

## Research Article



## Hepatoprotective Property of *Phyllanthus amarus* Alkaloid-Rich Fraction with Respect to Oxidative Stress Marker Enzymes

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### ABSTRACT

A disruption in the equilibrium between the generation of reactive oxygen species and antioxidant defense enzymes is referred to as oxidative stress. In the present study, we planned to identify the hepatoprotective effect of *Phyllanthus amarus* alkaloid rich fraction in wistar strain albino male rats. The hepatic damage was induced by the D-galactosamine and ameliorative effect was tested with alkaloid rich fraction of *P. amarus* by measuring oxidative stress markers such as G6PDH, LDH, SDH, MDH and GDH in the liver tissue. Activity levels of G6PDH, SDH, MDH and GDH were significantly decreased in D-galactosamine induced hepatitis rats when compare to normal control rat group, while their activities were significantly increased in hepatitis rat group that supplemented with alkaloid rich fraction of *P. amarus*. In contrast, LDH enzyme activity of liver was significantly increased in the hepatitis rat group when compare to normal control rats, while its activity was significantly decreased in hepatitis rats treated with alkaloid fraction. In conclusion, it is very clear that alkaloid fraction of *P. amarus* has hepatoprotective property with respect of decreasing oxidative stress by regulating oxidative stress marker enzymes. The isolation and identification of specific alkaloid compounds with hepatoprotective properties and anti-oxidative stress will require much further research.

**Keywords:** *Phyllanthus amarus*, oxidative stress, alkaloid rich fraction, hepatoprotective, medicinal plants.

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### INTRODUCTION

*Phyllanthus amarus* is medicinally important medicinal plant, belongs to the family *Euphorbiaceae*. It has been proved to treat many deadly diseases traditionally and pharmaceutically. In traditional herbal system, *P. amarus* has been used hugely in various regions around world to treat many adverse effects. In India, especially, this plant being used traditionally to treat anemia, appetizer, asthma, astringent, bronchitis, conjunctivitis, cough, diabetes, diarrhoea, diuretic, dropsy, dysentery, dyspepsia, eye disorders, fevers,, genitourinary disorders, gonorrhoea, hepatitis, itchness, jaundice, leucorrhoea, menorrhagia, oligogalactia, ringworm, scabies, skin ulcers, sores, stomachic, swelling, thirst, tuberculosis, tumor (abdomen), urogenital tract infections, and warts.<sup>1</sup> In addition, pharmaceutically this plant has been proved as anti-amnesic, antibacterial, anticancer, antifungal, anti-diarrhoeal, gastroprotective, antiulcer, analgesic, anti-inflammatory, anti-allodynic, anti-oedematogenic, antinociceptic, antioxidant, antiplasmodial and antiviral.<sup>1</sup>

Alkaloids are indeed organic molecules which mostly contain basic nitrogen atoms, those abundantly available in nature in the tissues of plants and others. This group also contains certain associated neutral molecules and weakly acidic constituents also present. Allo-securine, dihydrosecurinine, epibubbialine, isobubbialine, nor-securinine, phyllanthine,

Securinine, securinol and tetrahydrosecurinine alkaloids were isolated from the *P. amarus* through chromatographic methods.<sup>2,3</sup> In addition to that alkaloids Foo and Wong, were isolated 4-hydrosecurinine, 4-methoxy-nor-securinine, 4-methoxy dihydrosecurinine and 4-methoxytetrahydrosecurinine.<sup>4</sup> Hence, the present research paper design to separate alkaloids fraction from *P. amarus* and the same was tested for hepatoprotective activity with reference to oxidative stress enzymes.

G6PDH is a cytoplasmic enzyme present all organisms, a part in pentose phosphate pathway which produces NADPH (nicotinamide adenine dinucleotide phosphate) that is necessary for elimination of free radicals such as reactive oxygen species produced in cell.<sup>5</sup> This NADPH helps to protect the cells from oxidative stress caused by substances like H<sub>2</sub>O<sub>2</sub> by maintaining the level of glutathione in these cells and also maintains glucose through pentose phosphate pathway.<sup>6</sup> LDH is abbreviated as lactate dehydrogenase enzyme present in virtually most living tissue/cells. Generally, a dehydrogenase is a molecule-to-molecule hydride exchange enzyme. It is a biomarker of severe ailments and diseases since it is



