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Beneficial effects of two Mediterranean medicinal plants on blood, liver, and kidney toxicity induced by formalin in rats

Background: *Acacia nilotica* (*A.nilotica*) and *Retama raetam* (*R.raetam*), being in the Fabaceae family, are indigenous plants commonly found in the north and east Mediterranean region. These plants are used for treatment of hypertension and have hypoglycemic and diuretic effects. **Aim:** The present investigation was designed to evaluate the treatment efficacy of these medicinal plants against liver and kidney toxicity induced by formalin. **Methodology**: Twenty-four male Sprague dawley albino rats were obtained and divided into four groups (six per group). Group I was kept as control group while groups II, III, and IV were given a daily dose of formalin of 7.2 mg/kg for 2 weeks. However, groups III and IV were also nourished with a daily dose of 100 mg/kg and 20mg/kg of methanol extracts of *A.nilotica* and *R.raetam* respectively for 3 weeks. Later on, histo-pathological investigations were done. **Results:** Formalin caused a profound increase in serum glucose, red blood cells, hemoglobin, liver and kidney functions, serum malondialdehyde, plasma nitric oxide and, lipid profile. A significant decrease was exhibited in white blood cells count, serum proteins, blood superoxide dismutase and glutathione peroxidase. Administration of methanol extracts of *A.nilotica* and *R.raetam* restored the above parameters to the normal levels. **Conclusion**: Methanol extracts of *A.nilotica* and *R.raetam* have the ability to modify rodents' susceptibility to blood, liver and kidney toxicity as well as oxidative stress induced by formalin.

Keywords: *Acacia nilotica, Retama raetam,* Fabaceae, Formalin toxicity, Albino rat

INTRODUCTION

Ingestion of formaldehyde may cause burning in the digestive system and harmful effects to organ such as kidney and liver {1}. After 24 hours of administration of HCHO (50 or 100 mg/kg), hepatic metallothionein was increased by 15-fold {2} while 19 mg/l of HCHO resulted in a decrease of the sulfide production by 90% {3}. Formaldehyde dehydrogenase (FDH) activity was observed in the olfactory and respiratory mucosa and in the hepatic tissues of the rats. The presence of FDH and glutathione in the epithelial layer of the nasal cavity is a barrier to inhaled formaldehyde at low concentrations {4}. Oral administration of HCHO (80 mg/kg) by gastric tube led to a slight decrease in body weights, whereas the lymph node weights were significantly increased. The lymphoid organs were not influenced after 28 days {5}. Yasokawa *et al*. {6} suggested that membrane structure is a major target of methyl alcohol toxicity, while membrane's proteins were major targets of formaldehyde toxicity. *Acacia nilotica* and *Retama raetam* {7} are indigenous plants, found in the north and east Mediterranean region {8, 9}. These plants are used for various purposes e.g., treatment of hypertension {10}, reduction of hyperglycemia in diabetes {11}, treatment of carcinoma (COR-L23) {12} and, protection against toxicity induced by cadmium chloride in liver and kidney {13}. The present study was conducted to evaluate the possible treatment effects of the methyl alcohol extracts of *A.nilotica* and *R.raetam* plants against the harmful effects of formalin induced in liver and kidney of rats.

MATERIALS AND METHODS

Chemicals

Formalin consisting of 40% solution of formaldehyde (HCHO) and methanol (CH_3OH) were obtained from E. Merck, Darmstadt, Germany.

Experimental animals and treatments

The treatment doses used in this study were taken from the work done by Magrani et al., {14} and Singh et al. {15}. Male albino rats (24, 100±10g) used in this study, were divided into four groups (six per group). Food and water were available at all times to chosen animal subjects and they were kept under conventional conditions (temperature 20-22 ºC, humidity 60-70%, 12 h light: dark cycle) and fed with standard rodent chow. The experiments were carried out in accordance with the national regulations on animal welfare whereby ethical approval was obtained by the Institutional Animal Ethical Committee (IAEC) of National Research Centre (NRC), Cairo - Egypt. The subjects - albino rats were obtained from the animal house at NRC, Cairo - Egypt and housed in polypropylene cages. The rats were 10-12 weeks old at the onset of the experiments. The animals were divided into four equal groups (six per group). Group I, that served as control group received the same volume of distilled water while groups II, III and IV were given a dose of 7.2mg/kg per day of formalin [(HCHO) {16} and (CH_3OH)] dissolved in 1 ml distilled water through gavage for 2 weeks. Later on, group III was fed through gavage with a dose of 100 mg/kg per day {15} of *A. nilotica* methanol extract dissolved in 2 ml distilled water for 3 weeks. Group IV

