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# *Effect of Purslane Extract (Portulaca oleracea L.) on Hypoxic Rat Organs*

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**Abstract— Hypoxia occurs when the body experiences a lack of oxygen or an increase in cellular oxygen consumption. A lack of oxygen can affect the production of reactive oxygen species (ROS). High levels of ROS can cause various diseases and damage to the body. Antioxidant compounds can suppress this increase in ROS. The purslane plant (Portulaca oleracea L.) has the potential to be a source of antioxidants. This study aimed to examine the compounds and observe the effect of purslane plant extract on repairing and reducing organ damage in the lungs, hearts, and brains of hypoxic rats. Observation parameters for organ morphology were reviewed using organ index measurements and histopathology. The results indicate that two substances, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and 12-Methyl-E,E-2,13-octadecadien-1-ol, have the potential to be antioxidants, and the weight ratio of lung organs in each treatment did not differ significantly ($P > 0.05$). At doses of 150 and 300 mg/kgBW, the parameter of inflammatory cells in the hypoxia group was significantly different from the control group ($P < 0.05$). Based on the histology of the lung, heart, and brain organs of rats, it can be determined that hypoxic tissue in the control group does not undergo a repair process, resulting in necrosis, hemorrhage, and an increase in inflammatory cells.**

**Keywords—Portulaca oleracea L.; Antioxidant; Hypoxia; Inflammatory cells; Necrosis**

## I. INTRODUCTION

Hypoxia is characterized by a deficiency of oxygen or an increased oxygen demand at the cellular level, which can disrupt normal physiological processes (Uyun & Indriawati, 2013). This condition leads to the elevated production of reactive oxygen species (ROS), molecules that, in high concentrations, are linked to a variety of diseases such as cancer, asthma, pulmonary

fibrosis, bronchitis, and chronic obstructive pulmonary disease (Ferdian et al., 2020). The body's defense against this oxidative stress involves antioxidants, which mitigate the effects of ROS by donating electrons to free radicals, thereby inhibiting the chain reactions they cause (Julfitriyani et al., 2016). Normally, the human body is capable of neutralizing free radicals unless their levels become excessive (Werdhasari, 2014). Dietary intake of antioxidants, particularly flavonoid compounds found in tea, fruits, and vegetables, plays a crucial role in this defense mechanism (Simanjuntak, 2012).

Research has demonstrated the potential of plant extracts in modulating the body's response to hypoxia. For instance, rats treated with fig leaf extract exhibited modifications in catalase-induced hypoxia, suggesting that reducing catalase levels can mitigate hypoxic conditions. Similarly, studies utilizing Maja fruit extract (Aegle marmelos) and blackberry leaf extract (Rubus sp.) showed that these extracts could lower malondialdehyde (MDA) levels while enhancing antioxidant capacity in hypoxic rats, indicating their potential in compensating for antioxidant loss (Kelvin et al., 2019; Limanan et al., 2019).

The purslane plant (Portulaca oleracea L.) is known for its rich content of proteins, vitamins B and C, various antioxidant compounds such as saponins, carotene, alkaloids, flavonoids, coumarins, glycosides, and high levels of omega-3 fatty acids, positioning it as a potential source of natural antioxidants (Kardinan, 2007). This study aims to explore the compounds present in purslane plant extract and assess its effectiveness in repairing and reducing lung organ damage in rats subjected to a hypoxic model. By evaluating the protective effects of purslane extract against hypoxia-induced damage, this research could contribute significantly to the understanding of natural antioxidants in medical and nutritional applications.

## II. Research Methods

This study is an experiment in which purslane plants (*Portulaca oleracea* L.) are extracted for their active compounds. Over two months, the purslane root, stem, and leaf extracts (*Portulaca oleracea* L.) were made at the Biology Research Laboratory, Ahmad Dahlan University Yogyakarta. Purslane plant identification in the Biology Laboratory there under number 182/Lab.Bio/B/V/2021. The Ahmad Dahlan University Research Ethics Committee approved Ethical Clearance (EC) permission for this study with number 012106030 (KEP).

The instruments used in the study were a rotary evaporator, blender, microtome, micropipette, micropipette tip, surgical tool set, hypoxia cage, homogenizer, centrifuge, and EDTA tube. Ngawi, East Java, supplied the purslane (Portulaca oleracea L.). Alcohol, citrate buffer, distilled water, xylol, entering, PBS, hematoxylin, eosin, HCl, NaCl 0.9%, paraformaldehyde, acetone, and 70% ethanol were also used.

