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IN VITRO STUDY OF CYTOPROTECTION BY *ALOE VERA* LEAF GEL AGAINST APAP (AAP) MEDIATED OXIDATIVE STRESS IN RAT LIVER SLICES

Bhagyashree Patil

Department of Biotechnology Engineering,
Tatyasaheb Kore Institute of Engineering and Technology,
Warananagar, Tal. Panhala, Dist. Kolhapur, MS, Maharashtra

ABSTRACT

Present investigation was carried to find out efficacy of crude *Aloe vera* leaf gel in protecting hepatic cells against the oxidative stress mediated by varying concentrations of APAP (AAP) during different periods of time. APAP is non toxic in therapeutic doses but imparts toxicity when given singly in high doses. *Aloe vera* is a medicinal herb and the leaf gel is widely utilized for treating diseases due to medicinal properties associated with it. Hence in the present investigation, the leaf gel was studied. *In vitro* rat liver slice model was used to study and evaluate the hepatocyte protection offered by the crude gel of *Aloe vera* leaf. AST, ALT, ALP levels in the medium were studied as the markers of hepatocyte damage along with Glutathione and TBARS in the liver tissue slices. No significant toxicity was observed at the end of one hour of incubation, while there was dose dependent toxicity induction and leakage of enzymes in the media in presence of increasing doses of APAP and increasing time of incubation. The higher concentration of *Aloe vera* was more effective to protect the cells against APAP induced toxicity amongst the two different concentrations of *Aloe vera* tested for their protective efficacy. The toxicity studies of *Aloe vera* leaf gel showed that *Aloe vera* leaf gel is safe for hepatocytes and no toxicity outset was observed in terms of enzyme leakage and TBARS. *Aloe vera* leaf gel is effective in protecting the liver cells against APAP mediated oxidative stress and related damage in terms of the parameters studied in the present project.

Key words: APAP, AAP, *Aloe vera* gel, AST, ALT, TBARS, Glutathione, oxidative stress, antioxidant

Introduction:

N-Acetyl para aminophenol (APAP) or acetaminophen (AAP) is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is commonly used for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-inflammatory drugs (NSAIDs) or opioid analgesics, APAP is used also in the management of more severe pain like cancer pain.[1] While generally safe for human use at recommended doses, acute overdoses of APAP can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcoholism. APAP toxicity is the foremost cause of acute liver failure in the Western world, and accounts for most drug overdoses in the United States, the United Kingdom, Australia and New Zealand[2-5] A study conducted in 31 countries on over 200,000 children indicates that infants who are given APAP may be at an increased risk of developing asthma as children [6]

Medicinal plants form the backbone of traditional system of medicine in India. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds. Phytochemicals from medicinal plants serve

as lead compounds in drug discovery and design. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs [7].

Aloe vera L. (syn.: *Aloe barbadensis* Miller) is a perennial succulent plant belonging to the *Aloeceas* family which is a sub family of *Asphodelaceas*. Amongst the 400 *Aloe* species, *Aloe vera* is most widely accepted and used for various medical and cosmetic purposes. Various studies has revealed that *Aloe vera* leaf possesses many pharmaceutical activities including purgative, antibacterial, anticancer, antifungal and antioxidant [8]. The raw gel of Aloe vera contains water soluble and fat-soluble vitamins, minerals, enzymes, polysachharides. Phenolic compounds and organic acids [9]. It has been hypothesized that this heterogenous composition of Aloe vera leaf gel may contribute to the diverse pharmacological and therapeutic activities activities which have been observed for Aloe gel [10]

APAP and liver

Hepatotoxicity is the most remarkable feature of APAP overdose. Acute overdoses of APAP can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption. APAP toxicity is foremost cause of acute liver failure. [11]
Over dosage of APAP leads to the saturation of conjugation pathway leading to glutathione depletion and increase in the formation of toxic reactive metabolites. A high level of reactive metabolites increases the level of hepatotoxicity, with increased level of protein adducts formation, mitochondrial dysfunction and oxidative stress. The present study reveals the hepatoprotective activity of *Aloe vera* leaf gel against a well known hepatotoxin APAP.

It is established that following an oral therapeutic dose, a fraction of APAP is converted *via* the cytochrome p450 pathway to a highly toxic metabolite, N-acetyl-p-benzoquinone-imine (NAPQI), which is normally conjugated with glutathione and excreted in the urine as conjugates. Overdoses of APAP deplete glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction, and the development of acute hepatic necrosis. Also depletion of glutathione enhances the expression of tumor necrosis factor alpha (TNFa). TNFQ primes phagocytic NADPH oxidase to the enhanced production of oxygen free radicals and contributes to liver damage. In lieu of this, the present project was carried on *Aloe vera* leaf gel, which has been already proved to have antioxidative properties.

