Antioxidant biochemical enzyme activities of *Adansonia digitata* L. in ulcer induced experimental male Wistar rats

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Abstract: To investigate the conceivable antioxidant capacity of ***Adansonia digitata* L,** The present study focused on the alterations in antioxidant enzymatic activities. With a specific end goal to investigate the impacts of oxidant prevention agent protections on the procedure of ulceration, in all stomach tissues, the cell reinforcement levels were assessed. The level of Glutathione (GSH), Glutathione Reductase (GR), Glutathione s transferase (GST), superoxide dismutase (SOD), catalase (CAT), and Glutathione Peroxidase (GPx) were assessed in ulcer control, Standard and drug treated group. Aqueous and Alcoholic extracts of *Adansonia digitata L.* leaves (AAD and EAD) demonstrated a dosage dependant antioxidant action ($p<0.05$) in every anti ulcer model. Ethanol in ulcer incited rats created depletion in the accompanying enzymes, GSH, GR, GPx, GST, SOD and CAT. In every distinctive concentration raising from 100 to 200 mg/kg, AAD 200mg/kg has shown the basic and earth shattering effect in each and every enzymatic examination, This is just about demonstrating near to effect to the standard drug Ranitidine. Glutathione levels of AAD 200mg/kg have shown 5.560 ± 0. 380 compare with standard drug 6.632 ± 0.372 and GR levels as 28.73 ±0.947 where as standard has shown 30.14 ± 0.786. AAD 200 has shown significant increase in GPx levels also compared to ulcer rats. AAD 200 extract has also shown a significant increase in GST levels compared with EAD and AAD extracts. Our current study demonstrated the probable role of antioxidant enzymes in scavenging reactive oxygen species (ROS) associated with tolerance to gastric ulcer.

Keywords: *Adansonia digitata, anti oxidant,* GST, GR, GPx, SOD, Biochemical enzymes.

Introduction:

Gastric ulcer (GU) is one of the mainly common digestive system diseases with an elevated morbidity of about 5–10 % during human life span, being a major public health burden in the present century (A. Lanas and F.K.L. Chan 2017). though the etiology and pathogenesis of Gastric ulcer remains contentious, several studies have revealed that it is caused by the critical unevenness between mucosal invasive factors (such as long period consumption of NSAIDS) and the protective factors of gastric mucosa (especially prostaglandins level and antioxidant enzymes activity), resulting in disruption of the gastric mucosal defensive barrier thus causing to gastric ulcer (A. Woolf and R. Rose 2019). As well, ethanol induced stress has been well reported to ulcerate on gastric mucosa throughout the origin of highly cytotoxic free radicals (R.S. Aziz *et al.,* 2019) and (X. Wu *et al.,* 2019). Ethanol alters gastric secretary action, disturbs cell permeability and reduces gastric mucus, making gastric mucosal cells more susceptible by free

radicals (S. Byeon *et al.*, 2018) and (N. Sistani Karampour *et al.*, 2019). A number of medications such as antibiotics, antacids, proton-pump inhibitors (omeprazole) and H2 receptor antagonists (Ranitidine) are readily accessible for the treatment of ulcers. However, these agents are facing major problems due to their narrow efficacy against gastroschisis and severe side effects, for example, gynecomastia, hypoacidity, impotence, osteoporotic bone fracture, hypergastrinaemia and cardiovascular disease risks (O.I. Kulikova *et al.*, 2020) (K. Chakravarty and S. Gaur 2019). Hence, new drug candidates which may perhaps provide high efficacy and low toxicity are required valuable for the prevention and treatment of Gastric ulcer. conventional medicinal herbs and their ingredients have been used as the principal therapeutic agents in INDIA from times immemorial. *Adansonia digitata* L. (Malvaceae) is commonly known as monkey breed baobab tree. It is seemingly perpetual tree with all-purpose utilizations, which offers protection and make available food, clothing and medication just as crude material for some helpful things. The seeds, fruit pulp leaves, flowers, bark and roots of baobab are eatable and they have been studied by researchers for their precious properties (Jitin Rahul *et al.*, 2015)

