



HEPATOPROTECTIVE ACTIVITY OF ALKALOID RICH FRACTION SEPARATED FROM *PHYLLANTHUS AMARUS* WITH REFERENCE TO ENDOGENOUS ANTIOXIDANT ENZYMES

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ABSTRACT

Phyllanthus amarus has long been used as a medicinal herb. This plant has been used to cure a variety of severe ailments traditionally, and it has been scientifically proven to be safe and effective against a variety of ailments. The aim of this research was to see whether the separated alkaloid rich fraction from *P. amarus* has any hepatoprotective property. We successfully isolated the alkaloid fraction from this plant and tested it on male albino rats suffering with D-galactosamine induced hepatitis. We looked at endogenous antioxidant enzymes in the liver tissue to see whether alkaloid fraction has hepatoprotective activity. The results showed that antioxidant enzymes such SOD, CAT, GR, GPx, and GSH levels were considerably higher in the alkaloid rich fraction that treated to hepatitis group, but they were lower in the hepatitis alone rat group. In addition, in the hepatitis group, however, GST enzyme activity and lipid peroxidation in the form of MDA were significantly higher, but these values were also considerably decreased in the alkaloid rich fraction treatment of hepatitis rats. In conclusion, the alkaloid-rich fraction separated from *P. amarus* displays hepatoprotective effects in the liver via boosting endogenous antioxidant enzymes. In future, the mechanism of action of the alkaloid fraction of *P. amarus* must be determined, as well as the isolation of individual alkaloid compounds.

KEYWORDS: *Phyllanthus amarus*, Antioxidant enzymes, hepatoprotective, alkaloid-rich fraction, D-galactosamine

INTRODUCTION

Free radicals defined as “molecules or molecular fragments having one or more unpaired electrons in its outer orbitals”. Due to uncharged and short-lived molecules, they are very reactive in nature (Halliwell and Gutteridge, 1999). These reactive species are generated in the body during ATP generation through mitochondria and many other sites. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated in the cell due to the cellular redox process. These radicals attack the adjacent biomolecules such as carbohydrates, lipids, proteins and nucleic acids (Sisein, 2014). ROS are $\cdot O_2^-$ (superoxide anion), $\cdot OH$ (hydroxyl radical), $RO\cdot$ (alkoxyl radical), and $ROO\cdot$ (peroxyl radical), singlet oxygen (1O_2), hydrogen

peroxide (H_2O_2) and, Hypochlorous acid (HClO) while, RNS include $NO\cdot$ (nitric oxide), $NO_2\cdot$ (nitric dioxide), and $OONO^-$ (peroxynitrite) (Simon *et al.*, 2000).

An antioxidant is a molecule, donate an electron to a free radical and nullify its attack on biomolecule. Due to having free radical scavenging activity, these antioxidants inhibit the cellular damage (Halliwell, 1995; Ravi *et al.*, 2017). Our body build antioxidant mechanism in the form of enzymatic those include superoxide dismutase (SOD), Catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx). These enzymes scavenge the free radicals in the body and protect the tissue from damage. At the same time there were other antioxidants such as glutathione, Thioredoxin and glutaredoxin in the body to replenish active form of antioxidant enzyme or maintained reduced state (Brewer, 2011). In chronic disease condition, there is imbalance in free radicals and internal antioxidants, in such cases external supplementation of antioxidants should be given. These external supplementations of antioxidants are two types such as synthetic and natural. Synthetic antioxidants include BHT, BHA, Pyrogallate are very potential at very low concentration. But they are showing many adverse effects like cancer, intestinal problems etc. hence, now a day's people are aware about these side effects so, they looking forward to use natural antioxidants that did not exhibit side effects (Anbudhasan *et al.*, 2014). Natural antioxidants present in plants, food, marine sources etc. among all these, majorly phenols, flavonoids, terpenoids, Saponins, and tannins are act as potential antioxidants (Dong-Ping *et al.*, 2017).

P. 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, itches, jaundice, leucorrhoea, menorrhagia, oligogalactia, ringworm, scabies, skin ulcers, sores, stomachic, swelling, thirst, tuberculosis, tumor (abdomen), urogenital tract infections, and warts (Patel *et al.*, 2011). In addition, pharmaceutically this plant has been proved as anti-amnesic, antibacterial, anticancer, antifungal, anti-diarrhoeal, gastroprotective, anti-ulcer, analgesic, anti-inflammatory, anti-allodynic, anti-oedematogenic, antinociceptive, antioxidant, antiplasmodial and antiviral (Patel *et al.*, 2011).