James et al [12] reviews that the nitrated tyrosine residues as well as APAP adducts occur in the necrotic cells following toxic doses of APAP. Nitrotyrosine was postulated to be mediated by peroxynitrite, a reactive nitrogen species formed by the very rapid reaction of superoxide and nitric oxide (NO). Peroxynitrite is normally detoxified by GSH, which is depleted in APAP toxicity. It is suggested that NO may play a role in controlling lipid peroxidation and that

reactive nitrogen/oxygen species may be important in toxicity. The source of the superoxide has not been identified, but it was found that NADPH oxidase knockout mice were equally sensitive to APAP and had equal nitration of tyrosine suggesting that the superoxide is not from the activation of Kupffer cells. It was postulated that NAPQI-mediated mitochondrial injury may be the source of the superoxide.

Aloe vera: potencies: *Aloe vera* has wound-healing and antibiotic effects [13]. *Aloe vera* leaves are used in diseases of the eyes and enlargements of spleen and liver [14]. Moreover, its liver protective function via anti-oxidative and anti-inflammatory effects is well known [15, 16]

Concentrations of APAP: APAP is cytotoxic at concentrations above 0.6×10^{-3} M in the culture medium in isolated hepatocytes. Hence a range of cytotoxic concentrations of APAP 0.6×10^{-3} M, 0.8×10^{-3} M, 1.0×10^{-3} M, and 1.2×10^{-3} M was tested to evaluate the potency of *Aloe vera* leaf gel to protect hepatocytes against the APAP induced hepatotoxicity and/or oxidative stress.

Parameters studied: The liver function test parameters Aspartate Transaminase (AST, EC 2.6.1.1) and Alanine Transaminase (ALT, EC 2.6.1.2) and Alkaline phosphatase (ALP, EC 3.1.3.1) were studied in the culture medium. Leakage of these enzymes indicates possible hepatocyte damage. Alongwith these function tests thiobarbituric acid reactive substances (TBARS) and Glutathione were estimated in the liver slices to understand the possible damage to the lipids/membrane and status of the antioxidant glutathione, as APAP is a well known glutathione depletory.

Material and Methods

Plant material: *Aloe vera* (L) was obtained from a farm in Shiradwad, a small village in Kolhapur district MS India. It was grown for one year in a soil which was free from the use of chemical fertilizers. The leaf gel was obtained as described by Ramchandra and Rao [17] as modified traditional hand-filleting method. Gel was collected and used immediately when fresh after the removal

Chemicals: APAP was obtained from S D fine chemicals, Mumbai while the culture media used was M199 of Himedia. All other chemicals used were of analytical grade. Pathological diagnostic kits were purchased from AGAPPE Diagnostics, India.

Experimental Animals: Laboratory bred male albino rats obtained from the registered animal house of Tatyasaheb Kore College of Pharmacy. Animal was sacrificed giving deep ether anesthesia.

Experimental Procedure: Surgical procedures were carried out on fed rats under deep ether anaesthesia. The liver slices (LS) were prepared from the whole liver without distinction of lobes. LS were incubated in a shaking water bath (100 cycles/min) at 37 °C to randomize and avoid any variability between slices that may come from localization in liver lobes or size. After 1 h of preincubation, allowing fresh slices to recover, the slices were transferred to other vials containing fresh medium M199 and supplemented with different combinations of APAP. (0.6×10^{-3} M, 0.8×10^{-3} M, 1.0×10^{-3} M, and 1.2×10^{-3} M), and/or and *Aloe vera* leaf pulp (5 mg/ml and 10 mg/ml of

medium) for 1, 2, 4 and 6 hours. Unsupplemented control slices were used as control/s.

Assessment by Biochemical parameters

At the end of planned period of incubation, liver slices were removed from the vials, homogenized and utilized for assessment of TBA Reactive Substances [18] and glutathione contents [19]. However the medium was used to test the amount of leakage of alanine transaminase [20], aspartate transaminase [20] and alkaline phosphatase [21].