This plant utilized by the clans of Nallamala forest of Kurnool district of AP INDIA for the inflammation, ulcers, and in any event, for the treatment of gastritis and tonsils. In India it is found in Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Maharashtra, and other beach front districts of the nation. It is limited to hot, dry forest on stony, all around depleted soils, in ice free regions that get low precipitation. (Rajesh Kumar Mishra *et al.*, 2019). In this current research work, we have evaluated the antioxidant enzyme effectiveness of different extracts of *Adansonia digitata* using succession of in vivo biological assays.

Material & Methods:

Plant collection: The whole plant of *Adansonia digitata* L was collected from the Nallamala forest of Chittoor district region during the month of October. It is identified and authenticated by Dr. J vasundharamma, M.sc., Ph.D. Dept.of Botany, KVR Govt. College (W) (A), Kurnool.

Preparation of Extracts: The coarse powder was subjected to successive extraction in a soxhlet apparatus using different solvents such as Hexane, Ethyl acetate, Ethanol and Water. Each time before extracting with the next solvent, the plant material was dried in a hot air oven. Furthermore, extracts were filtered through Whatmann No.1filter paper and concentrated to the dry mass with the aid of rotary evaporator. The yield of each extract was measured and residues were stored in dark for further assay. Different extracts were labelled as **EAD** (for Ethanol extract) and **AAD** (for aqueous extract). Dried extracts of 20 mg/ml stock solution were prepared and different concentrations were used in various experiments.

Experimental Design: (Vogel HG and Vogel WH2002)

Male Wister albino Wister rats between 3 to 4 months of age and weighing 250-300g were procured from Albino Research & Training Institute, Bachupally, Hyderabad. Animals are maintained as per the guidelines of National Institute of Nutrition (NIN) Animal User's Manual. Animals were acclimatized for 7 days to our animal house, maintained at temperature of 20-24^{0}c.

The rats were grouped into 6 groups, Group I represents the control which has received Ethanol mg/Kg bw. Group II is Standard and has received the Ranitidine 20mg/kg bw as reference drug. Group III, IV is AAD with 100mg/kg bw, 200mg/kg bw. Group V & VI is

EAD with 100mg/kg bw, 200mg/kg bw. The induction of Ulcer was done as per the procedure of Dhasan and Jagadeesan, with slight modifications. (Dhasan and Jagadeesan, 2010).

statistical analysis:

All the full length experiments were performed in triplicates and experimental results were expressed as mean ± standard Error of mean (SEM) of three replicates. IC50 value (the concentration of the extracts required to scavenge 50% of radicals) was calculated for different extracts of *A. digitata*. Graph pad prism 8.4.3 (686) software was used for statistical analysis and to prepare the graphical representation of results.

Oxidative stress studies of stomach:

After partition of stomach, 10 % tissue homogenate was readied in 0.15 M Kcl by utilizing potter-elvehjem homogenizer at 40 C, and then centrifuged at 12,000 rpm for 45 min at 0-4oC. The obtained supernatant was disseminated into eppendorf tubes, marked and put away at - 20oC and utilized for enzyme analysis.

As we realize that Glutathione is vital for keeping up the diminishing capacity of cells Glutathione has a wide assortment of urgent physiological parts. As a matter of first importance, it is the focal individual from an intricate cancer prevention agent framework shielding the cell from oxidative stress (Alfonso Pompella *et al*., 2003). Glutathione Peroxidase dwells in the Cytosol and Mitochondrial matrix. It goes about as a compound shielding hemoglobin from oxidative devastation by H2O2 furthermore goes about as a "contraction cofactor" of mitochondria i.e. as a compound avoiding loss of contractibility of mitochondria under exceptional conditions. A known amount of enzyme preparation was allowed to react with H_20_2 in the presence of GSH for a specified time period according to the method of Rotsruck (1973) and remaining GSH was measured by Ellman's method (1959) as described for GSH estimation.

