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THE AMELIORATIVE EFFECTS OF GUM ARABIC ON HISTOPATHOLOGICAL AND PHYSIOLOGICAL ALTERATIONS INDUCED BY MELAMINE TOXICITY IN SWISS ALBINO MICE

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Abstract

Herbal medicine is experiencing a significant resurgence as natural approach to health and wellness. Gum Arabic, derived from the sap of Acacia trees, is gaining attention due to its numerous health benefits. This study investigates the protective effects of Gum Arabic against histopathological and physiological alterations induced by melamine toxicity in Swiss albino mice. Melamine exposure has been linked to various toxicological effects, particularly in kidney. The research involved administering Gum Arabic to mice subjected to melamine dose-1 and dose-2 according to their body weight, followed by assessments of physiological parameters and histological changes in kidney tissues. Results indicated that Gum Arabic significantly mitigated the adverse effects of melamine, evidenced by improved histopathological outcomes and restored physiological functions i.e weight gain. The findings suggest that Gum Arabic may serve as a potential therapeutic agent in counteracting melamine-induced toxicity, highlighting its role in enhancing health and reducing oxidative stress in affected tissues.

Keyword: Body Weight, Gum Arabic, Melamine, Nephrotoxicity, Physiological Changes.

1. INTRODUCTION

Melamine, a nitrogen-rich organic compound widely used in the production of plastics and fertilizers, has garnered significant attention due to its health implications. Melamine is often illegally added to food products to falsely enhance protein content, as it contains a high level of nitrogen.

This practice has led to severe health consequences, particularly in vulnerable populations such as children and the elderly [1]. Several studies have investigated how melamine is transmitted to edible animal products. In one significant study, Calitz et al [2] colleagues examined the absorption and excretion rates of melamine in sheep fed 69 grams of melamine daily.

Their findings revealed that melamine was indeed deposited in various tissues, including meat, liver, and kidneys, with an apparent absorption rate of 76.7%.

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Additionally, researchers from Stellenbosch University found that when laying hens were given melamine-contaminated feed for ten days, the melamine transferred to their eggs. Analysis of the eggs showed that melamine predominantly accumulated in the albumin, highlighting the potential for dietary melamine to affect multiple animal products [3].

Research indicates that melamine exposure can result in acute kidney injury, nephrolithiasis (kidney stones), and other renal complications. A notable incident in 2008 involved melamine-contaminated infant formula, which resulted in over 294,000 children being affected, with several fatalities reported due to kidney-related issues [4].

Melamine is a chemical compound that poses significant health risks due to its entry into the food chain primarily through contaminated food products and animal feed. It can leach into food from plastics and resins, particularly affecting dairy products, and can accumulate in animals that consume contaminated feed, which then transfers to humans through meat and dairy consumption.

Environmental contamination also contributes to its presence in crops and water sources [5]. Exposure to melamine has been shown to induce severe histopathological changes, particularly affecting renal and hepatic tissues, leading to compromised organ function and overall health deterioration [6].

Gum Arabic, derived from the exudate of *Acacia senegal* and *Acacia seyal,* has a rich history in folk and herbal medicine, particularly in Sub-Saharan Africa and the Middle East. Its applications span various health benefits, making it a significant component in traditional remedies. It is valued for its digestive health benefits, acting as a prebiotic to support gut bacteria and alleviate gastrointestinal issues.

Additionally, it promotes oral hygiene by reducing plaque and gingivitis, exhibits anti-inflammatory properties beneficial for conditions like arthritis, and supports cardiovascular health by improving lipid profiles. Topically, it aids in wound healing and skin irritations, while its antibacterial properties help prevent infections. Historically, it has been used as a nutritional source, showcasing its versatility in both traditional remedies and modern health practices [7].

Gum Arabic has been extensively studied for its health benefits particularly its antioxidant and anti-inflammatory properties. Research indicates that Gum Arabic can significantly reduce oxidative stress and inflammation, which are critical factors in the pathogenesis of various toxicities, including those induced by melamine. For instance, Nemmar et al demonstrated that Gum Arabic effectively ameliorates impaired coagulation and cardiotoxicity resulting from exposure to harmful substances, showcasing its protective capabilities in animal models [8].

The US Food and Drug Administration considers Gum Arabic (GA) to be among the safest dietary fibers [9]. In previous studies GA has been found to lower blood pressure, reduce plasma cholesterol levels in rats, promote dental remineralization, exhibit antimicrobial properties, and enhance intestinal absorption to counteract diarrhea [10]. A clinical trial with 40 end-stage renal failure patients on regular hemodialysis demonstrated that daily

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oral supplementation with 30 grams of Gum Arabic (GA) for 12 weeks significantly increased total antioxidant capacity and decreased levels of the inflammatory marker C-reactive protein. These findings indicate that GA has strong antioxidative and anti-inflammatory properties, which can help alleviate the chronic inflammation associated with kidney disease and dialysis.