Results:

One hour:

Table No.1 presents the results noted in the medium and liver slices (LS), at the end of one hour of incubation. Insignificant alterations in AST, ALT and ALP were observed in presence of concentrations of APAP and APAP + 5 mg /ml *Aloe vera* leaf gel (AVG-5) and APAP + 10 mg/ml *Aloe vera* leaf gel (AVG-10) except liver slices (LS) incubated with 1.2 X 10^{-3} M concentration of APAP (APAP-1.2).TBARS measured at the end of one hour showed marginally significant increase when the LS incubated with 1.0 X 10^{-3} M concentration of APAP (APAP-1.0) and a dose dependent increase was seen in LS incubated in presence of APAP-1.2 (Table No. 5). However LS incubated in presence of APAP-0.6 + AVG 10 exhibited a marginally significant decrease in TBARS. Glutathione contents of APAP controls of all concentrations studied in the present project showed significant reductions when compared with controls (Table No. 6).

Two hours:

After two hours period of incubation, AST levels of LS treated with two high concentrations of APAP showed marginally significant increase, while ALT appeared a more sensitive parameter showing significant increase in presence of APAP while in presence of APAP + AVG, ALT levels were reduced in a dose dependent manner, however AVG-10 significantly decreased ALT levels when compared with control (Table No. 2). TBARS measured at this time was significantly high in presence of APAP. AVG-5 showed decline in TBARS levels elevated by APAP only for two lower concentrations of APAP, while AVG-10 showed further reduction depending upon the concentration of APAP (Table No. 5). Significant decline in glutathione contents of LS were noted in presence of all concentrations of APAP. AVG-5 restored the levels near control when in combination with APAP-0.6. AVG-10 however restored the glutathione levels near controls (Table No. 6)

Four hours

When LS were incubated for four hours in presence of APAP, all concentrations showed significant increases in the levels of AST, ALT and ALP. AVG-5 as well as AVG-10 in presence of all concentrations of APAP showed declined levels of AST, ALT and ALP when compared with the controls. ALT and ALP levels were near normal in presence of AVG-5 while AVG-10 further declined them significantly in presence of APAP-0.6, APAP-0.8 and APAP-1.0. Other remained unaltered or near control (Table No. 3). TBARS measured at this stage were significantly high in presence of APAP while

in presence of AVG-5 and APAP-0.6 to 1.0, the levels were near control. In the LS incubated with APAP-1.2 + AVG-5, marginally significant levels of TBARS were observed. However in presence of APAP 0.6 to 1.2 and AVG-10 the LS showed marginally declined levels of TBARS (Table No. 5). Glutathione levels were highly depleted in presence of all concentrations of APAP. AVG-5 showed elevated levels of glutathione when compared with their respective APAP controls but were low when compared with controls. In presence of all concentrations of APAP and AVG-10 the LS showed the glutathione levels near or slightly above control (Table No. 6).

Six hours

Six hours incubation of LS in presence of APAP showed high levels of AST, ALT and ALP. AST levels were near normal in presence of APAP and AVG-5 as well as AVG-10. However for ALT only AVG-10 showed the levels near normal in presence of all concentrations of APAP. ALP levels were near normal in presence of APAP+AVG-5 but were further declined in presence of AVG-10 + APAP (Table No. 4). For TBARS and glutathione a trend similar to that observed at the end of four hours was repeated by the LS (Table No. 5 and 6).

Discussion:

APAP treatment significantly increased the serum enzyme levels, *viz* AST, ALT and ALP indicating chemical induced hepatocellular toxicity. Treatment with AVG restored the liver enzyme parameters showing a concentration dependent effect. Some enzyme leakage by the lower concentrations of APAP (0.6×10^{-3} M and 0.8×10^{-3} M) were reduced by the low concentration of AVG-5 although the toxicity mediated by two higher concentrations of APAP 1.0×10^{-3} M and 1.2×10^{-3} M, was prevented only by the high concentrations (AVG-10).

Aloe vera leaf gel also consists of some lipid soluble vitamin tocopherol [22].The protective effect may be the result of stabilization of plasma membrane thereby preserving the structural integrity of cell as well as the repair of hepatic tissue damage caused by APAP metabolites. The hepatoprotective effect of AVG can be attributed to the inhibition of reaction involved in the formation of reactive metabolites and possibly due to its radical scavenging activity as postulated by [14]

AVG effectively prevented APAP induced lipid peroxidation in liver cells (Table No. 5). In present study, elevated level of TBARS observed in APAP treated liver slices indicates excessive formation of free radicals and activation of lipid peroxidation system resulting in hepatic damage. The significant decline in the concentration of these constituents in the liver slices incubated with APAP and AVG indicate anti-lipid peroxidative effects of AVG.