To 0.5 ml phosphate buffer (0.2 M, pH 7.0 containing 0.4 mM EDTA, and 10 mM sodium azide), 0.2 ml enzyme source (0.4 mg – 0.8 mg protein), 0.2 ml GSH (2 mM) and 0.1 ml of H_2O_2 (0.2 mM) were added and incubated for 10 min at room temperature along with a control tube containing all reagents except enzyme source. The reaction was arrested by adding TCA (10 %), centrifuged at 4000 rpm for 5 min and GSH content in 0.5 ml of supernatant was estimated by following the method of Ellman's (1959). The activity was expressed as µg of GSH consumed /min / mg protein.

Glutathione Reductase (Enzyme Code: 1.6.4.2):

Glutathione Reductase is a enzyme which catabolizes the reduction of oxidized glutathione (GSSG) by NADPH to GSH. The activity of this enzyme was measured by the accompanying of oxidation of NADPH Spectrophotometrically with minor changes at 340 nm according to the technique for Pinto and Bartley (1969).

This assay system contains 0.5 ml of phosphate buffer (0.25 M, pH 7.4), 0.1 ml of EDTA (25 mM), 0.1 ml of NADPH (1mM), 0.96 ml of distilled water and 0.1 ml of enzyme source (150 µg proteins). The reaction is initiated by the addition of 0.024 ml of GSSG (50 mM). The decrease in the absorbance was recorded at

1 min interval for 5 min at 340 nm. The activity was expressed as µmol of NADPH oxidized/min/mg of protein by using millimolar extinction coefficient for NADPH of 6.22 cm^{-1} μmol^{-1}.

Glutathione-S-transferase (Enzyme Code: 2.5.1.18):

GST is a multifunctional complex protein found in numerous tissues and demonstrates augment specificity for natural hydro peroxides however not for H_2O_2 (Bruce *et al*., 1982). GST is well known to play a physiological role as potential alkylating agents in detoxification mechanism (Booth *et al*., 1961; Boyland and Chasseaud, 1969)

Glutathione-S-transferase activity was measured by observing the increase in the absorbance at 340 nm by using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate with slight modifications to the method of Habib *et al*., (1974).

The assay system contains 1.7 ml of sodium phosphate buffer (0.14 M, pH 6.5), 0.2 ml of GSH (30 mM) and 0.04 ml of enzyme source (40 µg of protein). The reaction was initiated by 0.06 ml of CDNB (0.01 M, dissolved in 50 % ethanol). The change in absorbance was recorded at 340 nm for 5 min and the linear absorption change was measured and then the activity was calculated by using mM extinction coefficient of CDNB-GSH conjugate as 9.6. The activity could be expressed as µmoles of CDNB-GSH conjugate formed/min/mg of protein.

Catalase (Enzyme Code: 1.11.1.6):

According to a Scientific American Special Report on Aging article March 2015, they produced more catalase, which is an antioxidant, lived longer. Catalase catalyses the breakdown of H_2O_2 to H_2O and O_2 and the rate of decomposition of H_2O_2 was measured spectrophotometrically at 240 nm by following the method of Beers and Seizer (1952).

The assay system contains 1.9 ml of sodium phosphate buffer (0.05 M, pH 7.0) and 1.0 ml of H_2O_2 (0.059 M, in buffer) and the reaction was initiated by the addition of 0.1 ml enzyme source (45 µg). The decrease in absorbance was monitored at 1 min interval for 5 min at 240 nm and the activity was calculated by using a molar absorbance coefficient of H_2O_2 as 43.6. The activity was expressed as milli moles of H_2O_2 decomposed/min/mg protein.