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-securinine, dihydrosecurinine, epibubbialine, isobubbialine, nor-securinine, phyllanthine, Securinine, securinol and tetrahydrosecurinine alkaloids were isolated from the *P. amarus* through chromatographic methods (Houghton *et al.*, 1996; Kassuya *et al.*, 2006). In addition to that alkaloids Foo and Wong, (1992) were isolated 4-hydrosecurinine, 4-methoxy-nor-securinine, 4-methoxy dihydrosecurinine and 4-methoxytetrahydrosecurinine. Hence, the present research paper design to separate alkaloids fraction from *P. amarus* and the same was tested for hepatoprotective activity with reference to antioxidant enzymes.

MATERIAL AND METHODS AND CHEMICALS

D-Galactosamine was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and all other chemicals obtained from Fisher Scientific (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India) and Qualigens (Mumbai, India).

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. 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 was provided male Wistar rats weighing 180 ± 200 g. The animals were kept under standard laboratory conditions by maintaining temperature $27 \pm 2^\circ\text{C}$, 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. Animals were divided into five groups of six rats each, after 7 days of acclimatization.

Group 1- Normal Control (NC): Rats were given saline for 21 days and were used as a control group.

Group 2- Alkaloid rich Fraction treatment (Aft): For 21 days, rats were administered an alkaloid-rich fraction of *P. amarus* (100mg/kg b/w).

Group III- Hepatitis treatment (Ht): 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 (Ht+Aft): Pre-treatment of alkaloid rich fraction (AF) 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 (Ht+SYt): 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.

All of the rats were killed and blood samples were taken after 21 days of therapy. The serum was separated and stored at -80 degrees Celsius for further examination. The liver tissue was taken immediately and stored in formalin for histological analysis.

Antioxidant enzymes

Superoxide dismutase

Activity levels of superoxide dismutase (SOD) in the liver tissue were evaluated using the method defined by Misra and Fridovich, (1972). In a chilled 50 mM phosphate buffer (pH 7.0), containing 0.1 mM EDTA, the liver tissue was homogenized to produce 5 % homogenate (weight / volume). Further, it was subjected to high-speed cooling centrifugation at 10,000 rpm for 10 min at -4°C . The obtained supernatant component was collected and used as follows for enzyme assay: 0.2 ml of the tissue supernatant was transferred to the carbonate buffer 1.76 ml (0.05 M, pH 10.2, comprising 0.1 mM EDTA) and then 40 μl of 30 mM epinephrine dissolved in 0.05 percent acetic acid was added into the mixture. The final resultant was analysed in Shimadzu UV-1800 Spectrophotometer for assessing the OD values at 480 nm for 4 min. Activity has been defined as the total amount of enzyme which inhibits the oxidation of epinephrine by 50 %, that is equivalent to total 1 unit.

Catalase

Activity of catalase (CAT) in experimental groups was measured using the Aebi, (1984) procedure, with minor modifications. With a cooled 50 mM phosphate buffer (pH 7.0), comprising 0.1 mM EDTA, the liver tissue was homogenized to give 5 % homogenate (weight / volume). In high speed cooling centrifuge, the homogenate was centrifuged at 10,000 rpm for 10 min at -4°C. The supernatant portion of the resulting centrifugation was used as the source of the enzyme. 20 µl of 100% Ethyl alcohol was transferred to 200 µl homogeneous tissue and then put in an ice bath for an incubation time of 30 min. The tubes were held at room temperature after 30 min, immediately 20 µl of Triton X-100 was added. In UV-Vis Double beam spectrophotometer, the cuvette containing 0.4 ml of phosphate buffer, 0.1 ml of tissue extract and 0.5 ml of 0.066 M hydrogen peroxide in phosphate buffer was placed and optical density values were read at 240 nm for 1 min. For assess the activity of Catalase the molar extinction coefficient of 43.6 M cm⁻¹ was used. One unit of catalase activity is equivalent to the moles of H₂O₂ degraded / mg protein/min.

Se-Dependant Glutathione Peroxidase

Se-dependent Glutathione peroxidase (Se-GSH-Px) activity was calculated using the slightly changed Flohe and Gunzler, (1984) method. In 50 mM phosphate buffer (pH 7.0), comprising 0.1 mM EDTA, 5% (w / v) of the liver tissue homogenate was prepared. In high-speed cooling centrifuge, the obtained homogenate was centrifuged at 10,000 rpm for 10 min at -4°C. The supernatant was chosen as enzyme resource for the assay. The reaction mixture comprises 0.1 ml of glutathionereductase (0.24 units), 0.1 ml of reduced form 0.01 MGS, 0.1 ml of 1.5 mM NADPH and 0.5 ml of phosphate buffer. The 0.1 ml of homogenous tissue has been added to the reaction mixture and incubated for 10 min at 37°C. After incubation, 50 µl of 12 mM t-butyl hydroperoxide has been added to the overall reaction and optical density of samples was measured at 340 nm for 3 min. The activity of Se-GSH-Px was estimated using a molar extinction coefficient of 6.22 X 10³ M cm⁻¹. One activity unit is equal to the mM of oxidized NADPH / mg protein / min. The enzyme activity was expressed in µM oxidized NADPH / mg protein / min.