accepted through gavage a dose of 20 mg/kg per day {14} of the *R. raetam* methanol extract dissolved in 1 ml distilled water for 3 weeks.

Plant material

Seeds of *A.nilotica* (7 kilograms) and *R.raetam* (5 kilograms) were collected and authenticated by National Research Centre, Cairo, Egypt and voucher specimens of plants were deposited at the herbarium of the National Research Centre.

Extraction and isolation

The dried powdered seeds of *A.nilotica* and *R.raetam* were separately defatted with methyl chloride (CH_3Cl) and followed by extraction with methanol-water mixture [CH_3OH:H_2O (7:3)] at room temperature. The two extracts were filtered, evaporated under reduced pressure and lyophilized (200g). Finally, the extracts were suspended in distilled water at concentration of 30mg in 1 liter. The usage of these two medicinal plants are related to their high concentrations in polyphenol compounds especially flavonoids constituents. The chemical constituents of *A.nilotica* include catechin, catechin 7-*O*-gallate, catechin 3'-*O*-gallate, catechin 4'-*O*-gallate, catechin, 7, 3'-di-*O*-gallate, catechin 7, 4'-di-*O*-gallate. On the other hand, genistein 8-C-glucoside, orobol 8-C-glucoside, apigenin 8-C-glucoside orobol, genistein, and apigenin are enlisted as chemical constituents of *R.raetam* {17, 18}.

Liquid chromatography/Mass spectra (LC/MS)

The phyto-chemical screening was performed on Varian 310-MS triple quadruple Mass spectrometer. All the analyses were detected in positive ion electro-spray ionization (ESI) mode, with selected reaction monitoring (SRM). The settings of ESI source were as follows: Spray voltage=5000V; Capillary temperature=300^{0}C; Sheath gas pressure (Spraying) =20 arbitrary units; Auxiliary gas pressure (de-solvating) =10 arbitrary units; Ion sweep gas pressure (Curtain) =5 arbitrary units. Aqueous ammonium formate (5 mM) and methanol were used as mobile phases. The mass spectrometer was operated in negative electrospray ionization mode, recording two transitions for each analytic and one for each internal standard. Eight major groups of compounds isolated from both *A.nilotica* and *R.raetam* were structurally characterized by LC-MS and the purities were all >98% as shown in Table I.

Lead accumulation in different body organs

The tissues from liver, kidney, spleen, stomach, testis, and ovary were homogenized in normal physiological saline solution (0.5 N NaCl) in the ratio of 1:4 w/v. The homogenized mixture was centrifuged for 5 minutes at 3000 r.p.m at 4^{o}C and the supernatants were used to determine formalin concentration by colorimetric method used by Fukumura et al. {19} as shown in Table II.

Biochemical Estimations

All reagent kits were used for rat serum and all chemicals were purchased from Bio-diagnostic Co., Egypt. WBCs and RBCs were determined by trunk's fluid {20}. Hemoglobin content was calculated and Serum glucose was estimated based on enzymatic method of Siest et al., {21}. Serum transaminases (AST and ALT) and Serum total bilirubins were measured. Furthermore, serum alkaline phosphatase (ALP), serum total protein, serum albumin (Alb), Serum globulin (Glob) and (Alb)/ (Glob), serum urea, serum creatinine and cholesterol were also estimated. Serum low-density lipoproteins (LDL) {22}**,** Serum high-density lipoprotein (HDL) {23} and Serum triglycerides were also measured. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) in blood were also estimated. A colorimetric assay was used for detecting lipid peroxidation (MDA) in serum while plasma nitric oxide generation was analyzed.