Superoxide dismutase (Enzyme Code: 1.15.1.1):

SOD activity was measured based on the capability of the enzyme to inhibit the oxidation of Pyrogallol. A customized procedure describe by Marklund and Marklund (1974) was adopted as followed by Soon and Tan (2002).

The assay system contains 2.1ml of phosphate buffer (50 mM, pH 7.8 containing 1 mM EDTA buffer), 0.02 ml of enzyme source (35 µg protein) and 0.86 ml of distilled water. The reaction was initiated by the addition of 0.02 ml of Pyrogallol (10 mM, in 0.01 N HCl). The change in the absorbance was monitored at 420 nm for 5 minutes. The percent of inhibition was calculated on the basis of comparison with an assay blank system. One unit of SOD was defined as the amount of enzyme required to inhibit the oxidation of Pyrogallol by 50 % in standard assay system of 3 ml. The activity was expressed as units/ minutes/mg protein.

Results & Discussion:

Estimation of Biochemical Enzymes:

With a specific end goal to investigate the impacts of oxidant prevention agent protections on the procedure of ulceration, in all stomach tissues, the cell reinforcement levels were assessed. The level of Glutathione (GSH), Glutathione Reductase (GR), Glutathione s transferase (GST), superoxide dismutase (SOD), catalase (CAT), and Glutathione Peroxidase (GPx) were assessed in ulcer control, Standard and drug treated group.

In the Enzymatic investigations of ulcer incited models, we have push ahead with the two sorts of extracts with various AAD surements. Watery and Alcoholic extracts of *Adansonia digitata L.* leaves (AAD and EAD) demonstrated a dosage dependant antioxidant action ($p<0.05$) in every anti ulcer model. Ethanol in ulcer incited rats created depletion in the accompanying enzymes, glutathione (GSH), Glutathione Reductase (GR), Glutathione Peroxidase (GPx), Glutathione S transferase (GST), Super Oxide Dismutase (SOD) and Catalase (CAT).

In our test ponder, the treatment with AAD and EAD hoisted the levels of GSH, GR, GPx, GST, SOD and CAT, In every distinctive concentration raising from 100 to 200 mg/kg, Among these, AAD 200mg/kg has shown the basic and earth shattering effect in each and every enzymatic examination, This is just about demonstrating near to effect to the standard pharmaceutical Ranitidine.

Glutathione and Glutathione reductase:

Glutathione lessened (GSH) secure cells against free radicals, peroxides and other deadly compounds (Hiraishi *et al*., 1994). Exhaustion of gastric mucosal GSH may realize the total of free radicals that can start layer harm by lipid peroxidation. In consonance with our study Abbas and Sakr (2013) and Mei *et al*. (2013), have reported depletion in GSH level in the gastric tissues. Ethanol induced ulcer rats demonstrated a critical lessening in GSH content with fundamentally diminished action of GR. *Adansonia digitata L.*treatment for 30 days in Ulcer rats results in an essentially increment in GSH substance and action of GR contrasted with Ulcer rats. In this manner *A. digitata* treatment for 30 days has given a halfway recuperation in GSH substance and action of GR in Stomach against Ethanol affected modifications.

Glutathione Peroxidase:

Ethanol induced Ulcer rats showed a significant decrease in activity of GPx, when compared to Standard group. Treatment with *A. digitata* for 30 days in Ulcer rats results in significant increase in the levels of GPx activity of stomach tissue. But in case EAD 100 and 200 extract doesn't shows significant increased levels of GPx. However AAD 200 has shown 8.478 ± 0.065 (**Table 3 & Fig 3**) significant increase in GPx levels compared to ulcer rats. The decreased activity GR resulted in de creased production of GSH, which is essential for GPx activity for detoxification of peroxides. Glucose-6-phosphate DH produces NADPH which is a substrate for regenerating GSH from its oxidized from GSSG.