### 2.1. Animals and Experimental Protocols

The study used 28 male Sprague-Dawley rats aged 8-12 weeks and weighing 150-250 grams that had been acclimatized for seven days. In each hypoxia vehicle, 8% O2 and 92% N2 were used to induce hypoxia (Ferdian et al., 2020). The test animals were divided into five groups and were made subject to the following treatments:

P1 = Normal (normoxia);

P2 = Hypoxia treatment for ten days without extract or drug administration;

P3 = Hypoxia treatment for ten days with Dexamethasone drug administration;

P4 = Hypoxia treatment for ten days with 150 mg/KgBW of purslane extract;

P5 = Hypoxia treatment for ten days with 300 mg/KgBW of purslane extract.

### 2.2. Extraction of Purslane

Purslane (*Portulaca oleracea* L.) plants were cleaned and air-dried for 14 days. The simplisia was pulverized with a homogenizer after drying. An acetone solvent (1:10) was used for extraction for three days. After that, the extract was filtered with filter paper and concentrated with a rotary evaporator.

### 2.3. Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS testing was conducted at the Laboratorium Penelitian dan Pengujian Terpadu Universitas Gadjah Mada (LPPT UGM). GC-MS analysis of purslane plant extract compounds was carried out using the HP-5MS UI tool. The operating time runs for 55.99 minutes under the following conditions: ion source temperature 200°C and MS transfer line temperature 250°C.

Helium gas acted as a carrier and was maintained at 260°C. The injection was performed at a flow-split ratio of 50 mL/min. The initial column temperature was programmed to be 50°C for one minute and increased by 5°C per minute up to 260°C The compound composition of purslane plant extract was identified by comparing the retention time and molecular weight detected by the mass spectrum with the mass spectral database from the National Institute of Standards and Technology (NIST) library (US).

### 2.4. Induction of Hypoxia and Observations

Male Sprague-Dawley rats aged 8-12 weeks were used in the study, with 30 animals weighing 150-250 grams. The test animals were placed in individual cages with temperatures ranging from 24-28°C and humidity levels ranging from 60-75% (Ridwan et al., 2012). For seven days, the animals were acclimatized, fed, and watered ad libitum. For 6 hours, the experimental hypoxia group was placed in a hood with 10% O2 and 90% N2 as the primary air source. Every 24 hours, the hood was cleaned, and standard laboratory food was provided ad libitum.

*Portulaca oleracea* L. root, stem, and leaf extracts were administered to groups of test animals as part of the treatment. The extract was administered orally with a syringe to the test animals at doses of 150 and 300 mg/kgBW/day, up to 3 ml, immediately after hypoxia induction. For ten days of treatment, the duration of extract administration was adjusted to the duration of hypoxia in each subgroup. Body weight was measured by weighing rats on days 1, 3, 6, 8, and 10 of treatment. On day 11, animals were sacrificed, and the organs were collected. The following formula was used to calculate the organs index:

$$\text{Organ Index} = \text{organ weight (g)} / \text{rat body weight (g)}$$

### 2.5. Histopathological Analysis

The organs were then immersed in Buffer Neutral Formalin (BNF) fixative solution for 24 hours before being embedded in paraffin blocks and stained with hematoxylin and eosin (H & E). Observations were made by comparing data on cell necrosis, hemorrhage, and inflammatory cell in tested animals both in treatment and control groups.

### 2.6. Statistical Analysis

The data were statistically tested using the SPSS application's Kruskal-Wallis test. The data were further analysed using the Kruskal-Wallis 1-way ANOVA (k samples) test with a 95% confidence interval ($\alpha = 0.05$).

## III. Results and Discussion

### 3.1. GC-MS Analysis of Purslane Extract

GC-MS analysis of purslane acetone extract (*Portulaca oleracea* L.) samples yielded 27 compounds (Figure 1). Ten were selected with the highest content of purslane acetone extract (Table 1).