produced after tissue injury.<sup>7</sup> LDH mediates the rearrangement of pyruvate and lactate, as well as NAD<sup>+</sup> and NADH, at the same time. Whenever O<sub>2</sub> is missing or in scarcity, it transforms pyruvate, the end result of glycolysis, to lactate, and it reverses the process during the Cori cycle in the hepatic tissue.<sup>8</sup> Succinate dehydrogenase, also known as respiratory complex II, is an enzyme complex present in the inner mitochondrial membrane of eukaryotic and several bacterial cells. In the TCA cycle, this enzyme transforms succinate to fumarate catalytically, and in the electron transport chain, it converts ubiquinone to ubiquinol.<sup>9</sup> SDH receives electrons and transfers them through its four subunits, SDH-A, SDH-B, SDH-C, and SDH-D, before continuing the electron transfer to electron transport chain's complex II. Further, the electrons of FADH<sub>2</sub> following SDH catalysis and ubiquinone are subsequently transported to complex III of electron transport chain, where they are used to continue the synthesis of the cell's energy moiety, adenosine triphosphate.<sup>10</sup> The MDH2 gene encodes malate dehydrogenase (MDH), which is found in mitochondria and is also known as malate dehydrogenase 2 in humans. Malate dehydrogenase uses the NAD/NADH cofactor complex in the TCA cycle of carbohydrate metabolism to catalyze the bidirectional oxidation of malate to oxaloacetate. MDH is indeed active in gluconeogenesis, or the conversion of simpler compounds into glucose.<sup>11</sup> Glutamate dehydrogenase (GDH) enzyme present in the mitochondrial matrix made up of six identical monomers that form hexameric forms. This enzyme catalyzes the reversible oxidative deamination of glutamate to alpha-ketoglutarate with the release of free ammonia as a by-product. This enzyme uses either NAD or NADP as a cofactor to complete this reaction.<sup>12</sup>

## MATERIAL AND METHODS

### Collection of plant material

*P. amarus* plant was collected in Tirupati, Andhra Pradesh, India in the month of August and September 2017. Botany taxonomist Dr. Madhava Chetty, Department of Botany, S. V. University has identified and authenticated it. The Department of Botany, S. V. University, Tirupati has held a voucher specimen No.2151 in the herbarium lab. Further, the plant material was washed with water and rinsed with sterile distilled water. It was subjected to shade dry after washing and powdered with a mechanical grinder. This material was further used for extraction of alkaloid rich fraction.

### Preparation of alkaloids rich fraction from *P. amarus*

Alkaloids rich fraction was prepared by the method of general acid–base extraction as described by Houghton and Raman, 1998<sup>13</sup>. Briefly, the dried plant powder of *P. amarus* soaked in methanol for 24 h with occasional stirring. After soaking, the mixture was filtered using Whatman No. 1 filter paper. The filtrate collected had been concentrated under reduced pressure using rotary evaporator. The concentrated crude extract has been

combined with 1 M HCl and purified for any precipitate removal. 2N NaOH was gradually applied to this filtrate to produce an off-white precipitate. Now the precipitate was divided into equal quantities of water and dichloromethane (DCM) layers. Separated, evaporated and measured the DCM layer contained alkaloids. Dragendorff's reagent (5 % bismuth nitrate, 4 % acetic acid, and 2 % potassium iodide) tested the separated alkaloids from crude. The rich alkaloid fraction yield was approximately 5 % (w/w).

### Animals and maintenance:

Indian Institute of Science (IISc), Bangalore provided male Wistar rats weighing 180± 200 g. The animals were kept under standard laboratory conditions by maintaining temperature 27 ± 2°C, natural light dark cycle such as photoperiod of 12 h light and 12 h, and humidity about 55–60%. The rats were maintained by providing regular pellet diet that was supplied by M / s Hindustan Lever Ltd., Mumbai and water ad libitum. Animal ethical committee approved the design of this study (Resolution No.10/08/a/CPCSCA/IAEC/SVU/09-10/ZOOL/KRS/Dt.25.09.2009). Animals were divided into five groups of six rats each, after 7 days of acclimatization.

### Grouping of animals:

**Group I- Normal Control (NC):** Rats received saline for 21 days and served as normal control.

**Group II- Alkaloid rich Fraction control (AF):** Rats were given alkaloid rich fraction of *P. amarus* (100mg/kg b/w) for 21 days.

**Group III- Hepatitis Control (H):** A single injection of D-galactosamine hydrochloride (800mg/kg b/w) was given intraperitoneally for the induction of hepatitis 48 hours before sacrifice.

