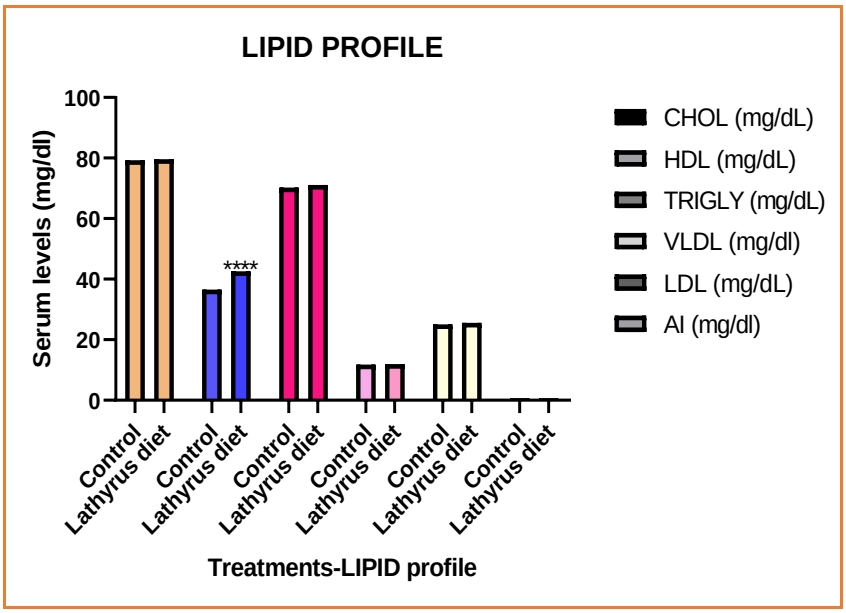

Figure 3: Lipid profiles recorded for control and *Lathyrus* diet groups on X-axis and serum levels (mg/dl) on Y-axis

In figure 3, CHOL stands cholesterol, HDL stands high density lipoprotein, TRIGLY stands triglycerides, VLDL stands very low-density lipoprotein, LDL stands low density lipoprotein and AI stands apo lipoprotein.

Unpaired t-test with Welch's correction of lipid profile

Whereas when degrees of freedom (df) considered value of cholesterol= 21.26 and Triglycerides=21.6 VLDL=13.19, LDL=13.15 and Apo lipoprotein=21.17 P-value was greater than 0.05 that were not significant but degrees of freedom (df) value of HDL=14.62 were having p-value HDL=<0.05 represents that HDL was significant denoted by **** (four asterisks) for HDL

For the true difference the 95% confidence interval in population has been known as **(-10.45, 1.61)** so we observed that the groups HDL with the 95% confidence interval above true difference range showed significance and remaining below range were not significant.

Marker enzymes

Cysteine Synthase (CS), used as a key regulatory enzyme involved in cysteine biosynthesis in plants [36-38]

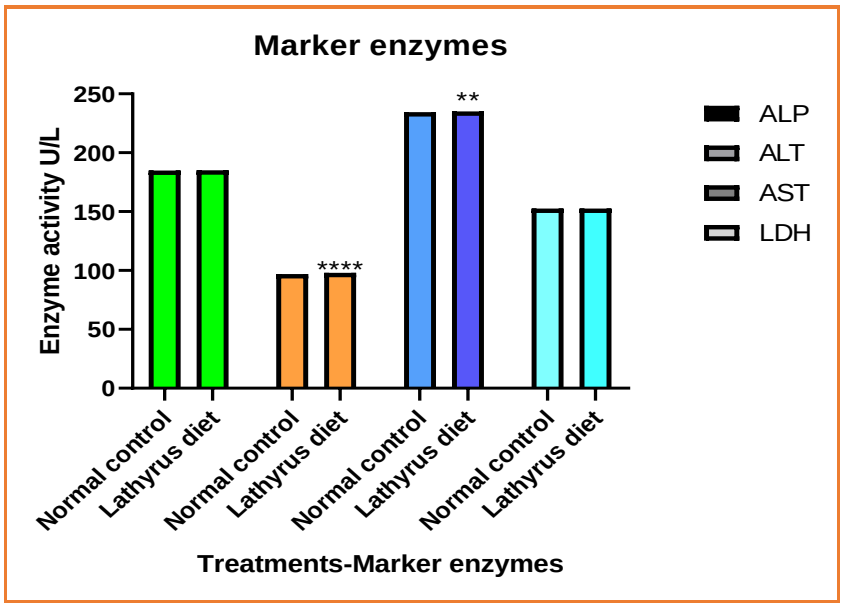

Figure 4: Marker enzymes recorded for control and *Lathyrus* diet groups on X-axis and enzyme activity (U/L) on Y-axis

Table 2. Results of t-test Unpaired with Welch's correction of lipid profile and marker enzymes for control and *Lathyrus* diet.