Given its longstanding use in traditional Sudanese medicine for treating chronic renal failure, GA appears to be a promising and safe therapeutic supplement for repairing kidney damage and improving outcomes in kidney disease patients [11]. Gum Arabic may play a crucial role in ameliorating the adverse effects of melamine toxicity. This study aims to investigate the protective effects of Gum Arabic on histopathological and weight alterations in Swiss albino mice subjected to melamine exposure.

2. MATERIALS AND METHODS

2.1 Chemicals

Melamine was obtained from Sigma-Aldrich Company. Gum Arabic was obtained from local market.

2.2 Experimental Design

A total of 100 healthy male Swiss albino mice (BALB/c), aged between 8 and 10 weeks and weighing between 20 and 32 grams, were utilized for this study. All mice were procured from a commercial source. The animals were housed in metal cages, each equipped with glass water bottles featuring stainless steel sipper tubes. The cages were lined with sawdust bedding, which was replaced biweekly. Environmental conditions were maintained at a temperature of 22 ± 2 °C and a humidity level of 50 ± 5%, with a 12-hour light and dark cycle. The mice were provided with standard rodent pellets and had continuous access to fresh water throughout the experimental period. Daily monitoring included weighing the mice and observing them for any signs of mortality or morbidity. Prior to the experiment, the animals were acclimatized to the laboratory environment for one week.

2.3 Melamine Dose Classification

LD50 of melamine was taken from literature i.e 3.3 g/kg per body weight [12]. Melamine dose for experimentation was categorized according to percentage of LD50:

Sub Lethal Dose 1: 25% of LD50 of melamine (0.825 mg/kg body weight)

Sub Lethal Dose 2: 50% of LD50 of melamine (1.65 mg/kg body weight)

2.4 Experimental Groups

After acclimatization, mice were randomly and equally distributed into five sub groups (20 animal each). Mice were observed daily for mortality and weighed at the start and end of study.

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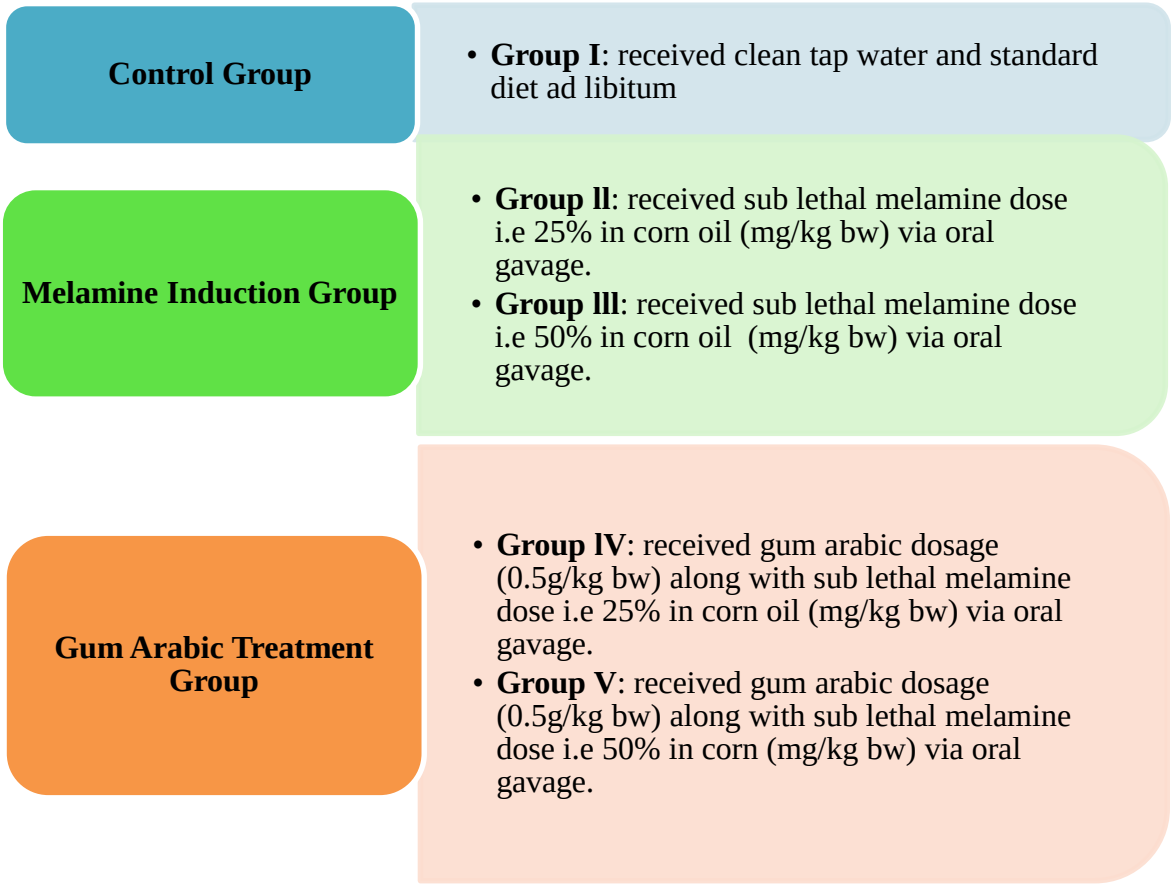