Glutathione-s-transferase:

This enzyme catalyses the reaction of such compounds with the (thiol) –SH group of GSH, thereby neutralizing their electrophillic sites and rendering the products more water- soluble. Treatment with *A. digitata* for 30 days in UI rats resulted in significant increase in GST activity in stomach tissue compared to standard UI rats. Here again AAD 200 extract has shown 4.685 ± 0.311 (**Table 4 & Fig 4**) a significant increase in GST levels compared with EAD and AAD 100 & 200 mg/kg extracts. Decreased GST in UI rats is indicates enhanced utilization of GSH for detoxification of toxic products. The decreased activity of GST may result in the involvement of deleterious oxidative changes due to accumulation of toxic products and acts as an indicator of neurotoxicity.

Superoxide dismutase and Catalase: CAT is heme protein which catalyses the reduction of hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals. H_2O_2 is considered a key metabolite because of its relative stability, its diffusion and its involvement in cell signaling cascade. CAT or GPx was the primary enzyme in the removal of H_2O_2. CAT decomposes H_2O_2 without generation of free radicals by minimizing one electron transfer whereas GPx needs GSH for decomposition. CAT resulted in protection against H_2O_2 mediated LPO. The O_2* has known to inactivate CAT. In ethanol induced ulcer rats the SOD & CAT levels were decreased significantly whereas in the pretreatment of *A. digitata* has shown the significant enhancement in the levels of SOD & CAT. Preferably AAD 200mg/kg has shown the remarkable increase in the SOD and CAT levels compared to Ethanol induced ulcer rats.

Our reports of increased SOD and CAT in ulcer rats was supported by earlier studies of (Li CY *et al.*, 2008) suggesting that, pretreatment with cyanidin 3-glucoside significantly increased the level of glutathione and activities of radical scavenging enzymes, such as superoxide dismutase, Catalase, and glutathione Peroxidase, in gastric tissue.

The significant cellular antioxidant enzymes (Superoxide dismutase)SOD and CAT(Catalase), contribute to the gastric oxidative/antioxidative balance. A decrease of both SOD and Cat activities in gastric mucosa of rats exposed to ethanol leads to the accumulation of ROS and consequently to an increase in MDA concentration. In our investigation, ethanol induced inhibition of SOD and CAT activities, suggesting an important role for these enzymes in the pathogenesis of gastric injury.

S.no.	Group	Glutathione (µg of GSH/g tissue)
1.	**Control**	**4.559±0.332**
2.	**Standard**	**6.632 ± 0.372**
3.	**AAD100**	**5.054 ± 0.376**
4.	**AAD200**	**5.560 ± 0. 380**
5.	**EAD100**	**5.037 ± 0.232**
6.	**EAD200**	**5.158 ± 0.338**
P Value		**P < 0.0001**

Table1. Determination Glutathione activity

S.no.	Group	Glutathione Reductase (µmoles of NADPH oxidized/min/mg protein)
1.	Control	24.36 ± 0.532
2.	Standard	30.14 ± 0.786
3.	AAD100	24.59 ± 0.799
4.	AAD200	28.73 ±0.947
5.	EAD100	24.45 ± 0.857
6.	EAD200	26.51 ± 0.571
P Value		P < 0.05

Table2. Determination Glutathione Reductase activity

S.no.	Group	Glutathione peroxidase (µg of GSH consumed/min/mg protein)
1.	Control	8.632 ± 0.192
2.	Standard	11.71 ± 0.110
3.	AAD100	7.879 ± 0.203
4.	AAD200	9.715 ± 0.113
5.	EAD100	8.478 ± 0.065
6.	EAD200	8.877 ± 0.052
P Value	P < 0.05	

Table 3: Determination Glutathione Peroxidase activity

S.no.	Group	Glutathione-s-transferase (µmoles of GSH-CDNB conjugate formed/min/mg protein)
1.	Control	2.596 ± 0.289
2.	Standard	5.067 ± 0.290
3.	AAD100	4.341 ± 0.269
4.	AAD200	4.685 ± 0.311
5.	EAD100	2.880 ± 0.305
6.	EAD200	3.090 ± 0.313
P Value	P < 0.05	

