A Systematic Perspective on the Metabolic Interplay between Gallic Acid and Paracetamol.

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Abstract:

Acetaminophen (Paracetamol; APAP) is a widely utilized over-the-counter analgesic and antipyretic medication. However, elevated doses or chronic exposure lead to acute centrilobular hepatic necrosis via the accumulation of its toxic reactive intermediate, N-acetyl-p-benzoquinone imine (NAPQI). Gallic acid (3,4,5-trihydroxybenzoic acid), a major naturally occurring polyphenol in plants such as *Terminalia belerica*, green tea, and red wine, exhibits potent antioxidant, anti-inflammatory, antimutagenic, and hepatoprotective activities. This review presents a comprehensive assessment of the individual metabolic pathways of acetaminophen and gallic acid, detailing phase I bioactivation, phase II conjugation (glucuronidation, sulfation, and methylation), and renal elimination. Furthermore, it delineates the combined metabolic interplay and mechanistic pathways through which gallic acid mitigates APAP-induced hepatotoxicity by suppressing Cytochrome P450 (CYP2E1) bioactivation, scavenging reactive oxygen species (ROS), restoring intracellular glutathione (GSH) reserves, stabilizing mitochondrial membranes, and regulating biochemical markers.

Keywords: *Gallic Acid, Acetaminophen, Hepatotoxicity, NAPQI, Phase II Conjugation, Glutathione, CYP2E1, Oxidative Stress.*

1. Introduction:

Acetaminophen (Paracetamol or APAP or N-acetyl p-aminophenol) is integrated into over 800 prescription and over-the-counter therapeutic formulations worldwide (Dowdy *et al.,* 2003). While safe within recommended clinical dosages, accidental or intentional overdose represents the single most common cause of drug-induced liver injury (DILI) and acute liver failure in developed countries (Prescott, 1983; McGill & Jaeschke, 2013). The primary lesion in paracetamol poisoning is acute centrilobular hepatic necrosis, driven by reactive metabolite accumulation, oxidative stress, depletion of intracellular adenosine triphosphate (ATP), and altered Ca2+ homeostasis.

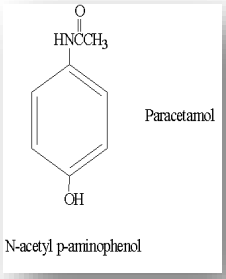


Fig.: Structure of Acetaminophen

Natural bioactive compounds that modulate drug-metabolizing enzymes and suppress oxidative stress offer significant therapeutic potential in protecting against chemically induced liver injury. Among naturally occurring polyphenols, gallic acid (3,4,5-trihydroxybenzoic acid) has attracted substantial scientific interest. Gallic acid is a natural phenolic acid obtained from the hydrolysis of tannins and represents up to 82% of total phenolics in dietary sources such as green tea and wine (Carlos

et al., 2001; Landrault *et al.,* 2001). It is a key active constituent of *Terminalia belerica* fruits, traditional botanical remedies, and various dietary items (Anand *et al.,* 1997; Hiroshi *et al.,* 2001).

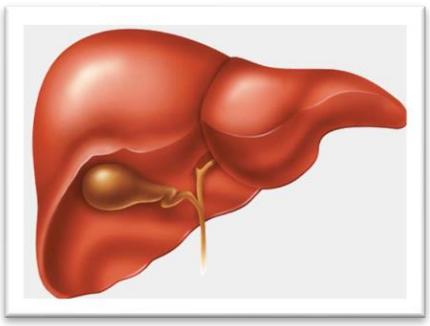


Fig.: **Normal Liver**

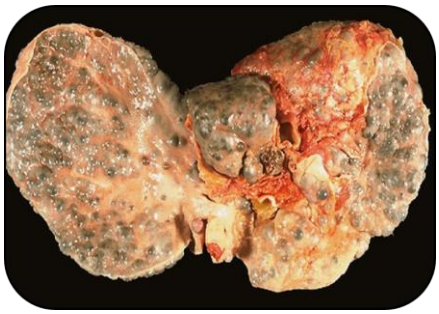


Fig.: Damaged Liver

This paper reviews the individual metabolic fates of paracetamol and gallic acid, evaluates their structural and functional bioactivities, and details the combined metabolic interactions that underpin the hepatoprotective efficacy of gallic acid against APAP-induced hepatotoxicity.