**Group IV- Hepatitis + Alkaloid rich Fraction treatment (H+AF):** Pre-treatment of alkaloid rich fraction of *P. amarus* (100mg/kg b/w) orally for 21 days and a single injection D-galactosamine hydrochloride (800mg/kg b/w) 48 hours before sacrifice.

**Group V- Hepatitis + Silymarin treatment (H+S):** Pre-treatment of standard drug silymarin (100 mg/kg b/w) orally for 21 days and a single injection D-galactosamine hydrochloride (800mg/kg b/w) 48 hours before sacrifice.

### Glucose-6-Phosphate dehydrogenase (G-6-PDH)

The behavior of glucose-6-phosphate dehydrogenase was assessed using the Mastanaiah et. al. (1978) method.<sup>17</sup> 10% (W / V) of homogeneous tissue was prepared in ice cold sucrose solution (0.25 M), centrifuged at 1000 g at 4°C for 15 minutes, and supernatant used as an enzyme source. The 2 ml reaction mix comprises 2 μ moles of INT, 100 μ moles of sodium phosphate buffer (pH 7.4), 0.3 μ mole of NADP and 20 μ moles of glucose-6-phosphate. The reaction was started by adding 0.5 ml of homogenous tissue containing 50 mg as an enzyme extract. The incubation was done at 37°C for 30 minutes, and the



reaction was then halted by adding 5 ml of glacial acetic acid. The developed formazan was extracted into toluene in 5 ml at 5°C. The color intensity was read at 495 nm versus the blank toluene. The G-6-PDH activity was represented as  $\mu$  moles of formazan formed/mg protein/hour.

#### Lactate dehydrogenase (LDH)

The activity of Lactate Dehydrogenase was determined by the Nachlas et. al., (1960),<sup>14</sup> and with minor modifications as suggested by Prameelamma and Swami (1975).<sup>15</sup> 10% (W / V) homogeneous liver tissue was prepared in an ice cold solution of 0.25 M sucrose, centrifuged for 15 minutes at 40C at 1000 g and supernatant used as enzyme source. The final 2 ml reaction mixture contained 40  $\mu$  moles of sodium lactate, 100  $\mu$  moles of phosphate buffer (pH 7.4), 0.1  $\mu$  mole of NAD and 4  $\mu$  moles of INT. The reaction was started by adding 0.2 ml of homogeneous 20 mg tissue as an enzyme resource and incubated at 37°C for 30 minutes, and the reaction was halted by adding 5 ml of glacial acetic acid. Zero time controls were retained by adding 5 ml of glacial acetic acid to the incubation solution led to the advent of the source enzyme. At 50C, the formazan developed was extracted into 5 ml of toluene overnight. The color formed was analyzed against the blank toluene at 495 nm in a Spectrophotometer. The activity of the enzyme was represented in  $\mu$  moles of formazan formed / mg protein / hour.

#### Succinate dehydrogenase (SDH)

The activity of SDH was determined by the Nachlas et. al., (1960),<sup>14</sup> and with minor modifications as suggested by Prameelamma and Swami (1975).<sup>15</sup> 10% (W / V) homogeneous liver tissue was prepared in an ice cold solution of 0.25 M sucrose, centrifuged at 1000 g at 40C for 15 minutes and obtained supernatant used as enzyme resource. The 2 ml reaction mix comprises 4  $\mu$  moles of INT, 100  $\mu$  moles of phosphate buffer (pH 7.0) and 40  $\mu$  moles of sodium succinate. The incubation was done at 37C for 15 minutes, and the reaction was then halted by adding 5 ml of glacial acetic acid. The following stages were followed same as designated for LDH. The SDH activity was represented in  $\mu$  moles of formazan formed / mg protein / hour.

#### Malate dehydrogenase (MDH)

The activity of MDH was determined by the Nachlas et. al., (1960),<sup>14</sup> and with minor modifications as suggested by Prameelamma and Swami (1975).<sup>15</sup> 10% (W / V) homogeneous liver tissue was prepared in an ice cold solution of 0.25 M sucrose, centrifuged at 1000 g at 40C for 15 minutes and obtained supernatant used as enzyme resource. The 2 ml reaction mix comprises 4  $\mu$  moles of INT, 100  $\mu$  moles of phosphate buffer (pH 7.0), 0.1  $\mu$  mole of NAD and 40  $\mu$  moles of sodium malate. The reaction was started by adding 0.2 ml of homogenous tissue containing 20 mg as an enzyme extract. The incubation was done at 37C for 15 minutes, and the reaction was then halted by adding 5 ml of glacial acetic acid. The following stages were

followed same as designated for LDH. The MDH activity was represented in  $\mu$  moles of formazan formed / mg protein / hour.