Glutathione Reductase

We assessed Glutathione reductase (GR) activity by making minor modifications to the Carlberg and Mannervik, (1985) method. To perform this assay, liver tissue was homogenized (5% w/v) in 50 mM phosphate buffer (pH 7.0) solution that containing 0.1 mM EDTA. At -4°C, the homogenate solution was centrifuged at 10,000 rpm for 10 min with high speed cooling centrifuge. The separate supernatant component was used as the source of the enzymes. A test tube comprising 50 µl of GSSG (20 mM) in a phosphate buffer (0.5 M, pH 7.0 containing 0.1 mM EDTA) and 0.8 ml of phosphate buffer was transferred to the cuvette containing NADPH (50 µl, 2 mM) in 10 mM Tris buffer (pH 7.0). The 0.1 ml tissue extract was added to the buffered solution of NADPH-GSSG and taken OD values for 3 min at 340 nm. The 6.22 X 10³ M cm⁻¹ molar extinction coefficient was used to assess GR activity which is equal to the mM of the oxidized NADPH / mg protein / min. Finally, the enzyme activity was expressed in NADPH oxidized / mg protein / min µ moles.

Glutathione -S- transferase:

Glutathione-S-transferase (GST) activity was measured at 340 nm with its likely substrate, 1-Chloro 2, 4-Dinitro Benzene (CDNB), following the Habig *et al.*, (1974) protocol. The liver homogenized in a 50 mM cold Tris-HCl buffer (pH 7.4) comprising 0.2 M sucrose, and content in the cooling centrifuge was centrifuged at 16,000 g for 45 at 4°C. Obtained supernatant was separated for further usage as enzyme resource. The 3 ml total volume reaction mixture comprises 100 µl of 30 mM CDNB, 100 µl of 30 mM GSH, 0.4 ml of source of enzyme and 2.4 ml of 0.3 M potassium phosphate buffer (pH 6.9). The reaction began with the inclusion of glutathione and the absorption spectrum against the reagent blank was viewed at 340 nm, and the activity was represented in µ moles of thioether-formed / mg protein / min.

Glutathione (GSH) content:

The amount of glutathione was calculated using the Theodorus *et al.*, (1981) procedure. The rat liver tissue was homogenized using homogenizer in 0.1 M ice cold phosphate buffer (pH 7.0) which comprising 0.001 M EDTA, then the protein content of tissue get sediment with the addition of 1 ml 5 % sulfosalicylic acid (w / v) and the components were centrifuged at 5000 g for 15 min at -40C. Upper supernatant solution was

separated and used as enzyme resource. The reaction mixture comprised 2.0 ml of 0.1 M potassium phosphate buffer in a complete volume of 2.5 ml, and other contents include 20 μ l of DTNB (1.5 mg / ml), 5 μ l of NADPH (4 mg / ml of 0.5 percent NaHCO₃), 20 μ l of glutathione reductase (6 units / ml) and necessary quantity of source of tissue. The reaction started with the addition of 0.41 ml of enzyme source and an OD change at 425 nm against the blank reagent was noted. The amount of glutathione was represented in the nano moles/gram wet weight of the tissue.

MDA content [Lipid Peroxidation (LP)]:

The amount of malondialdehyde (MDA) was assessed using the methodology stated by Ohkawa *et al.*, (1979). In 50 mM phosphate buffer (pH 7.0) comprising 0.1 mM EDTA, the hepatocellular tissue was homogenized (5 per cent w / v). further these samples were centrifuged in cooling centrifuge at 10,000 rpm for 10 min and obtained upper part was used for the assay. Tissue supernatant 0.4 ml into 0.1 ml of 8.1% SDS was transferred, vortexed and incubated at 37°C for 10 min. Added 0.75 ml of 20% acetic acid having pH 3.5 and 0.75 ml thiobarbituric acid (0.6%), and put in a heating water bath for 1 hour. Samples were allowed to refrigerate at ambient temperature. A 2.5 ml butanol: pyridine (15:1) mixture was applied, refluxed, and centrifuged for 5 min at 1000 rpm. The colored layer (1ml) was estimated at 532 nm, using standard 1,1,3,3-tetraethoxypropane. The values were expressed in the μ moles of malondialdehyde formed/gram tissue wet weight.