Lipid Profile	Control	L-ODAP	T-value	df	P-value	σ value	R^2	95% confidence level
CHOL	79.17±0.17	79.57±0.17	0.79	21.26	0.43	NS	0.03	-0.65 to 1.45
HDL	36.47±0.86	42.51±2.09	9.25	14.62	0.01	****	0.85	4.65 to 7.44
TRIGLS	70.16±1.15	70.99±1.32	1.63	21.6	0.12	NS	0.11	-0.22 to 1.88
VLDL	11.16±0.31	11.90±0.98	0.74	13.19	0.47	NS	0.04	-0.42 to 0.86
LDL	25.02±0.30	25.52±0.96	1.73	13.15	0.11	NS	0.19	-0.12 to 1.13

AI	0.68±0.030	0.682±0.03	0.21	21.17	0.83	NS	0.09	-0.022 to 0.03
ALP	184.84±0.74	185.05±0.17	0.61	20.90	0.55	NS	0.02	-0.50 to 0.92
ALT	97.03±0.47	97.95±0.53	5.34	22.00	<0.01	****	0.56	0.62 to 1.41
AST	234.54±0.3	235.11±0.73	3.44	12.00	0.04	**	0.49	0.53 to 2.35
LDH	152.55±0.78	152.6±0.94	0.15	21.30	0.88	NS	0.01	-0.68 to 0.79
ALP	184.84±0.74	185.05±0.17	0.61	20.91	0.55	NS	0.02	-0.50 to 0.92

Whereas considering degrees of freedom (df) value of ALP= 20.91 and LDH=21.3 having P-value greater than 0.01 were not significant but degrees of freedom (df) value of ALT=22 and AST=12 having p-value ALT=<0.0001 and AST=0.0049 represents that ALT and AST were significant denoted by **** (four asterisks) for ALT and ** (two asterisks) for AST

For the true difference the 95% confidence interval in population has been known as **(-10.45, 1.61)** so we observed that the groups ALT and AST with the 95% confidence interval above true difference range showed significance and the groups ALP and LDH below range were not significant.

We further tested blood samples for toxicology studies, where we observed that there is no difference in both control and *Lathyrus* diet fed rats for lipid profile and marker enzymes, which average body weight (Figure 1) of SD Strain rats showed that *Lathyrus* fed rats shown 3.8%, 7.65% and 9.42% decrease in mean average body weight when compared to control diet fed SD strain rats on Day 10, 20 and 30 respectively. From the overall results, we observed that on day-10 (Figure 2) the mean swimming ability time in control rat was 3.55±0.01min. and *Lathyrus* diet fed Rats was 4.4±0.01 min. On days 20 and 30, the swimming performance of *Lathyru*s diet fed rats (7.82±0.09 min., 8.07±0.015) observed more when compared to control (4.1±0.009 min., 8.07±0.015 min.) respectively.

In Lipid profile (Figure 3) we analyzed that HDL levels of control diet fed SD strain rats is 36.47±0.86 and *Lathyus* diet fed SD Strain rats is 42.51±2.09 which clearly showed that there was increase of HDL levels by 16.56% when rats served with control diet compared to rats served with *Lathyrus* diet. So, rise of HDL levels indicates that *Lathyrus* diet protects from hypertension and cardiac vasculature diseases. Thus, from these studies, we observed that *Lathyrus* diet may not possess any side effects apart from increasing the performance.

Discussion:

According to the forced rat swimming experiment, the *Lathyrus* diet group had better swimming performance than the control diet group, although having a lower average body weight gain. To improve performance in SD strain rats, we found that maintaining HIF-1α was the primary adaptive response to decreased oxygen absorption, leading to PKC activation. We found that PKC activation causes oxidative stress and decreased NO bioavailability, which L-Arginine must compensate for. *Lathyrus* diet contains L-homoarginine (HA), a non-protein amino acid discovered before the ODAP. Homoarginine, an arginase inhibitor, also improved the NO *lathyrus* diet group. The forced swimming experiment animal model with lathyrus diet may benefit hypoxic people. Research indicates that *Lathyrus sativus* includes β-ODAP, which

stimulates conservative protein kinase C and stabilises HIF-1α in normoxic conditions. Here are some mechanistic impacts from the more detailed research:

1. Binding of ODAP to protein kinase C (PKC): In previous research, ODAP coupled to PKC receptors as an agonist might activate PKC by starting a signalling cascade. PKC activation may lead to downstream actions that stabilise HIF-1α [39].