Decline in glutathione content in the liver slices incubated in presence of APAP and its levels towards near normancy/above normancy in the liver slices incubated with APAP + AVG also reveal anti-lipid peroxidative effect of AVG (Table No. 6). In the present study, decline in the level of glutathione observed in the liver slices incubated with APAP is a clear

manifestation of excessive formation of free radicals and activation of lipid peroxidation system resulting in tissue damage. The increase in the concentration of glutathione when liver slices were incubated with APAP + AVG indicate antioxidant effect of aloe vera leaf gel.

The results of studies of the antioxidant hepatoprotective property of AVG using rat liver slice model *in vitro* indicate the *in vitro* free radical scavenging activity of AVG is concentration dependent. The exact mechanism of radical scavenging activity of AVG is presently unknown. The superoxide anion scavenging activity of the AVG may be due to the direct scavenging of superoxide anion generated from NAPQI-mediated mitochondrial injury. Langmead and co-workers (2004) [23] report anti-oxidant effects of aloe vera leaf gel exhibited in two cell-free in vitro systems when the gel was incubated with inflamed colorectal mucosal biopsies. It is reported that the phenolic component found to be present in aloe vera leaf gel may be responsible for anti-oxidant effects which was able to scavenge superoxide radicals. So partly, the protective effect may be due to the scavenging the superoxide radical by the phenolic component.

The effect may also be mediated by trapping the electron released for the generation of superoxide anion or by reducing superoxide anion to a non-radical. Also there may be a possibility of removal of oxygen from the reaction mixture which cannot be ignored. The free radical scavenging and TBARS inhibiting activity might also be mediated through the ability of the AVG in scavenging the generated radicals or through the reductive efficiency of some components and/or biotransformed component present in the aloe vera gel, which possesses an ability to improve the bioavailability of co-administered vitamins in human subjects due to its absorption enhancing effects [24]. The AVG contains vitamin B1, B2, B6, C, β-carotene, choline, folic acid, α-tocopherol [22] Tocopherol is known as an antioxidant and can protect the cells from injury associated with LPO induced by APAP by lowering it however the tocopherol alone was found to be ineffective in reducing the LPO to normal levels [25]. So partial protection of the hepatocytes by the AVG may be due to small amounts of tocopherol present in the gel which may be contributing to enhance the membrane stability being lipid soluble. Additionally the protective efficacy of tocopherol is enhanced in presence of Ascorbic acid. Nwanjo and co-workers [26] have shown protective role of α-Tocopherol and ascorbic acid supplementation on Halofantrine - induced hepatotoxicity in rats which also involves oxidative stress. This may help to explain in part why the mechanism of protection by AVG is concentration dependent (both, the concentration of APAP and Aloe vera leaf gel)

The mechanism of protection against oxidative stress can either be the decreased production of free radical derivatives or due to the antioxidant activity of phenolic component present in the Aloe vera leaf gel or a cumulative effect of phenolic component, tocopherol and other vitamins, alongwith the components which are

responsible for increasing the bioavailability of the vitamins.

Although over 75 active ingredients from the inner gel of aloe vera have been identified, therapeutic effects have not been correlated well with each individual component [27]. Many of the medicinal effects of aloe leaf extracts have been attributed to the polysaccharides found in the inner leaf parenchymatous tissues [28, 29] but it is believed that these biological activities should be assigned to a synergistic action of the compounds contained therein rather than a single chemical substance. Hence it is possible that the anti-lipid peroxidative and anti-oxidant efficacy associated with AVG which is responsible for protection of liver slices against the APAP mediated oxidative stress may be a sum of activity of all/multiple bioactive components present in the gel rather than a single effective active component.

Aloe vera leaf gel contains three malic acid acylated carbohydrates: veracylglucans A, B, and C. All three compounds demonstrate anti-inflammatory effects [30]. As the APAP is known to have weak anti-inflammatory action [12] combination and co-treatment of APAP and *Aloe vera* leaf gel can be postulated as a suitable method where there is a need of relieving pain, fever as well as suppression of inflammation after proper understanding of mechanism of action of aloe vera leaf gel and APAP, as well as possible interactions between their biotransformed metabolites.