Statistical analysis

The data was presented in the form of means and standard deviations (SD). For the significance of the main effects (factors), treatments, and their interactions, variance analysis was performed using Duncan's multiple comparison tests among data using the SPSS (Version 15; SPSS Inc., Chicago, IL, USA) and M.S. Office, excel software. $P < 0.05$ was chosen as the threshold for statistical significance.

RESULTS AND DISCUSSION

In current study, SOD levels were significantly lower in the hepatitis group than NC, while SOD levels were significantly higher in alkaloid-rich fraction treated hepatitis rats when compare to Ht (Figure 1). Treatment of alkaloid-rich fraction to hepatitis rats demonstrated a greater improvement in SOD activity relative to hepatitis groups and a significant increase compared to standard drug Silymarin treatment. When compared to NC, there were no changes in the AFt group. SOD is a first-line antioxidant defense system, scavenging the hydroxy radical into hydrogen peroxide (Ighodaro and Akinloye, 2018). In many liver diseases, pathological symptoms associated with free radicals have been reported. Antioxidant therapy has been played possible benefits in rid of liver damage in acute and chronic diseases (Singal *et al.*, 2011). As per Zhang *et al.*, (2020) free radical generated oxidative stress is may be reason for hepatitis and liver damage.

Based on the results obtained in the present study, SOD is a key enzyme in the antioxidant enzymes because it scavenges the free radical induced oxidative stress in the hepatitis rats (Figure 1). AFt acted as antioxidant and inducer of antioxidant enzyme SOD. This fraction acted significantly on free radicals induced oxidative stress in hepatitis condition by increasing action of SOD. Similar results were also reported by Sinha *et al.*, (2007) that epicatechin at 15 mg/kg for 3 successive days exhibited hepatoprotective activity in radiation-induced oxidative stress in Swiss albino mice. The production of SOD is varying in different types diseases (Ivanov *et al.*, 2017). Likewise, in the present investigation the activity level of SOD is considerably higher in AF treated group when compared to hepatitis group

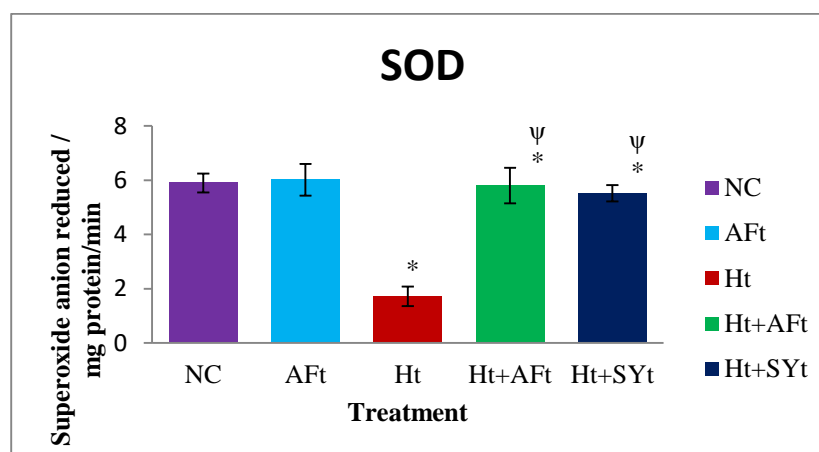


Figure 1: Effect of Alkaloid rich fraction of *P. amarus* (AFt), Hepatitis control (Ht), Hepatitis + Alkaloid rich fraction of *P. amarus* treated (Ht+AFt) and Hepatitis + Silymarin treated (Ht+SYt) on SOD activity in the hepatic tissue of male albino rats. The values are significant compared with normal control (* $p < 0.05$).

Treatment with AFt to the hepatitis rats protects the liver tissue damage by elevating antioxidant enzyme such as catalase (Figure 2). A catalase activity level in the hepatitis group was significantly decreased when compared to the normal control group. Treatment with AFt reduced the hepatitis by significant elevation of catalase when compared to Ht group. The results of AFt at taken dose were comparatively more than standard anti-hepatitis drug Silymarin. AFt alone not induce any changes in catalase activity when compare NC group. Results obtained in this study revealed that significant decrease of catalase activity in hepatitis rats; this may be due to accumulation of superoxide radicals and hydrogen peroxide in the liver tissue (Ismail *et al.*, 2010; Alavian and Showraki, 2016). Whereas, significant reduction of hepatitis was observed; this property of the AF was due to induction of antioxidants/ direct action on superoxide radicals and hydrogen peroxide.