#### Glutamate dehydrogenase (GDH)

Activity of glutamate dehydrogenase (GDH) was assessed using the Lee and Lardy (1965) procedure.<sup>16</sup> 5% (W / V) of homogeneous tissue was prepared in ice cold sucrose solution (0.25 M), centrifuged at 1000 g at 40C for 15 minutes, and supernatant used as an enzyme source. The 2 ml reaction mix comprises 2  $\mu$  moles of INT, 100  $\mu$  moles of phosphate buffer (pH 7.0), 0.1  $\mu$  mole of NAD and 40  $\mu$  moles of sodium glutamate. The reaction was started by adding 0.2 ml of homogenous tissue containing 10 mg as an enzyme extract. The incubation was done at 37C for 15 minutes, and the reaction was then halted by adding 5 ml of glacial acetic acid. The developed formazan was extracted into toluene in 5 ml. The color intensity was read at 495 nm versus the blank toluene. The GDH activity was represented as  $\mu$  moles of formazan formed/mg protein/hour.

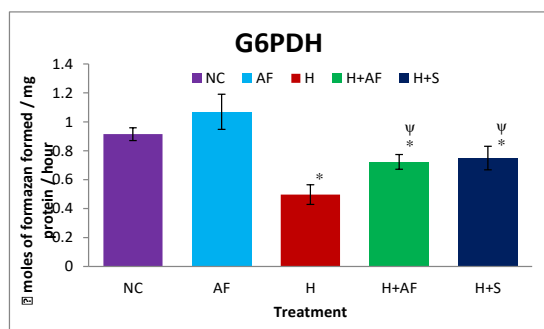
### RESULTS AND DISCUSSION

In the present study, the activity levels of G6PDH in alkaloid fraction treated hepatitis animals were recovered as compared to normal control rats. The G6PDH of the hepatitis control rat group was considerably lower than that of the other groups studied. Normal control rats given the alkaloid fraction, on the other hand, showed very minor increasing changes in G6PDH when compared to the normal control group. In hepatitis rats, silymerin, a widely available plant-derived medicine, was shown to be somewhat greater efficient than the alkaloid fraction in increasing G6PDH activity (Figure 1)

D-galactosamine is a hepatotoxin which induces the hepatic tissue damage by producing oxidative stress such as reactive oxygen species and reactive nitrogen species. In the oxidative stress, reactive oxygen species like superoxide radicals are generated through mitochondria which in turn converted into the hydrogen peroxide by the enzyme superoxide dismutase.<sup>18</sup> Further, these superoxide radicals converted into most dangerous hydroxyl radicals. D-galactosamine, on the other hand, causes liver damage in rats, which is linked to induction of lower levels of G6PDH by the hepatotoxin in rats. Due to lower activity of G6PDH, increased levels of oxidative stress were observed in hepatic tissue.<sup>19</sup> Alternatively, the functionality of G6PDH was reduced in the hepatic tissue of the current study's hepatitis rats. This implies that reduced conversion of glucose-6-phosphate to 6-phosphogluconate causes NADPH production in the HMP shunt to be diminished. Intern, the glycolytic and krebs cycle paths are harmed by this process as an alternative source of energy. When oxidoreductases in the mitochondria are inhibited, the energy supply for regular metabolic processes is reduced. The decreased amount of NADP might potentially be a factor in Dgalactosamine's inhibition of G6PDH.<sup>20</sup> Many research studies found that a toxin that induces the liver



toxicity causes the decline of G6PDH.<sup>21</sup> Similarity, in the present study, D-galactosamine also induced the hepatic damage and reduction levels of G6PDH.



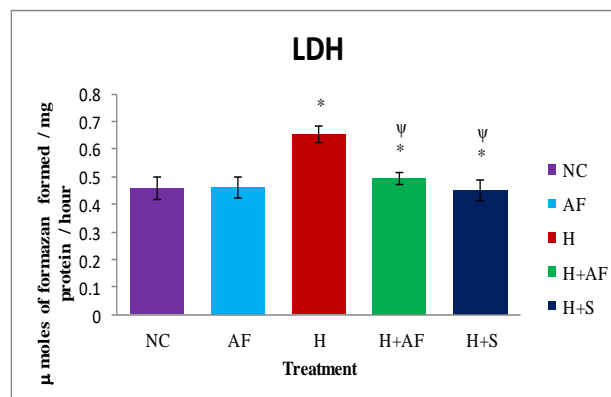
**Figure 1:** Effect of Alkaloid rich fraction on Glucose-6-Phosphate Dehydrogenase (G-6-PDH) activity in the hepatic tissue of male albino rats.