Table 4: Determination GST activity

S.no.	Group	Superoxide dismutase (U/min/mg protein)
1.	Control	4.788 ± 0.081
2.	Standard	7.569 ± 0.062
3.	AAD100	5.467 ± 0.063
4.	AAD200	7.327 ± 0.054
5.	EAD100	5.242 ± 0.070
6.	EAD200	5.568 ± 0.048
P Value		P < 0.05

Table 5: Determination SOD activity

S.no.	Group	Catalase (mmoles of H_2O_2 decomposed/min/mg protein)
1.	**Control**	**4.106 ± 0.338**
2.	**Standard**	**6.009 ± 0.388**
3.	**AAD100**	**5.015 ± 0.261**
4.	**AAD200**	**5.272 ± 0.302**
5.	**EAD100**	**4.220 ± 0.344**
6.	**EAD200**	**4.120 ± 0.339**
P Value		**P < 0.05**

Table 6: Determination CAT activity

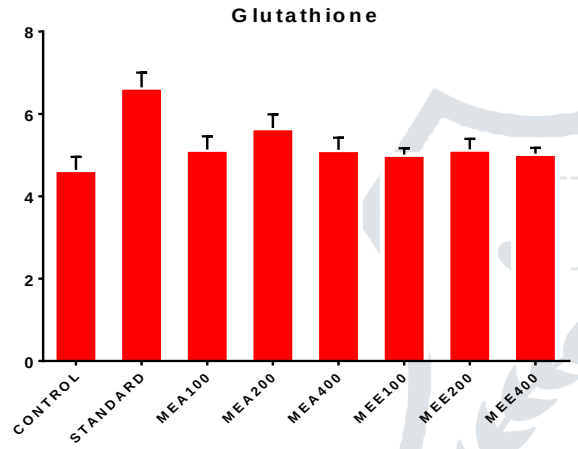

Fig 1: Graphical representation of GSH activity

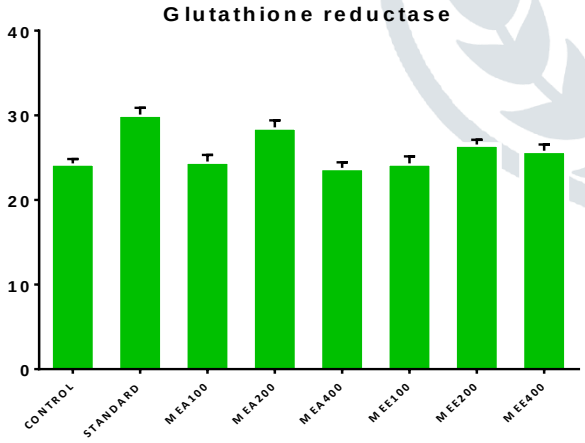

Fig 2: Graphical representation of GR activity