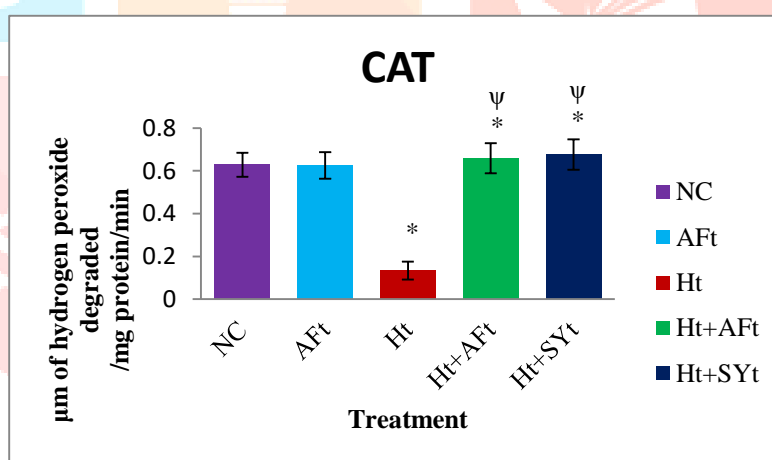


Figure 2: Effect of Alkaloid rich fraction of *P. amarus* (AFt), Hepatitis control (Ht), Hepatitis + Alkaloid rich fraction of *P. amarus* treated (Ht+AFt) and Hepatitis + Silymarin treated (Ht+SYt) on CAT activity in the hepatic tissue of male albino rats. The values are significant compared with normal control (* $p < 0.05$).

Catalase is a more popular and important antioxidant enzyme. It is activated at higher concentration of hydrogen peroxide but detoxification of hydrogen peroxide at lower concentration is associated with the GPx (glutathione peroxidase) enzyme (Sznarkowska *et al.*, 2017). Furthermore, elevation of catalase to detoxification of hydrogen peroxide is compensatory response to the disease condition or oxidative stress (Martins and English, 2014). Numerous pre-clinical studies have been conducted in patients suffering from dysfunction of liver using plant compound derivative preparations to protect the hepatic tissue. Among, Silymarin one of those drug, has been established for hepatoprotective property (Antonella *et al.*, 2013; Ghosh *et al.*, 2011). In the present study, treatment with AFt induced better elevation of catalase enzyme activity in concern of hepatoprotective activity than already existed drug Silymarin.

In the present study, GPx activity levels were significantly decreased in hepatic tissue of Ht rats when compared NC rats (Figure 3). The significant activity levels were increased in AFt treatment rats when compared to Ht rats. There was no significant changes were observed in pre-treatment of AFt when compared to normal control group. Interestingly, the GPx levels in the AFt treatment group were more than

the treatment with the standard drug Silymarin group i.e. Ht+SYt. Induction of GPx levels by the AFt in hepatitis rats was due to its free radical scavenging property or may be having a property that implication of GSH levels (Shanmugam *et al.*, 2017).

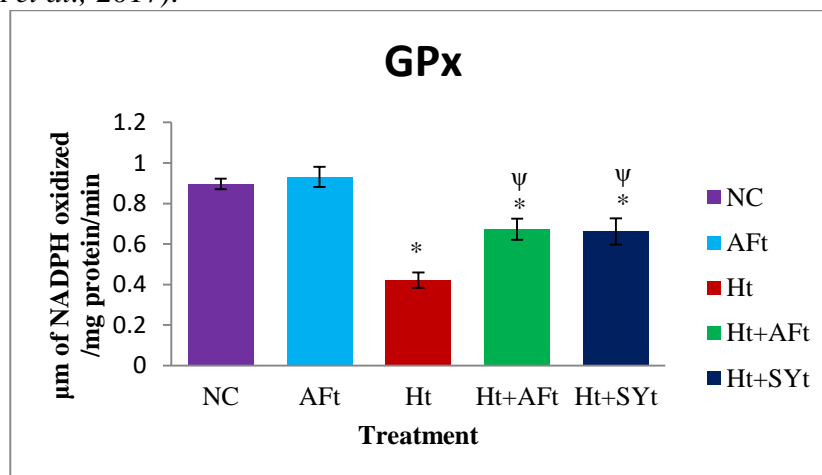


Figure 3: Effect of Alkaloid rich fraction of *P. amarus* (AFt), Hepatitis control (Ht), Hepatitis + Alkaloid rich fraction of *P. amarus* treated (Ht+AFt) and Hepatitis + Silymarin treated (Ht+SYt) on Se-GSH-Px activity in the hepatic tissue of male albino rats. The values are significant compared with normal control (* $p < 0.05$).