2. Pharmacological Profile and Bioactivity of Gallic Acid:

Gallic acid (3, 4, 5-trihydroxybenzoic acid) is an active principle of the fruit and it shows bile stimulating activities. Its derivatives are biologically active compounds, which are present in various plants (Kahkonen *et al.,* 1999; Lee *et al.,* 2000). It is especially abundant in green tea (Hiroshi *et al.,* 2001) and has antimutagenic (Shiraki *et al*., 1994), anticarcinogenic (Inoue *et al*., 1995) and anti-inflammatory activities (Siranoush *et al.,* 2001). It is a strong natural antioxidant (Khan *et al.,* 2000; Zheng and Wang, 2001). Effectiveness of this compound as antioxidants can be explained by hydrophobic binding to membrane lipids. It also showed a prooxidant effect by stimulating the copper dependent oxidation of low-density lipoprotein. 4-O-methyl gallic acid (4OMGA) has been reported as the main metabolite of gallic acid in rats (Shahrzad *et al*., 2001). Gallic acid and its alkylesters are used as food additives in order to scavenge reactive oxygen species and show cytotoxic and antiproliferative effects (Nakagawa *et al*., 1997). These effects of gallate compounds are presumed to be not due to the antioxidative property, but due to the prooxidant action by eliciting reactive oxygen species (Serrano *et al*., 1998). In a preliminary report gallic acid isolated from fraction of TB5 was evaluated for hepatoprotective efficacy (Anand *et al.,* 1997). In the present study we have analyzed gallic acid as hepatoprotective agent.

3,4,5-trihydroxybenzoic acid is a main constituent of *Terminalia belerica* and naturally occurring plant phenol obtained by the hydrolysis of tannins and shows some pharmacological activities (Anand *et al*., 1997). It is wide spread in plant foods and

beverages such as tea and wine and represented 82% total phenolics (Carlos *et al.,* 2001; Landrault *et al.,* 2001). In screening anticancerous agents, gallic acid is found to show cytotoxicity against all cancerous cells (Hiroshi *et al.,* 2001; Kouichi *et al*., 2000). It is a strong chelating agent and forms complex of high stability with iron (Li *et al.,* 2000). It has great interest in arteriosclerosis prevention (Abella and Chalas, 1997). It is known to induce apoptosis in cancer cells at lower IC 50 (values compared with values for normal cells) (Kazuto *et al.,* 2001). It is a structural unit of tannin, have antibacterial (Hisanori *et al.,* 2001), antimutagenic (Leva *et al.,* 2002), anticarcinogenic (Takamasa *et al.,* 2001; Miki *et al.,* 2001), anti-inflammatory (Siranoush *et al*., 2001), antimicrobial (Penna *et al.,* 2001), analgesic (Krogh *et al.,* 2000) and antiviral properties (Eisaku *et al.,* 2002).

Scientists investigated the antioxidant activity of gallic acid (Eduardo *et al.,* 2001; Naoko *et al*., 2001). It was observed that greater the number of hydroxyls linked to the aromatic ring, greater the antioxidant activity of the analyzed compounds (Dayani *et al.,* 2001). Increased length of the alkyl chain in gallate esters enhanced the inhibitory effect on the iron mediated lipid peroxidation. The concentrations of alkyl gallates required for the inhibition of the formation of thiobarbituric acid reactive substances were dependent on the hydrophobicity of the compounds (Ou-Boxin *et al.,* 2002). Reactive oxygen species (ROS) are known to be involved in various diseases including carcinogenesis, atherosclerosis and inflammatory disorders, cardiovascular disease, cancer diabetes and aging (Aloki *et al.,* 2001). Free radicals occur as a natural consequence of cell metabolism and also produces oxidative stress (Terasaka *et al.,* 2000). Antioxidant capacity of gallic acid against hydroxyl, azide and superoxide radicals has also been reported (Bors and Michel, 1999; Pulido *et al.,* 2000; Metelitza *et al.,* 2001).