A research found that an alkaloid-rich extract of *Litsea glutinosa* bark enhanced the activity of G6PDH in diabetic mice's liver.<sup>22</sup> The restoration of decreased GSH is the primary function of NADPH generated by G6PDH within liver. This protects liver tissue by detoxifying hydroxyl radicals, and hydrogen peroxide which are oxygen radicals generated in the liver.<sup>23</sup> Likewise, in the current study, an alkaloid-rich fraction and silymerin enhanced the activity of G6PDH in the hepatitis liver. At the same time, alkaloids from plant species also showed free radical scavenging activity.<sup>24</sup> On whole G6PDH reduction resulting free radicals were might also scavenged by the alkaloids of *P.amarus*, in the present study. Hence it is hypothesized that alkaloid rich fraction increased the G6PDH activity in the liver tissue by scavenging free radicals such as hydrogen peroxide and hydroxyl radicals.

LDH levels in the hepatitis group were considerably higher than NC, but LDH levels in alkaloid-rich fraction treated hepatitis rats were substantially lower than Hepatitis (Figure 2). Treatment of hepatitis rats with alkaloid-rich fraction resulted in a larger decrease in LDH activity compared to hepatitis groups, as well as a substantial decrease compared to standard medication Silymarin treatment. There were no significant differences in the AF group as compared to the NC group.

The livers plays a significant role in regulating the metabolism of carbohydrates by keeping glucose levels within a normal range and express a series of enzymes that are turned on alternatively or off probably depends on if the blood sugar levels take up or down. Especially, glycolysis of glucose in liver cells takes place in the cytosol under aerobic and anaerobic conditions.<sup>25</sup> In muscle and liver, during aerobic respiration, glucose in the cytosol is transformed to pyruvate, which then enters mitochondria for high energy generation, but during anaerobic respiration, pyruvate in the cytosol is transformed to lactic acid by the enzyme LDH, which then transforms NADH to NAD.<sup>26</sup> Again, to improve aerobic respiration, lactic acid is formed and enters the liver, where it is converted to

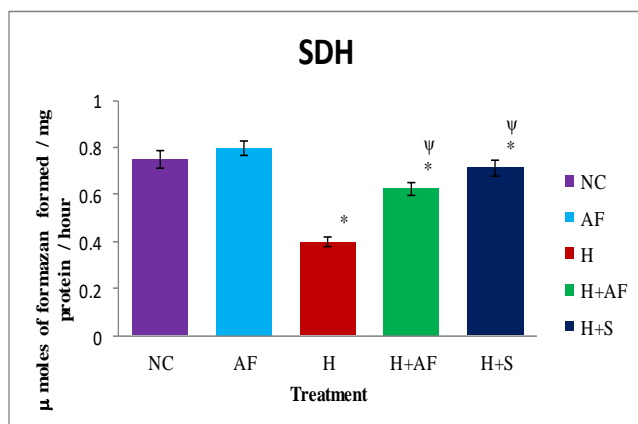
glucose through a process known as the cori cycle by LDH. Hence liver is a main organ for the regeneration of glucose under hypoxia.<sup>27</sup> Furthermore, liver failure has been linked to an increase in macrophage content, which causes endothelial cell destruction in sinusoids either direct or indirect through the production of cytokines. Hypoxia liver injury, commonly known as hepatitis, is a condition in which the liver's normal function is disrupted and the level of hypoxia-sensitive enzymes such as LDH rises.<sup>28</sup> Another reason is that hypoxia inducible factor-1 is a gene found in the liver that causes increased hypoxia in liver disorders such as cirrhosis, tumors, and fibrosis, and others.<sup>29</sup>



**Figure 2:** Effect of Alkaloid rich fraction of *P. amarus* on Lactate Dehydrogenase (LDH) activity in the hepatic tissue of male albino rats.