Figure 1: Experimental design of the study

2.5 Physiological and Behavioral Evaluation after Dosing

The general behavior of the mice was continuously monitored and recorded after dosing. It was done periodically during the first 24 h (with special attention given during the first 4 hours), and then daily thereafter, for a total of 28 days.

2.6 Anaesthesia and Scarification

Mice were anaesthetized after 28 days. The kidney from each mouse was carefully fixed in labeled jars according to their experimental groups, using a sufficient amount of 10% formalin. Formol saline was added to prevent organ decay.

2.7 Histopathological Examination

Standard histology laboratory techniques were employed to process the tissues. Parts of the removed mice kidneys were fixed in 10% buffered formalin, embedded in paraffin blocks using conventional paraffin-embedding techniques, processed into 5-µm-thick sections, stained with hematoxylin and eosin (H&E), and examined under a light microscope [13].

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3. RESULTS

The body weight changes were significant within the 28 days. The control group (G1) had an initial body weight ranging from 21 to 31 g, which increased to 25 to 35 g. In the MEL dose-1 group (G2), the initial body weight of 21 to 32 g decreased to 20.4 to 31 g. Similarly, in the MEL dose-2 group (G3), the initial body weight of 21 to 32 g decreased from 19.3 to 29.7 g. However, in the MEL dose-1 + Gum Arabic group (G4), the initial body weight of 21 to 32 g increased to 24.3 to 33.4 g, and in the MEL dose-2 + Gum Arabic group (G5), it increased to 23.2 to 34.2 g.

The control group (G1) exhibited normal behaviour and physiological responses, with no signs of distress, anxiety, or abnormal activity within 3 hours of dosing (Table 1). In contrast, the treatment group (G2), which received the first dose of melamine (MEL), displayed significant behavioural changes, including hyperactivity, increased anxiety, and excessive salivation (Table 2).

The treatment group (G3), after receiving the second dose of melamine (MEL dose-2), also showed hyperactivity and unusual behaviour of moving in circles (Table 3). Similarly, the treatment group (G4) exhibited restlessness, heavy breathing, and anxiety (Table 4). Lastly, the treatment group (G5) demonstrated a combination of salivation, anxiety, and hyperactivity (Table 5). These observations indicate that melamine toxicity induces various adverse physiological and behavioural effects in Swiss Albino mice.

The physiological changes observed in the treatment groups further highlighted the impact of melamine toxicity. In G2 (MEL Dose-1), 20% of mice exhibited anxiety and hyperactivity, 15% showed salivation and anxiety, and 65% were hyperactive as shown in fig 2. In G3 (MEL Dose-2), 15% of mice displayed salivation and anxiety, 20% showed hyperactivity and moving in circles, 20% exhibited anxiety and restlessness, 10% were anxious, and 35% were hyperactive (fig 3).

In G4 (MEL Dose-1 + GA), 35% of mice exhibited heavy breathing, 30% showed anxiety, and 35% were restless as observed in fig 4. In G5 (MEL Dose-2 + GA), 20% of mice exhibited heavy breathing, 50% showed anxiety, and 30% were restless as shown in fig 5. These findings suggest that Gum Arabic has a mitigating effect on some of the adverse impacts induced by melamine toxicity.

The outcomes of the current study showed no histopathological changes in the kidney of control (Fig. 6-A, 6-D) and melamine dose 1 group (Fig. 6-B). Similarly Gum Arabic consumption along with MEL dose-1 caused no significant change (Fig. 6-E).

On the other hand, majority of renal tubular epithelial cells were undergoing cellular swelling in kidney tissue of the of mice that were regularly given melamine dose-2 (Fig. 6-C). While Gum Arabic treatment confirmed its ameliorating nature when given along with MEL dose-2 (Fig. 6-F).