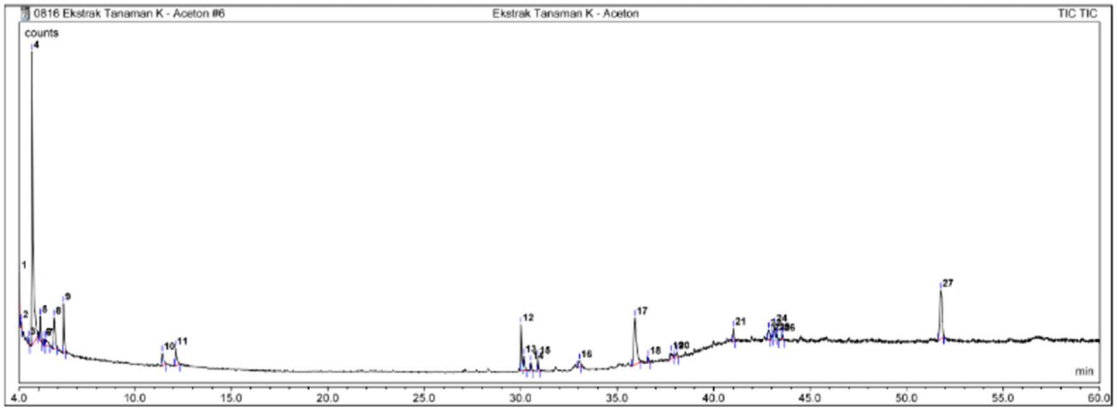


Figure 1. GC-MS chromatogram of purslane acetone extract

Compounds with antioxidant properties were 3,7,11,15-Tetramethyl-2-hexadecane-1-ol and 12-Methyl-E, E-2,13-octadecadienoic-1-ol. Tetramethyl-2-hexadecane-1-ol (3,7,11,15) exhibits antiradical and antibacterial properties (Pejin et al.,

2014). 12-Methyl-E, E-2,13-octadecadienoic-1-ol exhibits antihistamine, antioxidant, analgesic, anesthetic, antibacterial, anti-salmonella, and antiseptic effects (Lakshmi et al., 2018). Antioxidants can help to limit or avoid ROS production. High ROS levels in hypoxic situations can cause illnesses and injury such as asthma, allergies, and lung cancer (Parwata, 2015).

Table 1. Compound content of purslane plant acetone extract (*Portulaca oleracea* L.)

| Name | Chemical Formla | Molecular Weight | RT (min) | Peak Area (%) |
|---|---|---|---|---|
| 2-Pentanone, 4-hydroxy-4-methyl- | $C_6H_{12}O_2$ | 116 | 4.667 | 35.37 |
| Ethanol, 2-(9-octadecenyloxy)-, (Z)- | $C_{20}H_{40}O_2$ | 312 | 35.920 | 13.07 |
| Ethyl iso-allocholate | $C_{26}H_{44}O_5$ | 436 | 51.769 | 11.13 |
| 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | 296 | 30.012 | 4.91 |
| Bicyclo[4.2.0]octa-1,3,5-triene | $C_8H_8$ | 104 | 5.810 | 4.40 |
| 2-Pentanone, 4-methoxy-4-methyl- | $C_6H_{12}O_2$ | 130 | 6.303 | 4.14 |
| 4-Piperidinone, 2,2,6,6-tetramethyl- | $C_9H_{17}NO$ | 155 | 12.109 | 2.99 |
| Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3a,5a)- | $C_{29}H_{43}NO_3Si$ | 481 | 43.150 | 2.39 |
| Morpholine, 4-(1-cyclopentylpiperidin-4-yl)- | $C_{14}H_{26}N_2O$ | 238 | 11.418 | 2.11 |
| 12-Methyl-E,E-2,13-octadecadien-1-ol | $C_{19}H_{36}O$ | 280 | 30.151 | 2.02 |

### 3.2. Organ Index of Rats Lung, Brain and Heart

The results of the Kruskal-Wallis analysis showed some significant differences ($P > 0.05$) in organ treatment (Figure 2), which is believed to indicate a decrease in function and changes at the cellular level such as cell damage. The heart organ index to body weight of rats in P3 is significantly different from rats in P5. The brain organ index to body weight of rats in P1 and P4 differed significantly from P3 treatment. The lung organ index to body weight of rats in the P3 treatment compared to P4 and P5 did not differ significantly. This result suggests cell repair occurs in the P3, P4, and P5 lung organs.