Cellular GPx stimulates the intra cellular redox reaction. It has been proven and reported in many scientific publications. NADPH/NADP, thioredoxinred/thioredoxinox, GSH/oxidized glutathione (GSSG) and cellular GSH levels are 1000-fold raised than other redox couples under stress condition where GPx is also associated with this redox couples (Reddy *et al.*, 2001). In the present study, GPx levels were drastically increased in AFt treatment, it is clear that indirect increase of redox couple in the cell for retardation of ROS toxicity. Pathological symptoms of liver damage are improper function, activation of immune cells and steatosis. These events are closely associated with the oxidative stress induced by free radicals (Ramadori *et al.*, 2008).

Comparison with NC, GR activity in the hepatic tissue of Ht rats was significantly declined (Figure 4). Nonetheless, treatment with AFt in hepatitis rats significantly increased the GR function. In the case of silymerin chemotherapy, however, GR activity was slightly increased than the liver of the hepatitis rats treated with AFt. Overall, these results indicate the role of AFt in liver damage protection. Our findings were consistent with Chandra *et al.*, (2000), In fact they found that papaverine alkaloid could potentially reduce the hepatotoxicity caused by the ethanol by increasing the antioxidant enzyme glutathione reductase. In another study, alkaloid berberine was reported to have protected the liver tissue by elevating the antioxidant enzyme glutathione reductase under the toxicity of sodium nitrite in liver (Akhzari *et al.*, 2019).

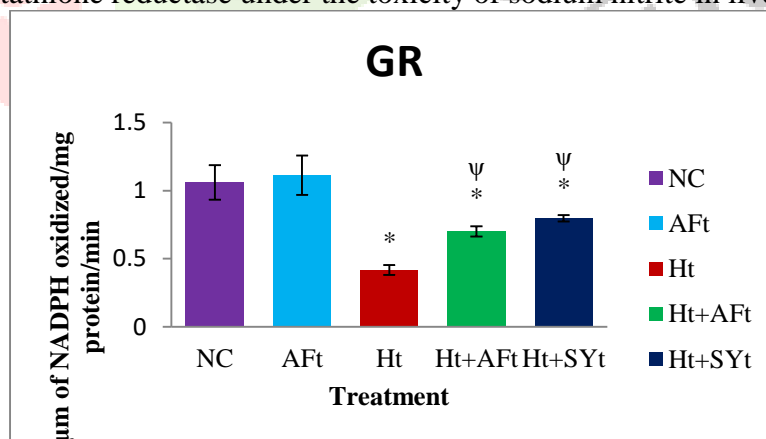


Figure 4: Effect of Alkaloid rich fraction of *P. amarus* (AFt), Hepatitis control (Ht), Hepatitis + Alkaloid rich fraction of *P. amarus* treated (Ht+AFt) and Hepatitis + Silymarin treated (Ht+SYt) on GR activity in the hepatic tissue of male albino rats. The values are significant compared with normal control (* $p < 0.05$).

In both studies they concluded that natural plant alkaloids have strong antioxidant activity and having capacity to elevate the antioxidant enzyme glutathione reductase. In the present study AFt of *P. amarus* also induced the elevation of glutathione reductase enzyme in D-galactosamine induced liver damage and this may be prevented the oxidative stress.

Compared with NC, GST activity in the liver tissue of Ht rats was considerably greater (Figure 5). AFt treatment in hepatitis rats however significantly decreased GST activity compared with NC rats. Nevertheless, in the case of Silymarin therapy, GST function was significantly decreased relative to the liver of the hepatitis rats treated by AFt. GST enzyme is capable of neutralizing these toxins or chemicals, thus increasing GST activity (Li *et al.*, 2015). If hepatic disease occurs in a high oxidative stress system for extended duration of time, therefore the condition actually appears to be chronic hepatic inflammation (Ivanov *et al.*, 2017). Likewise, we found a rise in liver protein that GST in current study, which suggests the presence of oxidative stress in liver cells owing to the induced illness.