Conclusion:

From the results, it can be concluded that the AVG prevents hepatocyte injury induced by APAP mediated oxidative stress in rat liver slices by neutralizing the oxidative stress for the concentrations studied in the present project. The hepatoprotective effect of aloe vera gel might be due to the presence antioxidant phenolic component or vitamin contents or a cumulative effect. The possible mechanism of hepatoprotective effect of AVG might be due to its anitoxidant effect. Further study is needed to identify and isolate the active principle of Aloe vera, which may be offering antioxidant and hepatoprotective properties . The present investigation encourages further studies to determine the active compounds that are responsible for the hepatoprotective effects and the mechanism of action involved in the antihepatotoxic effect. In vivo and detailed clinical studies are required before postulating use of the antioxidant activity of Aloe vera leaf gel against APAP mediated hepatotoxicity as well as for suggested co-treatment of APAP and Aloe vera leaf gel for reducing all, the pain, fever and inflammation.

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Table No. 1: *Aloe vera* leaf pulp (5 mg/ml and 10 mg /ml) mediated protective alterations in AST, ALT and ALP in presence of different concentrations of APAP at the end of 1 hr in rat liver slices (Values are expressed as Units/dl)

Sr. no.	Test (End of one hour incubation)	AST	ALT	ALP
1	Control	14.32 ± 0.53	16.05 ± 1.04	82.72 ± 6.29
2	APAP 0.6 x 10^{-3} M	15.52 ± 0.7	16.58 ± 0.69	86.42 ± 5.96
3	APAP 0.8 x 10^{-3} M	16.01 ± 0.62	17.16 ± 1.25	90.36 ± 8.04
4	APAP 1.0 x 10^{-3} M	16.49 ± 1.29	17.34 ± 1.11	90.31 ± 7.41
5	APAP 1.2 x 10^{-3} M	16.98 ± 0.92	17.92 ± 1.20[a]	91.24 ± 6.68[a]
6	APAP 0.6 x 10^{-3} M + AV 5 mg/ml	14.91 ± 1.09	15.39 ± 1.46	80.53 ± 3.88
7	APAP 0.8 x 10^{-3} M + AV 5 mg/ml	15.19 ± 0.56	15.87 ± 1.29	74.56 ± 3.28
8	APAP 1.0 x 10^{-3} M + AV 5 mg/ml	15.47 ± 1.01	16.35 ± 1.28	78.59 ± 3.85
9	APAP 1.2 x 10^{-3} M + AV 5 mg/ml	15.65 ± 0.88	16.83 ± 0.91	81.62 ± 3.18
10	APAP 0.6 x 10^{-3} M + AV 10 mg/ml	13.39 ± 0.8	14.04 ± 0.95[a]	82.09 ± 3.55
11	APAP 0.8 x 10^{-3} M + AV 10 mg/ml	13.51 ± 1.28	14.45 ± 0.87	83.52 ± 4.38
12	APAP 1.0 x 10^{-3} M + AV 10 mg/ml	13.59 ± 1.1	14.86 ± 0.78	76.95 ± 5.29
13	APAP 1.2 x 10^{-3} M + AV 10 mg/ml	14.3 ± 0.4	15.27 ± 0.84	79.53 ± 6.19
14	AV 5 mg/ml	14.71 ± 1.12	15.21 ± 1.13	80.95 ± 3.89
15	AV 10 mg/ml	14.33 ± 1.09	14.81 ± 0.83	80.25 ± 4.65

Values are mean ± SE of 6 sets *p – values : a < 0.05, b < 0.01, c < 0.001 vs. control*

Table No. 2: *Aloe vera* leaf pulp (5 mg/ml and 10 mg /ml) mediated protective alterations in AST, ALT and ALP in presence of different concentrations of APAP at the end of 2 hrs in rat liver slices (Values are expressed as Units/dl)