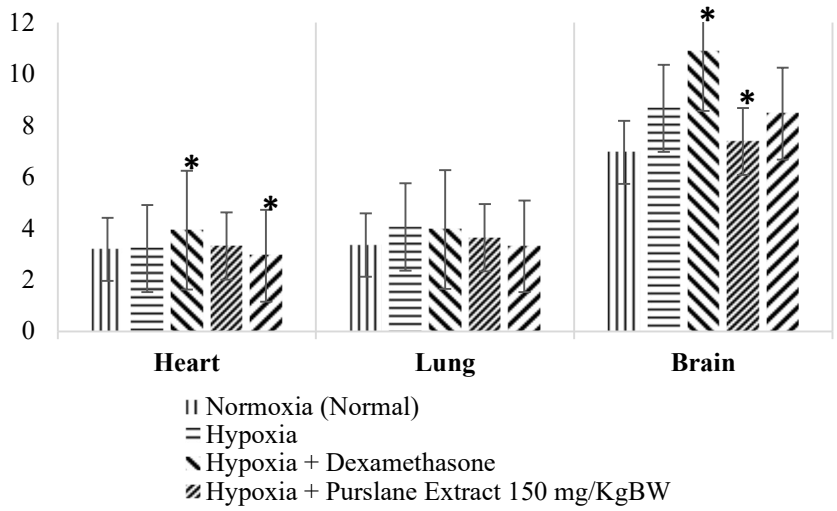


Figure. 2. Organ index of lung, brain, and heart on the 11th day of treatment

The fact that the organ index of rats in the P3 treatment tends to differ significantly from the P4 and P5 is suspected to be the function of dexamethasone, which is a drug from the group of corticosteroids that is a glucocorticoid that plays a role in the suppression of the immune system and has anti-inflammatory activity. Dexamethasone works by suppressing the formation, release, and activity of various inflammatory mediators and modifying the immune response (Santi, 2013).

### 3.3. Organ Morphology of Rats Lung, Brain and Heart

Hypoxia-induced organs (P2) tend to be paler than others, indicating a lack of oxygen supply carried by red blood cells through hemoglobin (Figure 3). Tissues that undergo necrosis and damage appear to be paler when compared to normal tissues around them. Hypoxia can also be seen in the results of blood gas analysis. The longer the duration of exposure, the greater the changes in the results of blood gas analysis, in addition to the transcription of genes through HIF-1α protein expression as the setting of oxygen homeostasis increases. Physiologically, hypoxia will lower the O2 pressure in the arteries, the CO2 pressure, and the O2 saturation due to the presence of metabolic stress due to hypoxia and reduced oxygen supply (Catherine & Ferdinal, 2018).

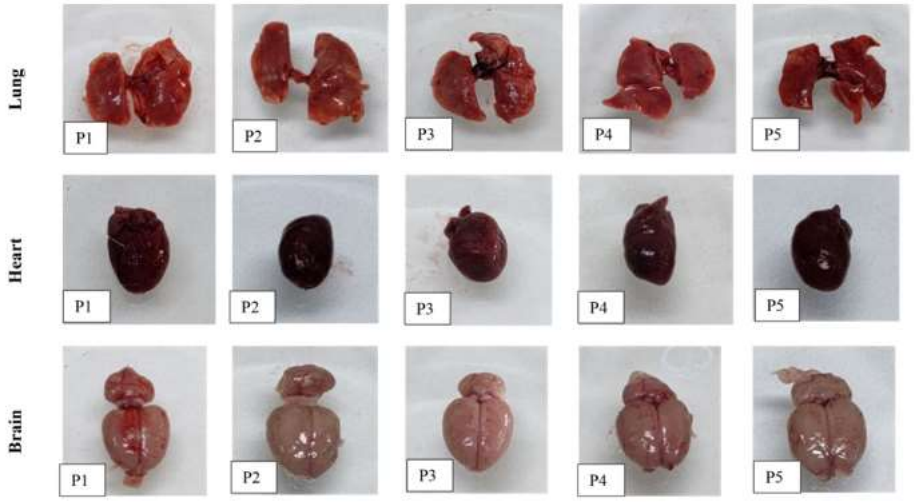


Figure 3. Organ morphology (from top to bottom: lung, heart, brain). P1: Normal; P2: Hypoxia without treatment; P3: Hypoxia + Dexamethasone; P4: Hypoxia + Purslane extract 150 mg/KgBW; P5: Hypoxia + Purslane extract 300 mg/KgBW