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Table 1: Particulars of Animals of Control Group (G1) within 3 hours of Dosing

Sr. No.	WEIGHT (g)		Period of Treatment (days)	Type of Treatment	Observations + Diagnosis
	Initial	Final			
1.	22	25	28	Nil	Normal
2.	24	29	28	Nil	Normal
3.	23	28	28	Nil	Normal
4.	28	34	28	Nil	Normal
5.	31	35	28	Nil	Normal
6.	25	30	28	Nil	Normal
7.	32	36	28	Nil	Normal
8	22	27	28	Nil	Normal
9.	21	27	28	Nil	Normal
10.	24	30	28	Nil	Normal
11.	23	30	28	Nil	Normal
12.	22	28	28	Nil	Normal
13.	29	34	28	Nil	Normal
14.	32	37	28	Nil	Normal
15.	23	27	28	Nil	Normal
16.	26	30	28	Nil	Normal
17.	21	26	28	Nil	Normal
18.	22	26	28	Nil	Normal
19.	24	29	28	Nil	Normal
20.	22	25	28	Nil	Normal

Table 2: Particulars of Animals of Treatment Group (G2)

Sr. No.	WEIGHT (g)		Period of Treatment (days)	Type of Treatment	Observations + Diagnosis
	Initial	Final			
1.	28	27.5	28	MEL dose-1	Hyper-active
2.	31	30.5	28	MEL dose-1	Hyper-active
3.	25	24.5	28	MEL dose-1	Hyper-active
4.	32	31	28	MEL dose-1	Hyper-active
5.	22	21	28	MEL dose-1	Hyper-active
6.	21	20.5	28	MEL dose-1	Hyper-active
7.	24	23.5	28	MEL dose-1	Anxiety + Hyper-active
8	23	22.7	28	MEL dose-1	Salivation + Anxiety
9.	22	21.4	28	MEL dose-1	Hyper-active
10.	29	28.3	28	MEL dose-1	Anxiety + Hyper-active
11.	22	21.2	28	MEL dose-1	Hyper-active
12.	24	23.6	28	MEL dose-1	Salivation + Anxiety
13.	23	22.7	28	MEL dose-1	Hyper-active
14.	22	21.4	28	MEL dose-1	Hyper-active
15.	24	23.5	28	MEL dose-1	Anxiety + Hyper-active
16.	32	31	28	MEL dose-1	Salivation + Anxiety
17.	23	22.6	28	MEL dose-1	Hyper-active
18.	26	25.5	28	MEL dose-1	Hyper-active
19.	21	20.4	28	MEL dose-1	Hyper-active
20.	22	21.5	28	MEL dose-1	Anxiety + Hyper-active

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Table 3: Particulars of Animals of Treatment Group (G3)

Sr. No.	WEIGHT (g)		Period of Treatment (days)	Type of Treatment	Observations + Diagnosis
	Initial	Final			
1.	22	20.5	28	MEL dose-2	Hyper-active
2.	24	22.4	28	MEL dose-2	Hyper-active + moving in circles
3.	23	21.8	28	MEL dose-2	Hyper-active+ moving in circles
4.	28	27	28	MEL dose-2	Hyper-active
5.	31	29.7	28	MEL dose-2	Anxiety
6.	25	23.6	28	MEL dose-2	Hyper-active + Moving in circles
7.	32	30.2	28	MEL dose-2	Anxiety + restlessness
8	22	20.7	28	MEL dose-2	Salivation + Anxiety
9.	21	20.3	28	MEL dose-2	Anxiety + restlessness
10.	24	22.4	28	MEL dose-2	Anxiety
11.	23	21.4	28	MEL dose-2	Anxiety + restlessness
12.	22	20.5	28	MEL dose-2	Salivation + Anxiety
13.	29	27	28	MEL dose-2	Hyper-active
14.	32	30	28	MEL dose-2	Hyper-active
15.	22	20.8	28	MEL dose-2	Anxiety + restlessness
16.	21	19.3	28	MEL dose-2	Salivation + Anxiety
17.	24	21.4	28	MEL dose-2	Hyper-active + moving in circles
18.	22	20.8	28	MEL dose-2	Hyper-active
19.	24	21.4	28	MEL dose-2	Hyper-active
20.	23	20.4	28	MEL dose-2	Hyper-active

Table 4: Particulars of Animals of Treatment Group (G4)