Sr. no.	Test (End of two hour incubation)	AST	ALT	ALP
1	Control	23.82 ± 0.93	19.74 ± 1.28	101.74 ± 8.95
2	APAP 0.6 x 10^{-3} M	24.86 ± 1.94	22.85 ± 1.20[b]	106.30 ± 8.18
3	APAP 0.8 x 10^{-3} M	25.59 ± 1.38	27.57 ± 1.63[c]	131.47 ± 8.68[c]
4	APAP 1.0 x 10^{-3} M	26.37 ± 1.92[a]	28.41 ± 2.70[c]	138.58 ± 7.62[c]
5	APAP 1.2 x 10^{-3} M	27.14 ± 1.00[a]	29.24 ± 2.37[c]	143.91 ± 6.33[c]
6	APAP 0.6 x 10^{-3} M + AV 5 mg/ml	23.84 ± 1.55	22.11 ± 1.72[a]	101.37 ± 4.97
7	APAP 0.8 x 10^{-3} M + AV 5 mg/ml	24.28 ± 1.36	22.80 ± 1.23[b]	107.17 ± 4.18
8	APAP 1.0 x 10^{-3} M + AV 5 mg/ml	24.73 ± 1.46	23.50 ± 1.72[b]	112.96 ± 6.66[a]
9	APAP 1.2 x 10^{-3} M + AV 5 mg/ml	25.03 ± 1.88	24.19 ± 1.57[c]	117.31 ± 8.09[b]
10	APAP 0.6 x 10^{-3} M + AV 10 mg/ml	21.74 ± 1.76	16.66 ± 0.93[b]	65.04 ± 5.14[c]
11	APAP 0.8 x 10^{-3} M + AV 10 mg/ml	21.61 ± 0.61	14.56 ± 0.86[c]	68.75 ± 6.12[c]
12	APAP 1.0 x 10^{-3} M + AV 10 mg/ml	21.74 ± 1.02	15.00 ± 1.43[c]	72.47 ± 2.97[c]
13	APAP 1.2 x 10^{-3} M + AV 10 mg/ml	22.86 ± 1.69	15.44 ± 1.25[c]	75.26 ± 5.34[c]
14	AV 5 mg/ml	23.53 ± 0.92	18.71 ± 1.22	99.57 ± 5.08
15	AV 10 mg/ml	22.91 ± 1.12	18.22 ± 0.51	98.70 ± 6.02

Values are mean ± SE of 6 sets *p – values : a < 0.05, b < 0.01, c < 0.001 vs. control*

Table No. 3: *Aloe vera* leaf pulp (5 mg/ml and 10 mg /ml) mediated protective alterations in AST, ALT and ALP in presence of different concentrations of APAP at the end of 4 hrs in rat liver slices (Values are expressed as Units/dl)

Sr. no.	Test (End of four hour incubation)	AST	ALT	ALP
1	Control	27.03 ± 1.76	21.5 ± 1.05	110.79 ± 8.64
2	APAP 0.6 x 10^{-3} M	31.67 ± 1.77[b]	29.16 ± 1.14[c]	123.3 ± 6.66[a]
3	APAP 0.8 x 10^{-3} M	37.72 ± 2.23[c]	40.64 ± 2.40[c]	146.17 ± 12.86[c]
4	APAP 1.0 x 10^{-3} M	38.87 ± 3.30[c]	41.87 ± 2.89[c]	145.7 ± 12.07[c]
5	APAP 1.2 x 10^{-3} M	40.01 ± 3.24[c]	43.11 ± 3.41[c]	152.84 ± 10.80[c]
6	APAP 0.6 x 10^{-3} M + AV 5 mg/ml	27.88 ± 0.78	23.77 ± 2.20[a]	121.9 ± 7.78[a]
7	APAP 0.8 x 10^{-3} M + AV 5 mg/ml	28.4 ± 1.33	24.67 ± 1.22[a]	129.43 ± 13.25[b]
8	APAP 1.0 x 10^{-3} M + AV 5 mg/ml	28.92 ± 2.14	24.57 ± 2.17[a]	136.97 ± 11.90[c]
9	APAP 1.2 x 10^{-3} M + AV 5 mg/ml	29.27 ± 1.14	25.47 ± 1.60[b]	142.62 ± 9.92[c]
10	APAP 0.6 x 10^{-3} M + AV 10 mg/ml	25.42 ± 1.25	20.39 ± 1.49	102.36 ± 3.15
11	APAP 0.8 x 10^{-3} M + AV 10 mg/ml	25.27 ± 1.49	20.67 ± 1.79	108.78 ± 5.58
12	APAP 1.0 x 10^{-3} M + AV 10 mg/ml	25.42 ± 1.75	20.92 ± 1.84	105.2 ± 9.26
13	APAP 1.2 x 10^{-3} M + AV 10 mg/ml	26.73 ± 2.17	21.68 ± 1.39	105.02 ± 5.07
14	AV 5 mg/ml	25.67 ± 0.77	20.01 ± 1.3	101.1 ± 5.74
15	AV 10 mg/ml	22.91 ± 0.87	18.22 ± 1.33[a]	98.7 ± 5.82[a]