### 3.4. Organ Histology of Rats Lung, Brain and Heart

Hypoxia in alveolar lung cells causes inflammation, which spreads throughout the body. The inflammatory process causes increased reactive oxygen species (ROS) and oxidative tissue damage. Inflammation is defined histopathologically by the infiltration of inflammatory cells (Smita et al., 2015). Inflammation draws plasma proteins and phagocytes to the injury site to isolate, destroy, or activate the incoming agent, clear debris, and prepare the tissue for healing (Corwin, 2008). Inflammation in all treatments is chronic inflammation caused by a lack of oxygen, leading to cell necrosis. One of the characteristics of cell necrosis is inflammation (Baratawidjaja, 2002).

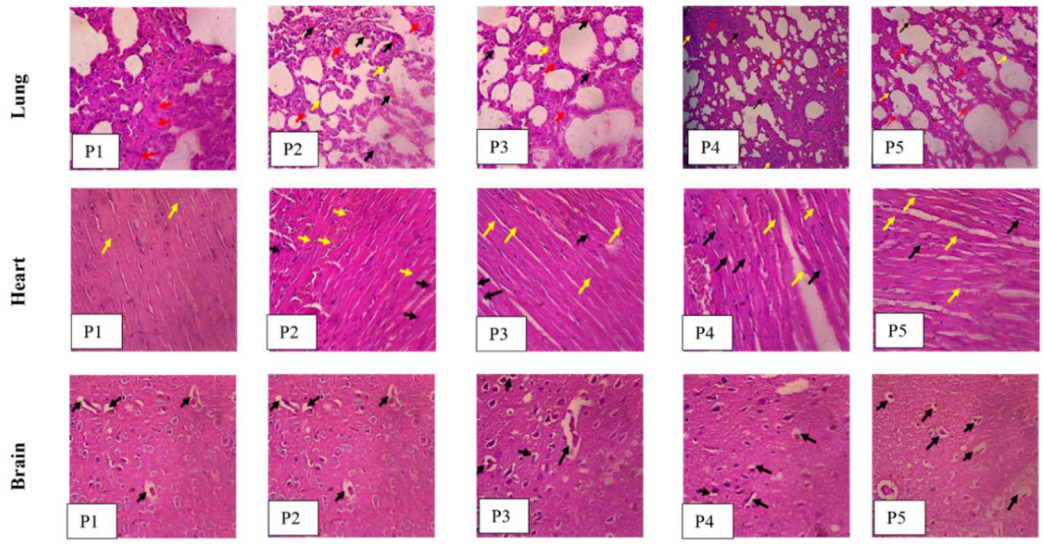


Figure 4. Ratio organ histology (from top to bottom: lung, heart, brain). P1: Normal; P2: Hypoxia without treatment; P3: Hypoxia + Dexamethasone; P4: Hypoxia + Purslane extract 150 mg/KgBW; P5: Hypoxia + Purslane extract 300 mg/KgBW

Hypoxia can cause cell necrosis and hypertrophy, which are visible in the heart cells. The results of microscopic observations (Figure 4) in all groups exposed to hypoxia showed necrosis that characterized by swollen cell membranes, pink cytoplasm, and thinner epithelium compared to the normoxia group. When the supply of oxygen decreases and causes reactive oxygen species (ROS) in the cell, it will decrease in the integrity of the cell wall so that there is swelling (hypertrophy), then the nucleus will shrink and can also be destroyed or disappear (Cut, 2016). Hypoxia can cause edema that led to the brain damage. Edema is a swelling produced by fluids and some cells that move from the bloodstream to the interstitial tissue (Muhsi et al., 2020). Membrane destabilization can interfere with ion transport activity within cells. This causes the damage of cell membranes to result in a leakage by the entry of extracellular fluid into the cytoplasm. The cytoplasm will increase the cell size, which causes metabolic activity to be inhibited and attempts to cause cell death (Harahap, 2017).