When compared to the normal control rats, the D-Galactosamine induced hepatitis rat group had higher levels of LDH in this research. Our findings are consistent with a recent study that after two injections of D-Galactosamine, LDH levels in liver fibrosis rats increased dramatically as compared to normal rats.<sup>30</sup> In a similar manner, CCL4-induced hepatic damage in Kunming mice produced the same effects, such as an increase in LDH levels.<sup>31</sup> In this research work, D-galactosamine-induced hepatitis rats treated with an alkaloid fraction obtained from the *p.amarus*, on the other hand, had lower LDH activity levels in rats when compare to hepatitis group. Similar results were reported in previous study that, *Acanthus ilicifolius* plant contained alkaloid *Acanthus ilicifolius* alkaloid A synthesized and tested for hepatoprotective activity in CCl4 induced hepatic damage in rats. Where they found that a decreased level of LDH was observed in alkaloid treated rats, an indication that alkaloid protected the liver cell from the hepatotoxin.<sup>31</sup> Our results correspond those of another study, which found that an alkaloid fraction extracted from *Hygrophila auriculata* leaves protected liver tissue against CCL4 toxicity by lowering LDH levels at various doses.<sup>32</sup> Based on the findings and their agreement with prior research, it is clear that the alkaloid-rich fraction of *P. amarus* is an excellent source of hepatoprotective alkaloids. Furthermore, it is believed that *P. amarus* alkaloids protected liver tissue by lowering hypoxia levels and reducing the expression of the hypoxia inducible factor-1 gene and LDH in the liver.

SDH levels in the hepatitis group were considerably lower than NC, but SDH levels in alkaloid-rich fraction treated hepatitis rats were substantially higher than Hepatitis (Figure 3). Treatment of hepatitis rats with alkaloid-rich fraction resulted in a larger increase in SDH activity compared to hepatitis groups, as well as a little bit decrease compared to standard medication Silymarin treatment. There were no significant differences in the AF group as compared to the NC group.



**Figure 3:** Effect of Alkaloid rich fraction of *P. amarus* on Succinate dehydrogenase (SDH) activity in the hepatic tissue of male albino rats

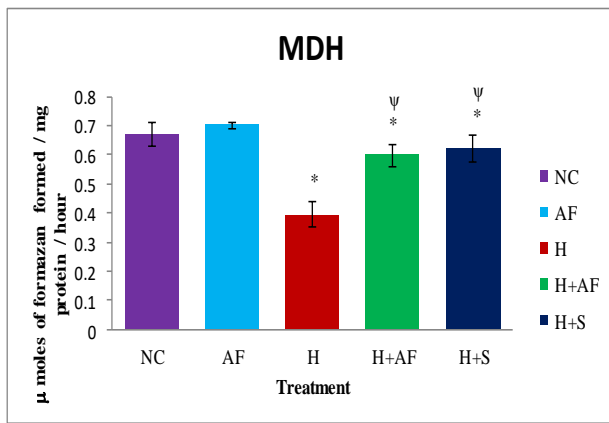
Studies on the worm *Caenorhabditis elegans* supported the theory that perhaps the SDH performs a particular role in mitochondrial oxygen management. An oxygen-hypersensitive worm was found to have a particular SDH mutation instead of a complete class of mitochondrial respiratory mutants.<sup>33</sup> Mutations in any of the SDH genes cause the complex II to breakdown completely in humans. Complex II can thus be ignored as a cause of further superoxide generation because it is not present. As a result, scientists believe that superoxide excessive production, which has been linked to carcinogenesis in humans and hypersensitivity to oxygen in mutant worms, is due to the SDH's inability to appropriately diminish the UQ pool, which is required to withstand oxidative stress.<sup>34</sup> The deficiency of SDH activity would not only lead to lower ATP production—a situation that may also be caused by a severe Complex I or Complex IV defect—but it will also deprive the Reaction Center of the only dehydrogenase activity capable of keeping the Ubiquitin pool at a high reduction status. This might lead to a decrease of the respiratory chain's anti-oxidant ability, as well as oxidative stress.<sup>34</sup> On other hand, Succinate, a tricarboxylic acid cycle intermediary involved in mitochondrial ATP production, has been identified as a key signaling molecule in a number of diseases. When succinate concentrations are high, the major ROS generating site in Complex II, FAD in SDHA, is unable to produce ROS. The mechanism of succinate-mediated suppression of ROS generation at FAD is that succinate may prevent FAD from receiving oxygen.<sup>35</sup> The stimulation of human GPR91, which really is accountable for liver damage, is induced by succinate. Furthermore, succinate is a substrate for the SDH enzyme,

which is diminished in many liver disorders, resulting in an increase in ROS, which leads to liver damage.<sup>36</sup> Similarly, decreased SDH levels were observed in the liver cancer.<sup>37</sup> In the present study, D-galactosamine induced hepatitis rats exhibited decreased levels.