The structure activity relationship study revealed that methylation of the phenolic hydroxyl group and esterification of the carboxyl group, markedly reduces cytotoxic activity (Li *et al*., 1999). It showed the specificity between cancerous and primary hepatocytes thus posses no

cytotoxicity against primary cultured rat hepatocytes and macrophages (Inoue *et al.,* 1995). Isuzugawa *et al*., (2001) confirmed that gallic acid generates hydrogen peroxides, which is a causal substance in the induction of apoptosis, *via* superoxide anion stemming from oxidation of gallic acid itself. Rajalakshmi, (2001) suggested that acute administration of gallic acid even at a dose as high as 5 g/kg body weight did not produce any sign of toxicity. Further, no appreciable change was noted in

the various biochemical parameters such as transaminases as well as many serum constituents such as protein, cholesterol, urea and bilirubin.

Gallic acid derivatives are biologically active compounds widespread in plant foods and medicinal flora (Kahkonen *et al.,* 1999; Lee *et al.,* 2000). Structurally characterized by three hydroxyl groups attached to an aromatic ring, gallic acid acts as a powerful antioxidant and free-radical scavenger (Khan *et al.,* 2000; Zheng & Wang, 2001). Structure-activity relationship (SAR) investigations demonstrate that a greater number of free hydroxyl groups linked to the aromatic ring directly enhances antioxidant potency (Dayani *et al.,* 2001).

3. Metabolism of Acetaminophen (Paracetamol):

Under normal therapeutic dosing, paracetamol is predominantly metabolized in the liver via Phase II biotransformation pathways. Approximately 50-60% undergoes glucuronidation via UDP-glucuronosyltransferases (UGTs), and 25-35% undergoes sulfation via sulfotransferases (SULTs) (Prescott, 1983; McGill & Jaeschke, 2013). These non-toxic hydrophilic conjugates are rapidly cleared by the kidneys and excreted in urine. A minor fraction (~5-10%) is metabolized by hepatic Cytochrome P450 enzymes primarily CYP2E1, with contributions from CYP3A4 and CYP1A2 to form the highly reactive, electrophilic intermediate N-acetyl-p-benzoquinone imine (NAPQI). Under physiological conditions, NAPQI is immediately detoxified through conjugation with reduced glutathione (GSH) by glutathione S-transferase (GST), producing non-toxic mercapturic acid and cysteine conjugates that are safely eliminated in urine.