Sr. No.	WEIGHT (g)		Period of Treatment (days)	Type of Treatment	Observations + Diagnosis
	Initial	Final			
1.	24	26.3	28	MEL dose-1 + GA	Anxiety
2.	23	25.4	28	MEL dose-1+ GA	Restlessness
3.	22	24.6	28	MEL dose-1+ GA	Breathing heavily
4.	29	31.5	28	MEL dose-1+ GA	Restlessness
5.	32	33.4	28	MEL dose-1+ GA	Anxiety
6.	22	24.5	28	MEL dose-1+ GA	Restlessness
7.	21	24.3	28	MEL dose-1+ GA	Restlessness
8	24	27.2	28	MEL dose-1+ GA	Restlessness
9.	22	25.8	28	MEL dose-1+ GA	Anxiety
10.	24	26.5	28	MEL dose-1+ GA	Anxiety
11.	22	25.4	28	MEL dose-1+ GA	Breathing heavily
12.	24	26.6	28	MEL dose-1+ GA	Breathing heavily
13.	23	25.5	28	MEL dose-1+ GA	Anxiety
14.	28	30.6	28	MEL dose-1+ GA	Breathing heavily
15.	31	33.4	28	MEL dose-1+ GA	Restlessness
16.	25	28.7	28	MEL dose-1+ GA	Restlessness
17.	32	35.4	28	MEL dose-1 + GA	Anxiety
18.	22	25.2	28	MEL dose-1+ GA	Breathing heavily
19.	21	24.4	28	MEL dose-1+ GA	Breathing heavily
20.	22	25.3	28	MEL dose-1+ GA	Breathing heavily

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Table 5: Particulars of Animals of Treatment Group (G5)

Sr. No.	WEIGHT (g)		Period of Treatment (days)	Type of Treatment	Observations + Diagnosis
	Initial	Final			
1.	29	30.4	28	MEL dose-2 + GA	Salivation + Anxiety
2.	32	33.5	28	MEL dose-2+ GA	Hyper-active
3.	22	23.8	28	MEL dose-2+ GA	Hyper-active
4.	21	23.2	28	MEL dose-2+ GA	Salivation + Anxiety
5.	24	25.6	28	MEL dose-2+ GA	Hyper-active
6.	22	24.5	28	MEL dose-2+ GA	Salivation + Anxiety
7.	24	25.8	28	MEL dose-2+ GA	Hyper-active
8	22	24.6	28	MEL dose-2+ GA	Anxiety + Hyper-active
9.	24	26.8	28	MEL dose-2+ GA	Hyper-active
10.	23	25.7	28	MEL dose-2+ GA	Hyper-active
11.	24	24.5	28	MEL dose-2+ GA	Hyper-active
12.	23	25.9	28	MEL dose-2+ GA	Hyper-active
13.	22	24.8	28	MEL dose-2+ GA	Anxiety + Hyper-active
14.	31	32.8	28	MEL dose-2+ GA	Anxiety + Hyper-active
15.	25	27	28	MEL dose-2+ GA	Anxiety + Hyper-active
16.	32	34.2	28	MEL dose-2+ GA	Hyper-active
17.	22	24.7	28	MEL dose-2+ GA	Hyper-active
18.	21	23.7	28	MEL dose-2+ GA	Hyper-active
19.	22	24	28	MEL dose-2+ GA	Hyper-active
20.	31	32.8	28	MEL dose-2 + GA	Hyper-active

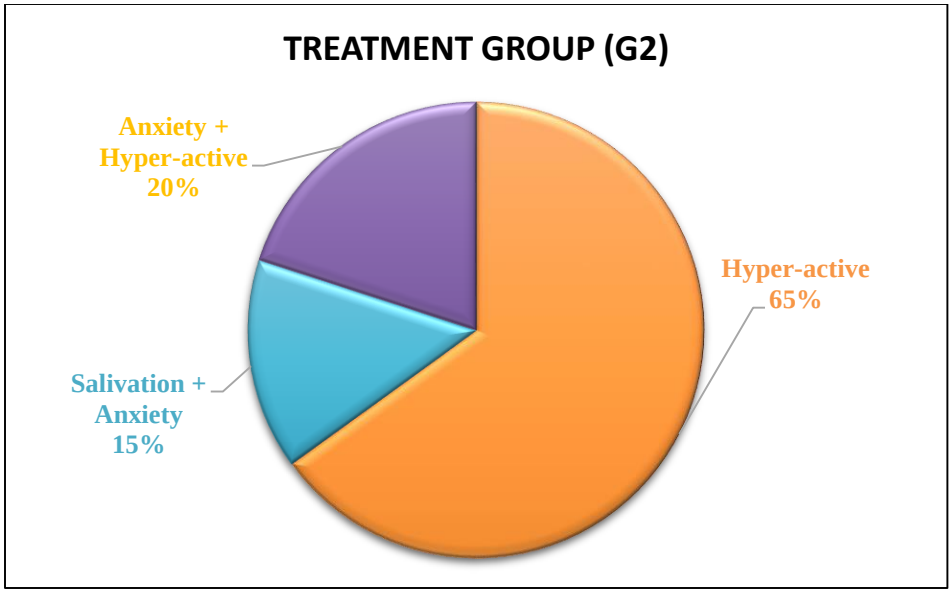

Figure 2: Physiological changes in Treatment Group (G2) within 3 hours of giving MEL dose-1