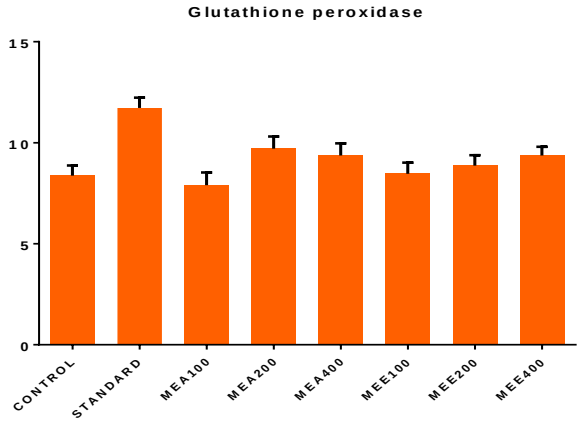

Fig 3: Graphical representation of GPx activity

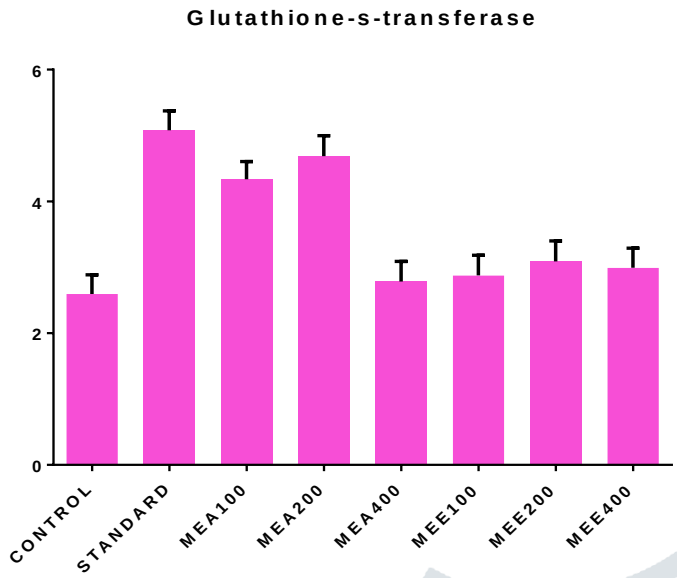

Fig 4: Graphical representation of GST activity

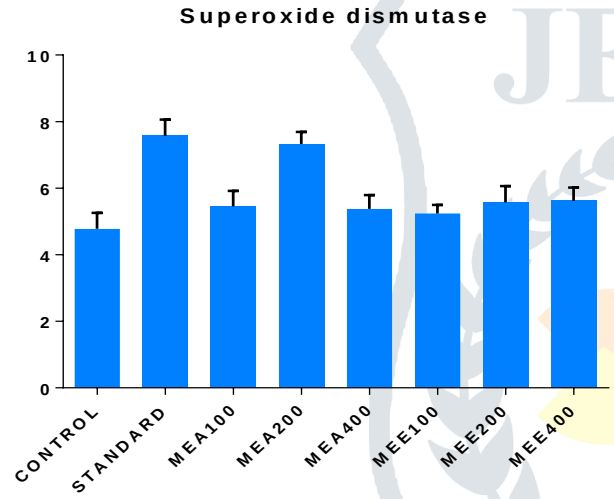

Fig 5: Graphical representation of SOD activity

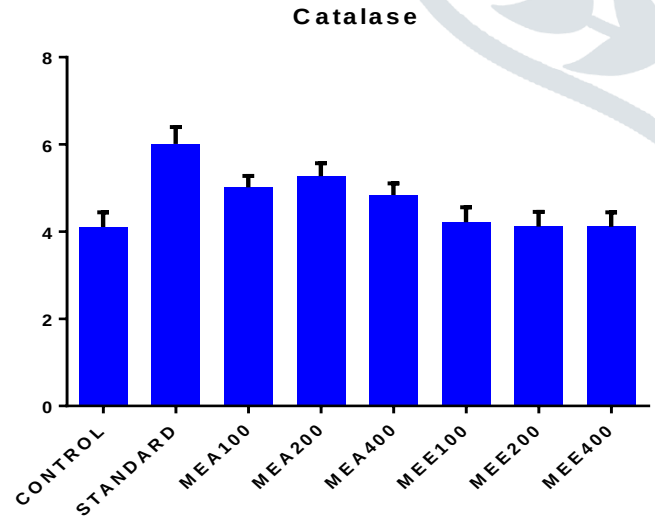

Fig 6: Graphical representation of CAT activity

Conflict of Interests:

"The authors declare that they have no conflict of interests."

Authors' contributions:

BD framed the study and procedure, carried out all the Experimental tests, analyzes the data and drafting of the manuscript. NBS & CMA offered technical support and involved in the drafting and review of the manuscript. Both the authors read carefully and approve the final manuscript.

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