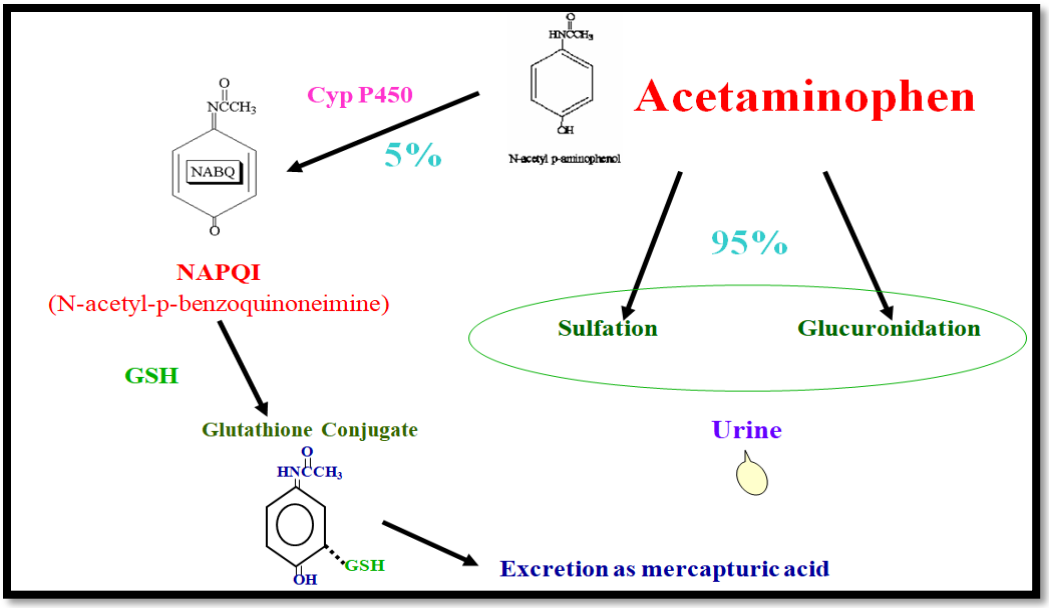


Fig.: Metabolism of Acetaminophen

In APAP overdose, the therapeutic glucuronidation and sulfation pathways become saturated. Consequently, an increased proportion of APAP is shunted into the Phase I CYP2E1 pathway, generating excessive NAPQI. Once hepatic GSH stores are depleted by >70-80%, unbound NAPQI covalently