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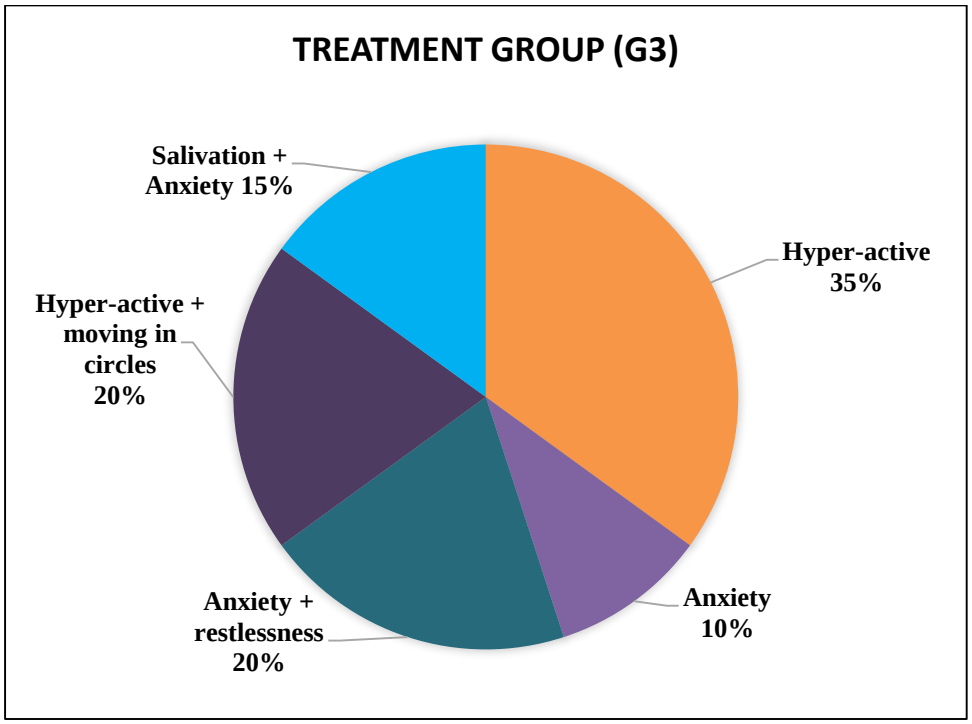

Figure 3: Physiological changes in Treatment Group (G3) within 3 hours of giving MEL dose-2

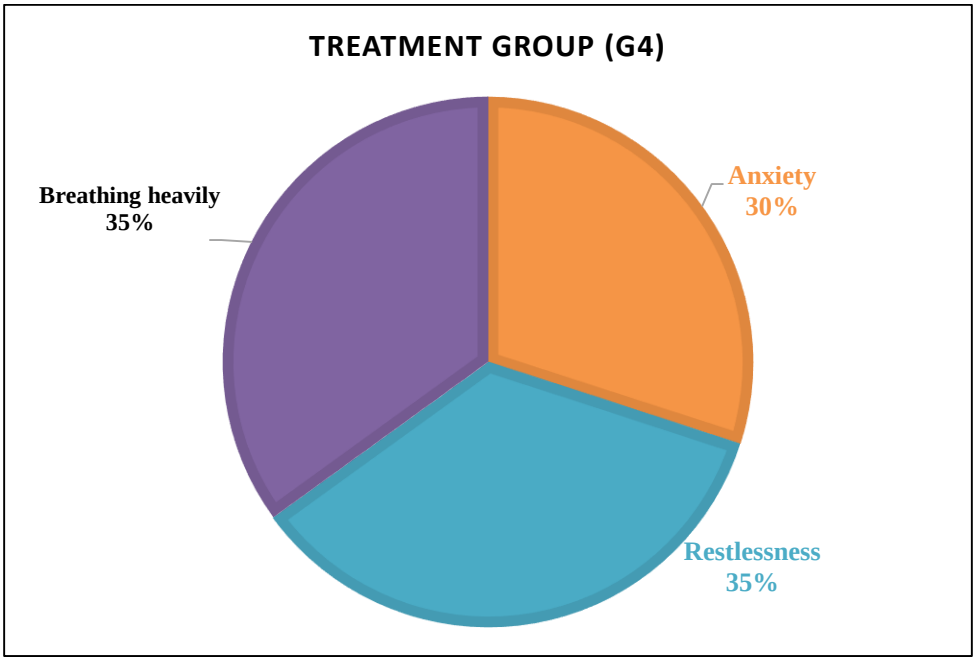

Figure 4: Physiological changes in Treatment Group (G4) within 3 hours of giving MEL dose-1 + GA