In contrast, plant compounds such as phenols, flavonoids and alkaloids are pharmaceutically active in reducing liver diseases by inducing increase levels of SDH. SDH levels were found to be higher in the alkaloid-rich fraction obtained from *Phyllanthus amarus* treated hepatitis rats in the current investigation. Our results were correlated with a study that the hydroalcoholic extract of *Chrysanthemum balsamita*, *Calendula officinalis* herbs were increased the SDH activity in the CCl<sub>4</sub> induced hepatotoxicity in the albino rats. These herbs were contained rich amount of alkaloids.<sup>38</sup> Along with, the similar results were observed in another study that a single dosage of 120 mg/kg of monocrotaline, a pyrrolizidine alkaloid, induced the increased levels of SDH in rats after 12 days.<sup>39</sup> Hence, it is hypothesize that alkaloid fraction of this study protected the liver tissue from the D-galactosamine induce hepatotoxicity by inducing SDH, decreasing oxidative stress.

MDH levels in the hepatitis group were considerably lower than NC, but MDH levels in alkaloid-rich fraction treated hepatitis rats were substantially higher than Hepatitis (Figure 4). Treatment of hepatitis rats with alkaloid-rich fraction resulted in a larger increase in MDH activity compared to hepatitis groups, as well as a somewhat lower when compared to standard medication Silymarin treatment. There were slight differences in the AF group was observed as compared to the NC group.

It has been reported that mitochondrial destruction is an initial and distinctive hallmark of alcohol liver diseases, and that it plays a role in liver damage etiology. Distended mitochondria with disoriented cristae have been seen in electron microscopy investigations, and experimental animal research have indicated that giving high amounts of ethanol to rats reduces the functional capacities of hepatic mitochondria by a modest but substantial amount.<sup>40</sup> Decrease levels of hepatic enzymes, notably the mitochondrial enzyme MDH, are commonly observed in alcoholics, even when steatosis is the sole histopathological abnormalities and this has supported the idea of mitochondrial damage.<sup>41</sup> It was well understood that higher amounts of free radicals cause mitochondrial damage. Reduced levels of mitochondrial enzymes have been linked to mitochondrial damage in liver disorders. In the same manner, decreased activity levels of MDH have been found in D-galactosamine induced hepatitis rats because of D-galactosamine induces the liver damage by increasing free radicals.<sup>42</sup> In the current investigation, reduced levels of MDH were detected in the D-galactosamine induced hepatitis rat group, which was consistent with previous findings.



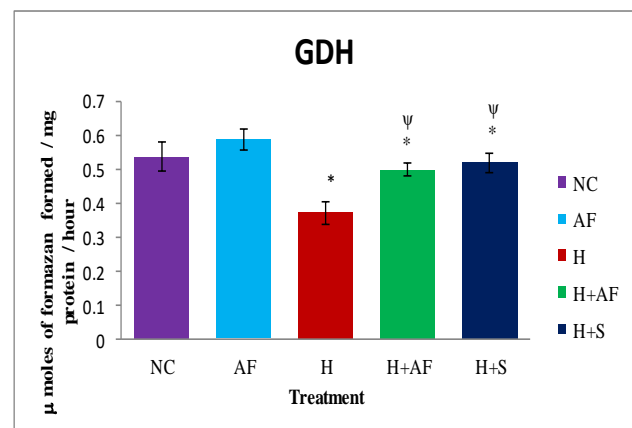
**Figure 4:** Effect of Alkaloid rich fraction of *P. amarum* on Malate dehydrogenase (MDH) activity in the hepatic tissue of male albino rats

Phytochemical secondary metabolites are abundant in medicinal plants. Phenols, flavonoids, and alkaloids are among the most commonly used medicinal formulations. Several investigations have discovered that plant alkaloids and alkaloid fractions protect liver tissue against chemically induced liver damage. The alkaloid fractions isolated from *Murraya koenigii* leaves ethanol extract protected liver tissue infected with chemicals like paracetamol and CCl<sub>4</sub>.<sup>43</sup> In another study, *Sarcococca saligna* plant alkaloids such as alkaloid-C, holaphylline and sarcovagine-D were reported to exhibit hepatoprotective properties in CCl<sub>4</sub>-induced hepatotoxicity.<sup>44</sup> The alkaloid fraction isolated from *p. amarum* also protected the hepatic tissue of male albino rats infected with D-galactosamine hepatitis in the current study. Furthermore, plant alkaloids have a number of significant pharmacological characteristics, one of which is free radical scavenging or antioxidant activity. In a study, fangchinoline and Cepharanthine from the *Stephania rotunda* plant scavenge free radicals such as DPPH, ABTS, and superoxide radicals *in vitro* antioxidant assays.<sup>45</sup> Hence, it is very clear that alkaloid fraction of this study also might be scavenges the free radicals in hepatitis rats. At the same time alkaloid fraction protected liver tissue under the hepatitis by increasing the MDH levels in this study. Furthermore, silymarin also exhibited highest hepatoprotective property by increasing MDH levels. Hence, alkaloid fraction from *Phyllanthus amarum* is protected liver tissue by preventing free radicals, mitochondria damage and increasing MDH.