binds to cellular cysteinyl sulfhydryl groups on proteins and mitochondrial macromolecules. This induces severe oxidative stress, loss of mitochondrial membrane potential, ATP exhaustion, and widespread centrilobular hepatic necrosis (Prescott, 1983; Dowdy *et al.,* 2003).

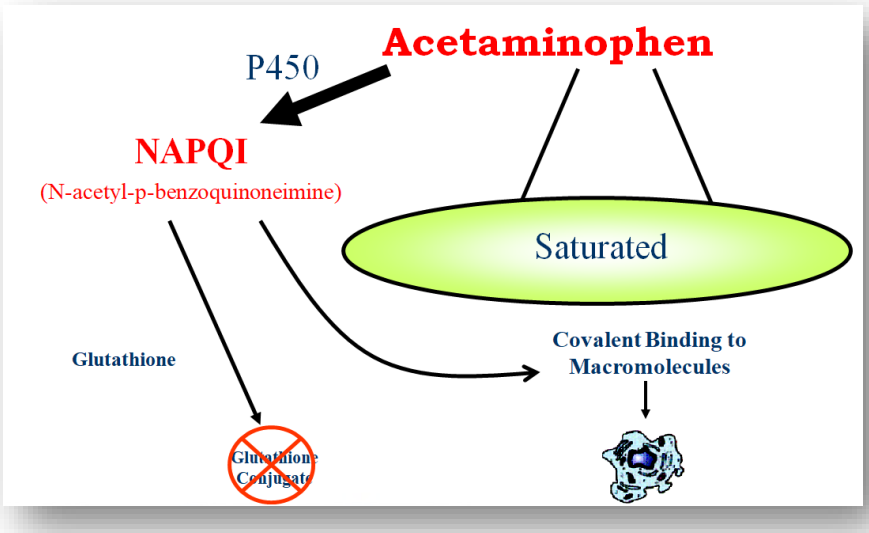
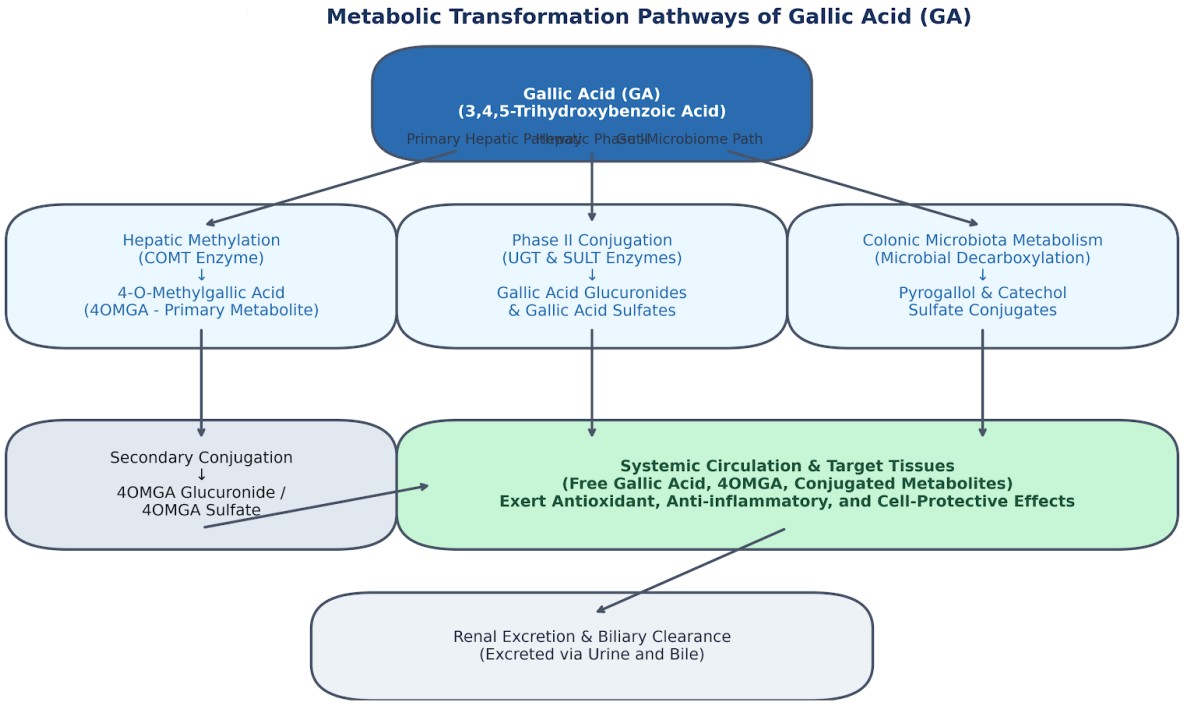