2. Activation of phosphoinositide pathway: Further, activated PKC could act on the phosphoinositide pathway, this could lead to an increase in intracellular calcium concentrations. Elevated intracellular calcium served as a crucial signal for HIF-1α stabilization [40].

3. Activation of calcium-dependent signaling pathways: In addition, elevated intracellular calcium may activate calmodulin and CaMK-dependent pathways. These routes may combine to improve HIF-1α stability and activity [41].

4. Inhibition of prolyl hydroxylase domain (PHD) Activity: Recent research indicates that prolyl hydroxylase domain (PHD) proteins regulate HIF-1α, leading to its ubiquitination and proteasomal destruction. ODAP inhibits PHD activity, limiting HIF-1α degradation and stabilising it, according to our lab colleagues [22, 42].

5. Homoarginine augmentation: The inclusion of homoarginine, an uncommon amino acid in Lathyrus sativus, increased PKC activity and enabled ODAP to stabilise HIF-1α. Homoarginine may help PKC respond more strongly to ODAP activation [43].

6. Modulation of reactive oxygen species (ROS): Further analysis showed that ODAP reduces ROS production. Low ROS levels can reduce PHD protein activity, leading to HIF-1α stabilisation [6].

7. HIF-1α-mediated regulation of body weight: We found that stabilising HIF-1α in swimming rats increased oxygen transport, metabolism, energy utilisation, and weight reduction. Swimming rats treated with ODAP may have reduced body weight due to this mechanism [44].

8. High-density lipoprotein (HDL) regulation: Our analysis found that stabilising HIF-1α regulates lipid metabolism, boosting HDL production and reversing cholesterol transfer. Swimming rats treated with ODAP have greater HDL levels, which may protect the heart [45].

Homoarginine regulates blood pressure, according to our research. Studies demonstrate that reduced homoarginine levels increase blood pressure, a major risk factor for cardiovascular disease. Homoarginine relaxes blood arteries, enhancing dilation and blood flow. Vasodilation reduces artery constriction, lowering blood pressure and maintaining cardiovascular health [18].

We found that increasing homo arginine levels may treat cardiovascular disorders. NO produced from homo arginine, is essential to cardiovascular function. There may be therapeutic implications:

1. Enhancing nitric oxide production: In increasing homoarginine levels boosted NO generation, which vasodilated. Vasodilation lowers blood pressure, improves blood flow, and reduces heart load. This vasodilation may help hypertension and coronary artery disease [17].

2. Endothelial dysfunction: Homoarginine-derived NO is essential for endothelial function. We found that lower NO production in endothelial dysfunction can impair blood vessel dilatation

and promote inflammation. We also found that increasing homoarginine availability restored endothelial function, helping treat atherosclerosis and vascular disorders [46].

3. Endothelial nitric oxide synthase (eNOS) dysfunction: Low homoarginine levels may impair eNOS activity and NO generation, causing endothelial dysfunction and cardiovascular disease. We found that increasing homo arginine levels could overcome this constraint and increase eNOS activity and NO generation, which could help treat eNOS dysfunction-related cardiovascular illnesses [47].

4. Anti-inflammatory effects: NO from homoarginine was anti-inflammatory. It may impede platelet, leukocyte, and smooth muscle growth. We found that lowering homo arginine levels may lessen inflammation in cardiovascular disorders such atherosclerosis and myocardial infarction [48].

5. Anti-thrombotic effects: NO from homoarginine inhibited platelet aggregation and adhesion, lowering thrombus risk. Thus, we found that raising homoarginine levels boosted NO's anti-thrombotic properties, which is useful for deep vein thrombosis, stroke, and peripheral artery disease. Further study is needed to discover the best technique and hazards of modulating homo arginine levels for cardiovascular disease therapy [49].

CONCLUSION

In conclusion, ODAP and hArg have different impacts on cardiovascular health, but their interaction shows the delicate balance needed for good cardiovascular function. ODAP, present in *Lathyrus sativus* plants, may cause cardiovascular excitotoxicity causing hypertension and atherosclerosis. Arginine derivative hArg reduces the risk of cardiovascular disease by protecting the heart and blood vessels. Understanding the complex interactions between these chemicals and their biological processes may help create cardiovascular disease treatments.

We concluded that the swimming rat experiment with high HDL and low body weight shed light on ODAP and homoarginine's involvement in cardiovascular health. HDL, or "good cholesterol," helps remove excess cholesterol from artery walls, preventing heart disease. However, low weight reduces cardiovascular risk factors including hypertension and dyslipidaemia. We also believe that hypoxic persons benefit more from *Lathyrus* diet. Thus, *Lathyrus sativus* may boost performance at high altitude as a food supplement.

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CONFLICT OF INTEREST

No

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