Values are mean ± SE of 6 sets

p – values : a < 0.05, b < 0.01, c < 0.001 vs. control

Table No. 4: *Aloe vera* leaf pulp (5 mg/ml and 10 mg /ml) mediated protective alterations in AST, ALT and ALP in presence of different concentrations of APAP at the end of 6 hrs in rat liver slices (Values are expressed as Units/dl)

Sr. no.	Test (end of four hour incubation)	AST	ALT	ALP
1	Control	29.42 ± 1.49	31.45 ± 0.88	162.12 ± 9.89
2	APAP 0.6 x 10^{-3} M	33.46 ± 1.79[a]	36.42 ± 1.71[b]	169.38 ± 12.03[a]
3	APAP 0.8 x 10^{-3} M	34.51 ± 2.16[b]	37.55 ± 2.78[b]	179.06 ± 12.71[b]
4	APAP 1.0 x 10^{-3} M	37.55 ± 2.62[c]	38.69 ± 1.51[c]	188.74 ± 9.81[c]
5	APAP 1.2 x 10^{-3} M	38.60 ± 0.93[c]	39.83 ± 1.95[c]	196.00 ± 11.56[c]
6	APAP 0.6 x 10^{-3} M + AV 5 mg/ml	29.22 ± 1.11	30.16 ± 1.78	150.69 ± 11.00
7	APAP 0.8 x 10^{-3} M + AV 5 mg/ml	29.76 ± 1.99	31.10 ± 2.15	159.30 ± 10.35
8	APAP 1.0 x 10^{-3} M + AV 5 mg/ml	30.31 ± 1.73	32.04 ± 2.60	167.91 ± 9.40
9	APAP 1.2 x 10^{-3} M + AV 5 mg/ml	30.68 ± 1.81	32.98 ± 2.41	174.37 ± 10.29
10	APAP 0.6 x 10^{-3} M + AV 10 mg/ml	26.65 ± 1.73	25.57 ± 1.66[b]	130.72 ± 9.02[b]
11	APAP 0.8 x 10^{-3} M + AV 10 mg/ml	26.49 ± 1.88	26.37 ± 1.48[b]	138.19 ± 10.92[a]
12	APAP 1.0 x 10^{-3} M + AV 10 mg/ml	26.65 ± 0.75	27.17 ± 1.60[a]	145.66 ± 12.96[a]
13	APAP 1.2 x 10^{-3} M + AV 10 mg/ml	28.02 ± 1.32	27.97 ± 2.66	151.26 ± 6.20
14	AV 5 mg/ml	28.84 ± 2.13	29.82 ± 1.97	158.66 ± 11.27
15	AV 10 mg/ml	28.08 ± 1.10	29.03 ± 1.89	157.28 ± 8.02

Values are mean ± SE of 6 sets *p – values : a < 0.05, b < 0.01, c < 0.001 vs. control*

Table No. 5: *Aloe vera* leaf pulp (5 mg/ml and 10 mg /ml) mediated protective alterations in TBARS in presence of different concentrations of APAP at various time durations in rat liver slices (Values expressed as µ mols /gm wet wt of tissue ± SE)

Test / Incubation period	One hour	Two hour	Four hour	Six hours
Control	29.47 ± 2.04	32.85 ± 1.58	36.36 ± 2.85	37.93 ± 2.68
APAP 0.6 x 10^{-3} M	30.93 ± 2.21	38.10 ± 1.72[b]	41.86 ± 3.59[a]	43.66 ± 3.37[a]
APAP 0.8 x 10^{-3} M	31.09 ± 2.04	40.00 ± 1.59[c]	45.34 ± 2.93[c]	42.07 ± 2.76[a]
APAP 1.0 x 10^{-3} M	32.69 ± 2.15[a]	59.56 ± 1.67[c]	65.45 ± 2.20[c]	68.26 ± 2.06[c]
APAP 1.2 x 10^{-3} M	33.12 ± 3.16[b]	64.15 ± 2.45[c]	70.49 ± 2.58[c]	73.51 ± 2.42[c]
APAP 0.6 x 10^{-3} M + AV 5 mg/ml	30.60 ± 2.15	34.36 ± 1.67	37.76 ± 2.04	39.38 ± 1.92
APAP 0.8 x 10^{-3} M + AV 5 mg/ml	29.83 ± 2.99	33.50 ± 2.32	36.81 ± 2.61	38.39 ± 2.46
APAP 1.0 x 10^{-3} M + AV 5 mg/ml	30.85 ± 2.09	34.64 ± 1.62	38.07 ± 2.21	39.70 ± 2.08
APAP 1.2 x 10^{-3} M + AV 5 mg/ml	32.55 ± 2.02	36.55 ± 1.57[b]	40.16 ± 2.38[a]	41.89 ± 2.23[a]
APAP 0.6 x 10^{-3} M + AV 10 mg/ml	25.50 ± 2.08[a]	28.14 ± 1.62[a]	31.27 ± 2.72[a]	32.82 ± 2.56[a]
APAP 0.8 x 10^{-3} M + AV 10 mg/ml	26.52 ± 2.56	29.78 ± 1.99	32.72 ± 2.79[a]	34.13 ± 2.62[a]
APAP 1.0 x 10^{-3} M + AV 10 mg/ml	25.50 ± 1.84	28.64 ± 1.43[a]	31.47 ± 2.21[a]	32.22 ± 2.08[a]
APAP 1.2 x 10^{-3} M + AV 10 mg/ml	29.58 ± 2.70	33.22 ± 2.10	36.50 ± 2.80	38.07 ± 2.64
AV 5 mg/ml	28.56 ± 1.96	30.43 ± 1.52	35.34 ± 2.18	37.77 ± 2.05
AV 10 mg/ml	29.11 ± 1.99	30.10 ± 1.78	33.31 ± 1.53	36.73 ± 1.44