Fig.: Acetaminophen Toxicity and Cell Damage

4. Metabolism of Gallic Acid:

Following ingestion, gallic acid undergoes rapid absorption in the gastrointestinal tract and extensive biotransformation in the liver and gut mucosa. Shahrzad *et al.* (2001) established that 4-O-methylgallic acid (4OMGA) is the main circulating metabolite of gallic acid in both humans and rats, catalyzed by catechol-O-methyltransferase (COMT). Phase II enzymatic processes further convert gallic acid and 4OMGA into glucuronide and sulfate conjugates. In addition, colonic microflora hydrolyze complex gallotannins into free gallic acid, which can undergo microbial decarboxylation to pyrogallol, followed by systemic absorption and secondary sulfation. Free gallic acid, 4OMGA, and their respective Phase II conjugates circulate systemically to exert cellular antioxidant actions before being excreted via renal filtration and biliary secretion.

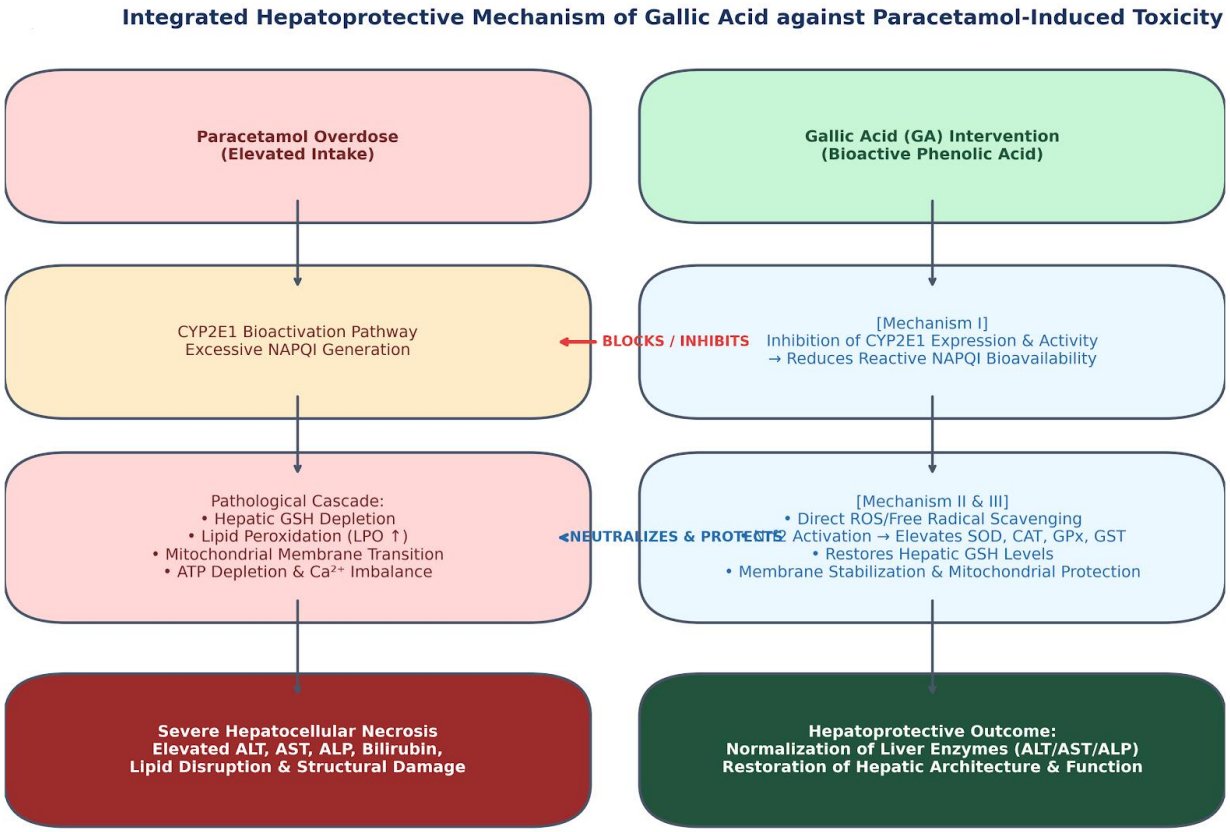


5. Possible Mechanism of Action and Hepatoprotective Mechanisms of Gallic acid:

Co-administration or post-treatment with gallic acid significantly mitigates acetaminophen-induced hepatotoxicity through three synergistic mechanisms:

1. Inhibition of Bioactivation (CYP Modulation): Gallic acid and phenolic constituents interfere with Phase I Cytochrome P450 enzymes specifically inhibiting CYP2E1 expression and catalytic activity.

This suppresses the bioactivation of APAP into reactive NAPQI, keeping NAPQI within levels manageable by endogenous detoxifying systems.



2. Radical Scavenging & GSH Preservation: Gallic acid directly neutralizes reactive oxygen species (ROS) such as hydroxyl and superoxide radicals, inhibiting lipid peroxidation (LPO). Furthermore, gallic acid activates the Nrf2 signaling pathway, upregulating antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and replenishing hepatic glutathione pools (Bors & Michel, 1999; Zand *et al.,* 2022).

3. Membrane Stabilization & Biochemical Normalization: Gallic acid exhibits cell-membrane-stabilizing properties, preventing mitochondrial permeability transition and maintaining structural hepatocyte integrity. Post-treatment significantly normalizes elevated serum biomarkers including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, urea, creatinine, cholesterol, and triglycerides (Anand *et al.,* 1997; Kumar & Singh, 2024).