### 3.5. Quantitative Analysis of Necrosis, Hemorrhage and Inflammatory Infiltration of Rats Lung

Based on the One-Way Anova quantitative analysis of inflammatory cell infiltration in P2, a significant difference was obtained compared to the P1 ($P > 0.05$), implying that the tissue does not heal, causing cell necrosis to increase. Meanwhile, there was no significant difference between the P3 and P2 treatments. As a result, improvements in inflammatory cells can be seen in histopathology preparations. The same results were obtained when P2 was combined with P4 and P5. Because of its high antioxidant content, purslane plant extract (Portulaca oleracea L.) at 150 and 300 mg/KgBW can reduce the effects of free radicals caused by hypoxia (Limanan et al., 2019). While the hemorrhage data revealed that the P2 treatment with P4 and P5 produced significantly different outcomes, bleeding or haemorrhage is indicated by red blood cells outside the blood vessels, injury to the alveolar capillaries, arterioles, and venules causing a buildup of red blood cells in the alveolar space, resulting in lung bleed (Ichsantya et al., 2017).

Table 2. Necrosis, hemorrhage and inflammatory infiltration of lung histopathology in each treatment group

| Group | Necrosis | Hemorrhage | Inflammatory Infiltration |
|---|---|---|---|
| P1 (Normoxia) | 0,059 ± 0,011[a] | 4,486 ± 1,708[a] | 7,619 ± 2,509[a] |
| P2 (Hypoxia Without Treatment) | 0,773 ± 0,043[c] | 25,551 ± 2,989[c] | 12,362 ± 1,667[c] |
| P3 (Hypoxia + Dexamethasone) | 0,387 ± 0,021[b] | 10,638 ± 0,782[b] | 11,056 ± 1,486[b] |
| P4 (Hypoxia + Purslane Extract 150 mg/KgBW) | 0,423 ± 0,027[b] | 13,007 ± 3,335[b] | 11,136 ± 2,834[b] |
| P5 (Hypoxia + Purslane Extract 150 mg/KgBW) | 0,422 ± 0,016[b] | 10,672 ± 2,693[b] | 9,315 ± 1,910[ab] |

### 3.6. Quantitative Analysis of Necrosis, Hemorrhage and Edema of Rats Heart and Brain

Hypoxia The presence of free radicals causes the tissue's initial response to metabolic disorders that occur in an organ, in this case, an increase in ROS due to decreased oxygen levels in the cell. Only One Way Anova quantitative analysis of cell necrosis and hypertrophy revealed that cells in P2 differed significantly from that in P1. Tissue damage can progress to degenerated cells (reversible) and irreversible cell necrosis. Hypoxic conditions in cells that occur during cell degeneration can cause necrosis (Cut, 2016). Based on statistical analysis, edema occurs in P2 is significantly differences ($P > 0.05$) to P4 group. The edema seen in this study is suspected to be vasogenic edema. Vasogenic edema is caused by damage in the tight junction of the endothelium blood barrier brain due to physical disorders or the release of vasoactive compounds. As a result, the intravascular protein and fluid go out into the extracellular space (Ismy & Fahmi, 2020).

Table 3. Necrosis, hemorrhage and edema of heart and brain histopathology in each treatment group

| Group | Heart | | Brain |
|---|---|---|---|
| | Necrosis | Hemorrhage | Edema |
| P1 (Normoxia) | 68,80 ± 9,20[a] | 55,60 ± 10,21[a] | 48,40 ± 8,73[a] |
| P2 (Hypoxia Without Treatment) | 152,40 ± 4,88[b] | 111,40 ± 5,13[b] | 154,80 ± 4,97[b] |
| P3 (Hypoxia + Dexamethasone) | 101,60 ± 8,20[c] | 77,40 ± 5,03[cd] | 80,60 ± 4,04[d] |
| P4 (Hypoxia + Purslane Extract 150 mg/KgBW) | 133,60 ± 6,27[d] | 81,40 ± 2,88[c] | 75,00 ± 9,00[d] |
| P5 (Hypoxia + Purslane Extract 150 mg/KgBW) | 109,20 ± 6,42[c] | 70,00 ± 4,30[d] | 58,40 ± 7,54[c] |

## IV. Conclusion

Purslane (Portulaca oleracea L.) acetone extract has been shown in quantitative studies to recover lung, brain, and heart function in hypoxic rats, with the main advantages seen in treated groups with 300 mg/KgBW of purslane.

## V. Acknowledgment

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