Values are mean ± SE of 6 sets *p – values : a < 0.05, b < 0.01, c < 0.001 vs. control*

Table No. 6: *Aloe vera* leaf pulp (5 mg/ml and 10 mg /ml) mediated protective alterations in Glutatione in presence of different concentrations of APAP at various time durations in rat liver slices (Values expressed as total Glutathione in liver µg per gm wet wt of liver)

Test / Incubation period	One hour	Two hour	Four hour	Six hours
Control	34.40 ± 2.76	35.02 ± 1.81	34.59 ± 1.76	34.21 ± 2.51
APAP 0.6 x 10^{-3} M	29.44 ± 2.48a	26.19 ± 1.96c	25.78 ± 1.91c	26.36 ± 2.72c
APAP 0.8 x 10^{-3} M	28.77 ± 2.85b	23.69 ± 1.82c	23.33 ± 1.77c	22.89 ± 2.52c
APAP 1.0 x 10^{-3} M	27.08 ± 2.13c	22.22 ± 1.91c	21.06 ± 1.86c	22.40 ± 2.64c
APAP 1.2 x 10^{-3} M	26.46 ± 2.50c	18.43 ± 2.81c	16.80 ± 1.29c	25.92 ± 1.89c
APAP 0.6 x 10^{-3} M + AV 5 mg/ml	31.85 ± 1.98	32.65 ± 1.91	29.13 ± 1.86b	29.60 ± 2.65a
APAP 0.8 x 10^{-3} M + AV 5 mg/ml	31.10 ± 2.54	29.53 ± 2.65c	28.36 ± 2.58b	25.71 ± 2.68c
APAP 1.0 x 10^{-3} M + AV 5 mg/ml	29.21 ± 2.14c	24.66 ± 1.85c	23.80 ± 1.80c	25.16 ± 2.57c
APAP 1.2 x 10^{-3} M + AV 5 mg/ml	29.64 ± 2.31	20.45 ± 1.80c	18.99 ± 1.75c	24.11 ± 2.49c
APAP 0.6 x 10^{-3} M + AV 10 mg/ml	35.59 ± 2.64	35.84 ± 1.85	37.13 ± 1.80	38.30 ± 2.56a
APAP 0.8 x 10^{-3} M + AV 10 mg/ml	35.72 ± 2.70	34.43 ± 2.28	35.89 ± 2.22	37.66 ± 2.16a
APAP 1.0 x 10^{-3} M + AV 10 mg/ml	33.55 ± 2.14	33.41 ± 1.63	34.88 ± 1.59	33.26 ± 2.26
APAP 1.2 x 10^{-3} M + AV 10 mg/ml	34.04 ± 2.72	33.22 ± 2.40	33.24 ± 2.33	32.55 ± 2.32
AV 5 mg/ml	35.08 ± 2.12	35.89 ± 1.74	35.76 ± 1.70	36.02 ± 2.42
AV 10 mg/ml	37.12 ± 1.48	37.03 ± 1.77	36.36 ± 1.98	36.78 ± 2.45

Values are mean ± SE of 6 sets

p – values : a < 0.05, b < 0.01, c < 0.001 vs. control