In a study Aniya and Amders, (1989) were aimed to explore the effect of enzymatically generated reduced oxygen metabolites on the intervention of liver microsomal GST activity in order to know the enzyme's probable physiological control system. The results of this study clearly stated that the microsomal GST is triggered by reduced oxygen species such as H_2O_2 and superoxide anion. As a result, metabolic advancement which produces high amounts of ROS can trigger microsomal GST, most likely by oxidizing the enzyme's sulfhydryl group. Moreover, this enhanced catalytic activity of GST can aid to protect the cells against oxidizing damage (Aniya and Amders. 1989). The similar manner, in the present investigation, the activity of GST in the liver tissue of Ht rats was significantly changed compared with NC. But at the other side, when compared with NC, AFt of *P. amarus* treatment in hepatitis induced rats' decreased GST behaviour in a notable way. Alternatively, GR activity was significantly decreased when treated with Silymarin than the liver of the hepatitis rats treated with AFt. Hence, AFt protected the hepatic tissue from oxidative stress by increasing GST.

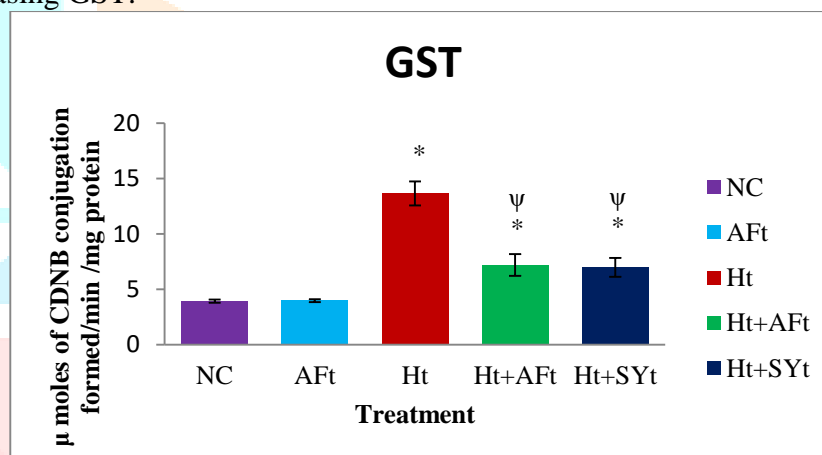


Figure 5: Effect of Alkaloid rich fraction of *P. amarus* (AFt), Hepatitis control (Ht), Hepatitis + Alkaloid rich fraction of *P. amarus* treated (Ht+AFt) and Hepatitis + Silymarin treated (Ht+SYt) on GST activity in the hepatic tissue of male albino rats. The values are significant compared with normal control (* $p < 0.05$).

In this study, the content of GSH in the hepatic tissue of Ht rats was significantly reduced as compared to NC (Figure 6). AFt treatment to hepatitis rats, therefore, has the GSH content remarkably similar to NC. Nevertheless, in silymerin therapy, GSH level was significantly lower than the liver of the hepatitis rats treated with AFt. In a study, *Phaleria macrocarpa* studied for the hepatoprotective role in CCl₄ induced toxicity. The obtained results found that extract protected the liver fibrosis by increasing the GSH content. Finally that research group concluded that this plant has rich amount of alkaloids and others they may be responsible for the hepatoprotective activity (Sundari *et al.*, 2018). In another study, 4-hydroxy-2(3H) benzoxazolone, a alkaloid compound isolated from *Acanthus ilicifolius* tested for hepatoprotective activity in CCl₄ induced hepato toxicity in rats. This compound has been protected by increasing GSH content to the hepatic tissue by reducing the oxidative stress caused by CCl₄ (Liu *et al.*, 2013). Recently, Akhzari *et al.*, (2019) tested berberine for hepatoprotective property in Sodium nitrite induced hepatotoxicity in Sprague-Dawley rats. Berberine is an alkaloid generally present in *Berberis* species. The results revealed that this alkaloid protected the liver tissue by nullifying oxidative stress of sodium nitrate by increasing GSH content. In the present study, our results also revealed that AFt treatment protected the D-Gal induced hepatotoxicity in hepatitis rats by increasing GSH content whereas GSH content was significantly decreased in hepatitis alone rats.