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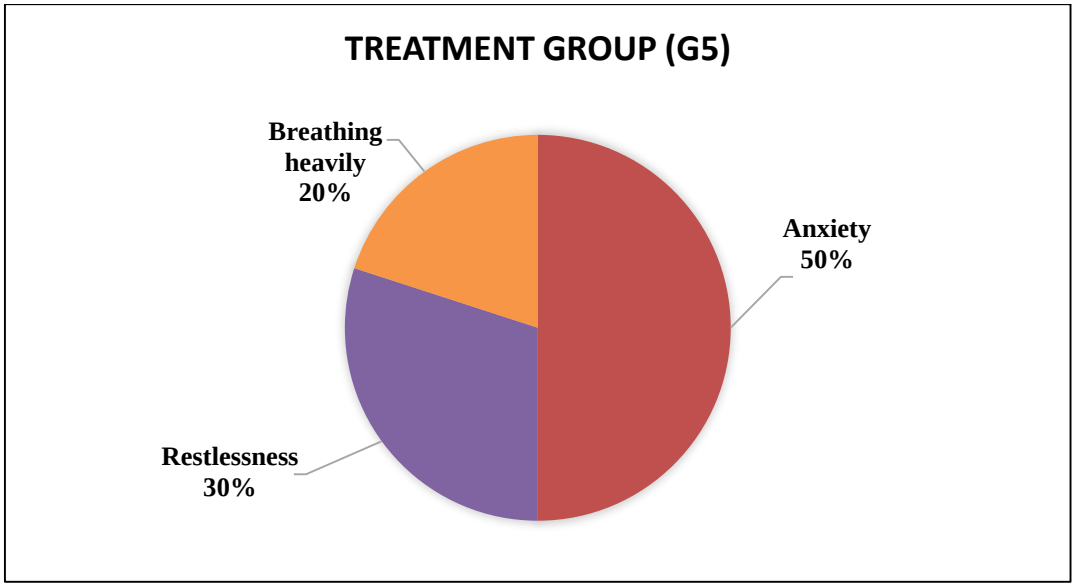

Figure 5: Physiological changes in Treatment Group (G5) within 3 hours of giving MEL dose-2 + GA

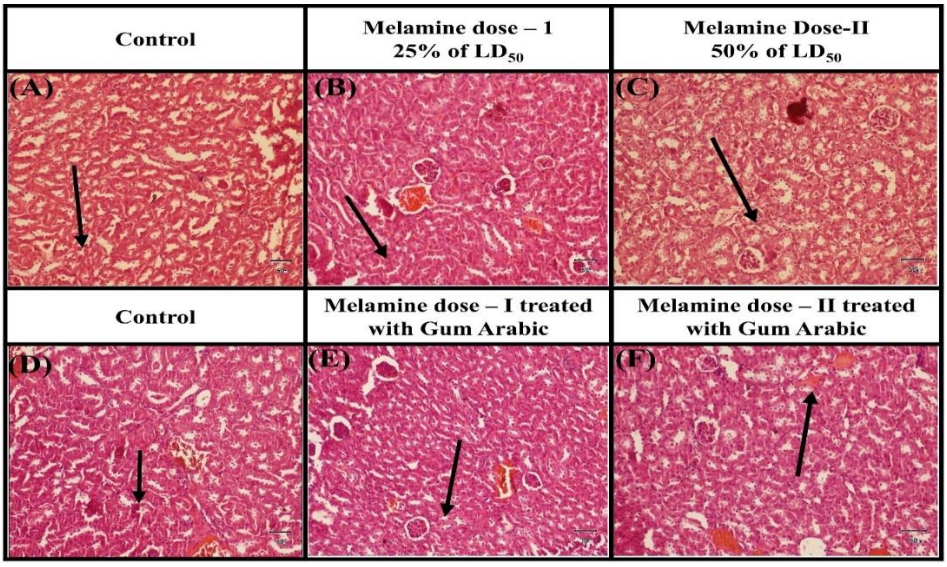

Figure 6: (A and D) Kidney section of control group showing regular structure of renal corpuscles and tubules (B) kidney tissues showing normal histological structure at induction of 25% LD_{50} of Melamine. (C) kidney tissues can be seen normal after being treated with Gum Arabic + 25% LD_{50} of Melamine. (E) renal section of mice intoxicated with 50% LD_{50} Melamine showed dramatic pathological changes and cellular swelling. (F) renal section of mice treated with GA extract along with 50% LD_{50} melamine administration showed repair i.e lesser swelling

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4. DISCUSSION

Melamine is an industrial chemical widely used in the production of plastics, laminates, and coatings. Its inadvertent presence in food products, however, has raised significant health concerns, particularly in relation to kidney damage. Melamine ingestion causes the formation of insoluble crystals in the kidneys which disrupts the excretion of waste products leading to systemic toxicity and ultimately renal failure, this renal dysfunction can lead to anorexia and malnutrition, contributing to weight loss. This makes weight alterations, an important parameter monitored in melamine toxicity studies [14].