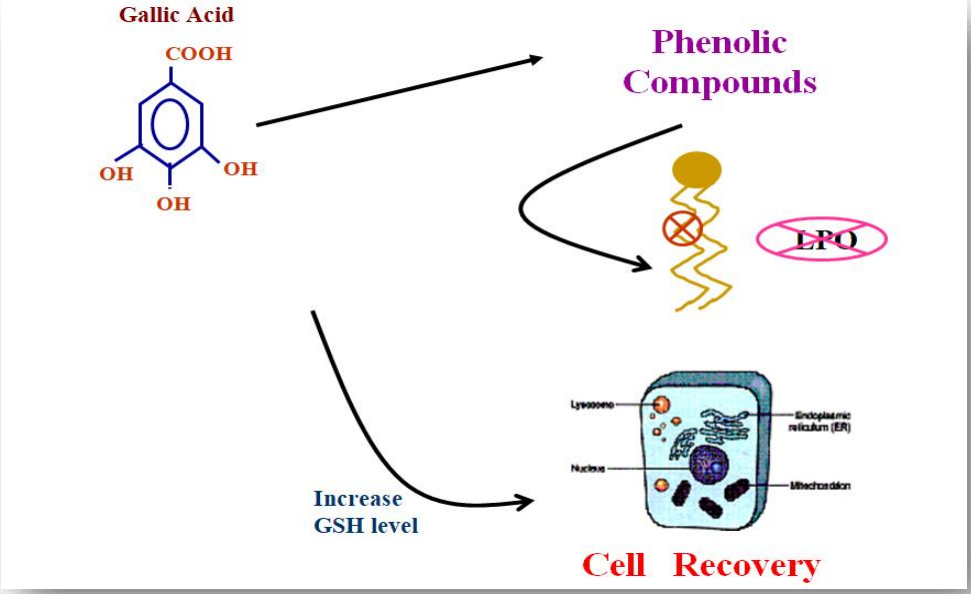


Fig.: Action and Hepatoprotective Mechanisms of Gallic acid

The acute toxicity produced by APAP was associated with a variety of biochemical abnormalities and these could usually be attributed to the release of intracellular constituents into the circulation. In addition, toxicants directly inhibit cellular proliferation, to induce oxidative stress, depleted adenosine triphosphatase level and alter Ca^{2+} homeostasis. Mitochondrial damage was a prominent feature and this was associated with early depression of mitochondrial function as shown by reduced respiration, energy metabolism and ATP content. The possible mechanisms of hepatoprotective action of TB extract may be due to their antioxidant activity as indicated by decrease in lipid peroxidation and increase of glutathione contents. Rest of the biochemical and histopathological parameters studied indicate the structural and functional integrity of the cells and provide further support to proposed mechanism of action. Results obtained strongly suggested that therapeutic agents in post treatment significantly inhibit the raised AST, ALT, ALP, urea, creatinine, bilirubin, cholesterol and triglycerides levels through modulation of phase I & phase II detoxification enzymes and elevation of antioxidant enzyme levels by inhibiting LPO.

6. Conclusion:

The acute toxicity produced by APAP overdose is intrinsically linked to GSH depletion, excessive NAPQI accumulation, oxidative failure, mitochondrial injury, and structural disruption of centrilobular liver architecture. Phenolic compounds, specifically gallic acid, exert profound hepatoprotective effects through a tripartite mechanism:

• Mechanism I: Inhibition of specific CYP isoenzymes (primarily CYP2E1) responsible for APAP bioactivation into NAPQI.

• Mechanism II: Direct membrane stabilization, prevention of lipid peroxidation, and radical scavenging.

• Mechanism III: Direct neutralization/conjugation of reactive metabolites, activation of Nrf2-mediated Phase II detoxification, and rapid restoration of cellular GSH levels.

These findings demonstrate that gallic acid serves as a highly effective natural therapeutic agent for protecting against and ameliorating acetaminophen-induced liver toxicity.

7. References

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