GDH levels in the hepatitis group were significantly lower than NC, but GDH levels in alkaloid-rich fraction treated hepatitis rats were substantially higher than Hepatitis (Figure 5). Treatment of hepatitis rats with alkaloid-rich fraction resulted in a larger increase in GDH activity compared to hepatitis groups, as well as a somewhat lower when compared to standard medication Silymarin treatment. There were slight differences in the AF group was observed as compared to the NC group.

The current study indicates that in hepatitis rats, GDH activity was significantly reduced. The reduction in GDH

function is due to the enzyme's inactivation by higher ammonia levels (product-inhibitor), which reduces the enzyme's kinetic effectiveness.<sup>46</sup> Drug injuries, ischemia injuries, metabolic illness, toxins and viral infections, are some of the causes of hepatic necrosis. Liver necrosis induced the damage of mitochondria which induce the release of mitochondrial enzymes. In the present study, increased mitochondrial membrane fluidity and subsequent mitochondrial enlargement may affect the activity of the mitochondrial enzyme GDH, which in turn may affect the state of mitochondrial NAD depletion in hepatitis. The reduction in GDH activity implies that during the hepatitis condition, the mechanisms of deamination and amination that regulate ammonia toxicity in the kidney are also disrupted.<sup>47</sup> In GDH knockout mice, however, a lack of GDH in liver leads to greater in circulatory ammonia and a reduction in the detoxification mechanism into urea, altering ammonia metabolism. The same study discovered that a lack of GDH in the liver causes metabolic changes such as changes in gluconeogenesis, the use of lipids instead of carbohydrates for energy generation, and an influence on food intake circadian rhythms.<sup>48</sup> Hence it is very supportive to the present investigation that decreased levels of GDH in hepatitis rats may induces the all the above said alterations in rats body.



**Figure 5:** Effect of Alkaloid rich fraction of *P. amarum* on Glutamate dehydrogenase (GDH) activity in the hepatic tissue of male albino rats.

Alterations in GDH activity is caused by ethanol exposure to progressive mitochondrial modification and degradation. The reduction in GDH activity is due to the enzyme's inactivation by higher ammonia levels, which reduces the enzyme's functional effectiveness. Besides, high ammonia and lactate levels, according to another study, inhibit GDH function.<sup>46</sup> The elevated LDH seen in this study is consistent with the fact that lactate limits GDH action. Means hepatitis rats exhibited elevated LDH which intern increase lactate, such lactate inhibits the GDH.

Many medicinal plants have been studied *in vitro* and *in vivo* for their hepatoprotective efficacy against CCl<sub>4</sub>, d-galactosamine, paracetamol-induced liver damage, and viral-infected hepatitis. These plants have been prevented

hepatic damage by inhibiting oxidative stress caused by chemicals and virus. plants with antioxidant property is major agent in reducing oxidative stress whereas this property is associated with phenols, flavonoids, terpenoids, alkaloids and may other plant secondary metabolites.<sup>49</sup> Plant extracts, separated fractions and isolated compounds protects the liver damage from chemicals and others. In this connection, plants having liver protection by increasing MDH also studied. For example, *phyllanthus niruri*, *Croton hypoleucus*, *Vicia calcarata Desf* have been protected liver damage by reducing oxidative stress and increasing MDH in animal models.<sup>50</sup> Similarly, alkaloid rich fraction isolated from *P. amarus* and silymerin, in this study, were also protected the liver tissue from oxidative stress by increasing MDH levels. Hence, these fraction and compounds are suggestive agents to treat hepatitis associated with oxidative stress.

### CONCLUSION

The findings of this study reveal that the alkaloid fraction of *P. amarus* is a rich source of oxidative stress relievers which enhance oxidative stress marker enzymes in hepatic tissue under hepatitis conditions. In future, the mechanism of action of the alkaloid fraction must be determined, as well as the isolation of individual alkaloid compounds is necessary to be done.

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