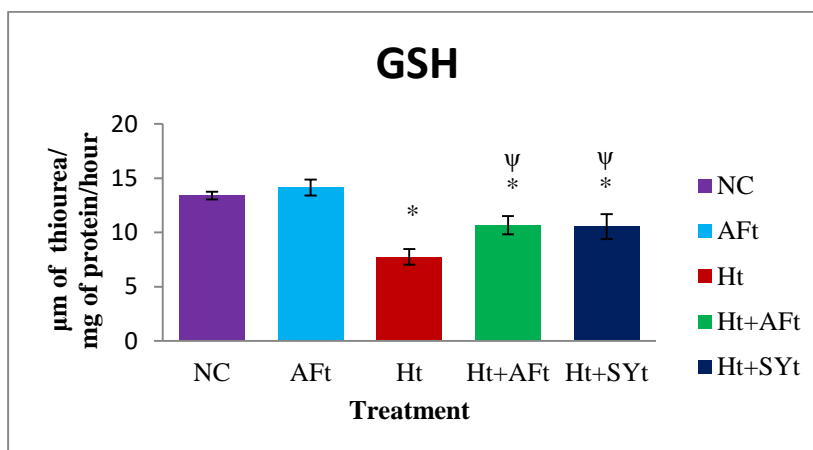


Figure 6: Effect of Alkaloid rich fraction of *P. amarum* (AFt), Hepatitis control (Ht), Hepatitis + Alkaloid rich fraction of *P. amarum* treated (Ht+AFt) and Hepatitis + Silymarin treated (Ht+SYt) on GSH activity in the hepatic tissue of male albino rats. The values are significant compared with normal control (* $p < 0.05$).

In the current study, the content of MDA in liver tissue of experimental groups NC, AF, Ht, Ht+AFt and Ht+SYt were compared and the results were summarized in (Figure 7). However, when compared with NC, AFt treatment in hepatitis rats reduced MDA contents in a slight way. Even so, in Silymarin treatment, the MDA level was slightly higher than the liver of the hepatitis rats treated with AFt. There are multiple lines of validation articles on the incidence of MDA in hepatic inflammation, which are accurate and stable to our outcomes (Atiba *et al.*, 2016; Li *et al.*, 2015; Vuppalachchi *et al.*, 2011). MDA is used frequently to assess oxidative stress in tissue specimens as well as being a reactive aldehyde which can covalently bind to biomolecules, such as proteins, lipids and DNA, thus producing epitopes of MDA (Rahal *et al.*, 2014). Such MDA epitopes act as mediators of illness of inflammation and hypothesize on their insight into etiology of illnesses (Del-Rio *et al.*, 2005). Significantly improved lipid peroxidation consequently joins many afflictions with an inflammatory condition, as stated by the identification of lipid peroxidation end production in various diseases (Ayala *et al.*, 2014). Finally, due to irrepressible development or oversupplied removal of lipid-peroxidation products during oxidative stress, Malondialdehyde epitopes could be differentiated as just a diagnostic tool for oxidative stress in liver provocative diseases.

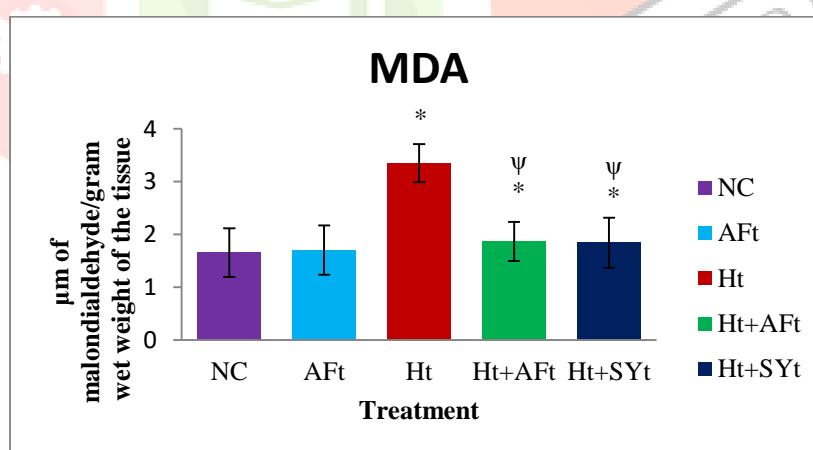


Figure 7: Effect of Alkaloid rich fraction of *P. amarum* (AFt), Hepatitis control (Ht), Hepatitis + Alkaloid rich fraction of *P. amarum* treated (Ht+AFt) and Hepatitis + Silymarin treated (Ht+SYt) on MDA content in the hepatic tissue of male albino rats. The values are significant compared with normal control (* $p < 0.05$).

In this study, the MDA content in the hepatic tissue of hepatitis rats was markedly increased when compared with NC. AFt medication in hepatitis rats however slightly reduced MDA content compared to NC. In the Silymarin treatment, however, MDA levels were slightly higher than the liver of the hepatitis rats treated with AFt. There are various lines of confirmatory article on the incidence of MDA in hepatic inflammation which is solid and valid with our results.

CONCLUSIONS

The findings of this study reveal that the alkaloid fraction of *P. amarus*, a rich source of antioxidants, can protect oxidative stress by enhancing antioxidant enzyme activity and lowering lipid peroxidation in hepatic tissue under hepatitis conditions. In future, the mechanism of action of the alkaloid fraction must be determined, as well as the separation of individual alkaloid compounds.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

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