Similarly, Puschner et al [15] observed significant weight loss in animals exposed to high doses of melamine likely due to renal toxicity, reduced food intake, and overall systemic stress. Likewise, a study conducted by Dobson et al. [16] investigated the effects of melamine on Sprague-Dawley rats. The study reported that rats exposed to high levels of melamine exhibited significant weight loss compared to the control group. This weight loss was attributed to decreased food intake, gastrointestinal distress, and renal dysfunction induced by melamine. The formation of melamine-cyanurate crystals in the kidneys was particularly noted as a critical factor leading to anorexia and subsequent weight loss in these animals. In current study, melamine supplementation at dose-1 and dose-2 caused significant weight loss in albino mice (table 2 & 3). These findings are parallel with [12], [17], [18]

The mechanisms by which melamine influences weight loss are complex and multifaceted, involving its toxic impact on organs and metabolic processes. Studies have shown that melamine induces oxidative stress by generating reactive oxygen species (ROS). This oxidative damage can impair cellular functions and contribute to weight loss by damaging tissues and organs critical for nutrient metabolism [12]. Zhu & Kannan[17] suggested that prolonged exposure to melamine can result in weight reduction in experimental animals due to chronic kidney damage, which leads to decreased nutrient absorption and overall health deterioration. In current study, Gum Arabic consumption along with melamine supplementation at dose-1 and dose-2 caused significant weight gain in experimental mice (table 4 & 5). These results are supported by [19] and [20].

Gum Arabic (GA) contains natural antioxidants that neutralize reactive oxygen species (ROS) and reduce oxidative stress. This protective effect prevents oxidative damage to kidney cells and tissues, ultimately supporting normal growth and weight gain [21]. Gum Arabic acts as a prebiotic, promoting the growth of beneficial gut bacteria [22]. GA can modulate metabolic pathways, improving energy balance and storage [23]. GA can improve digestive health by enhancing gut motility and preventing the absorption of harmful substances [10]. This improvement in gut microbiota, reduced gastrointestinal disturbance and decreased oxidative damage can enhance nutrient absorption and utilization, leading to better overall nutrition and weight gain in animals exposed to toxic substances.

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Melamine exposure can lead to significant histological changes in the kidneys, with the severity and nature of these changes depending on the dose. In current study melamine ingestion at dose-1 (fig 6-B) caused no significant change in kidney tissues as compared to control group (fig. 6-A). On the other hand, melamine dose-2 caused notable alterations in kidney tissues i.e, majority of renal tubular epithelial cells were undergoing cellular swelling (fig. 6-E). These histological evidences are consistent with [24], [25], [26]. Tian et al [26] examined long-term exposure effects of melamine on renal vascular function and histopathological changes in rats. It discussed the dose-dependent effects of melamine on renal blood flow and function, highlighting the formation of kidney stones and renal fibrosis. On the other hand, Gum Arabic consumption by Swiss albino mice significantly ameliorated the histological changes induced by melamine dose-1 and dose-2 supplementation. These outcomes are supported by [27], [28], [29], [30], [31].

A histopathological study of the kidneys from the different groups by El-Tahir et al [28] showed that gum Arabic mitigated the damage caused by gentamycin. Coagulative necrosis, haemorrhage, and reduced cellularity were not observed when gum Arabic was co-administered with gentamycin. A study was conducted by Hammad et al [29] on male Wistar rats weighing 211–230 g at the time of unilateral ureteric obstruction (UUO). The supplementation of Gum Arabic before and during the extended period of ureteric obstruction seemed to mitigate the UUO-induced changes in oxidative stress markers, the gene expression of certain inflammatory and fibrotic cytokines, and histological characteristics. The curresnt study highlighted the multifaceted protective effects of gum Arabic on kidney histology in the context of melamine toxicity. By reducing inflammation and oxidative stress, GA helped in preserving kidney structure.

5. CONCLUSION

Melamine ingestion caused significant body weight and histological changes in swiss albino mice. The primary mechanisms involve renal toxicity, reduced feed intake, metabolic disruption, and oxidative stress. Gum Arabic effectively mitigates the harmful impacts of melamine by restoring kidney tissue architecture and cellular damage. The administration of Gum Arabic presents a promising approach to counteract the deleterious effects of melamine toxicity warranting further investigation into its mechanisms of action and therapeutic interventions.

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