Blood Sampling and Handling

Blood samples were collected from retro-orbital plexus of rats using capillary tubes into clean centrifuge tubes {24}. One part of blood sample was collected on EDTA as anticoagulant for blood parameters, SOD and GPx. The other part of the blood sample was allowed to coagulate and centrifuged at 4000 r.p.m. for 15 minutes to separate blood serum. Separated serum was stored at -20^{o}C for the determination of liver and kidney functions as well as lipid profile.

Histo-pathological examination

Specimens of liver and kidney were fixed in 10% neutral formalin solution, and then processed for routine technique for embedding in paraffin. Blocks were sectioned at a thickness of 5 μm and stained with haematoxylin and eosin for histo-pathological examination.

Preparation of homogenate liver tissue

0.5 gm of liver and kidney tissues were dissolved in 2.5 ml of tris buffer solution, then homogenated in the homogenizer for 30 minutes. Later, they were centrifuged for 20 minutes at 7.000 r.p.m and separated from the supernatant, which was used to determine the tissue antioxidant activity in the same manner as of blood sample.

Statistical analysis

Statistical calculations were carried out with SPSS 10 for Windows software package (Statistical). Results were expressed as the mean ± S.E.M. of 6 independent experiments. Student's *t*-test was used for statistical analyses; P values ≤ 0.05 were considered to be significant.

RESULTS

The data presented in Table I reveal the chemical constituents of methanol extract from both *A.nilotica* and *R.raetam*. The table illustrates that flavonoids are the major constituents of both plants.Table II shows that animals treated with formalin have the highest accumulation of formalin in liver and kidney (9.2 and 9.0×10^{-6} mg/kg) as compared to gonads (testis 6.8×10^{-6} mg/kg and ovary 7.5×10^{-6} mg/kg). The data illustrated in table III present that formalin is responsible for a highly significant increase ($p<0.01$) in red blood cells count, hemoglobin content and serum glucose. A remarkable decrease ($p<0.01$) in white

blood cells count as compared to that of control group was also seen. Animals treated with formalin and *A.nilotica* and *R.raetam* plants cancel out the formalin mediated increase in red blood cells count, hemoglobin content, and serum glucose. Both plants counteract the formalin decreasing effect on white blood cells as compared to the formalin treated rats. The analysis of the results in table III shows that formalin caused a highly significant increase ($p<0.01$) in serum AST, ALT and bilirubin levels and a significant increase ($p<0.05$) in ALP level. While serum protein, albumin and globulin significantly increased ($p<0.05$), albumin/globulin ratio showed a highly significant decrease ($p<0.01$) when compared to the control group. Animals treated with formalin and methanol extract of *A.nilotica* or *R.raetam* counteract the formalin-induced increase in AST, ALT, ALP, and bilirubin as compared to normal rats. Formalin revealed a significant increase ($p<0.05$) in serum urea and creatinine values as compared to the control group. Animals treated with formalin and *A.nilotica* and *R.raetam* showed insignificant reduction ($p>0.05$) as compared to those treated with formalin only (table III).formalin induced a highly significant increase ($p<0.01$) in serum cholesterol, LDL, triglycerides and HDL in normal rats. Administration of *A.nilotica* and *R.raetam* plants to formalin treated rats turned the above parameters towards the normal values (table iii). A significant decrease ($p<0.05$) in blood SOD and GPX, and a significant increase ($p<0.05$) in serum MDA and plasma no were recorded after formalin administration compared to the control group. The administration of *A.nilotica* and *R.raetam* to formalin treated rats returned all the blood antioxidants to normal levels when compared with the control group (table IV). The reviewing of the data in table IV showed that formalin caused a significant decrease ($p<0.05$) in liver SOD and GPX; and a significant increase ($p<0.05$) in liver MDA and no compared to the control group. Administration of *A.nilotica* and *R.raetam* to formalin treated rats cancel out the decrease in liver SOD and GPX, and the increase in liver MDA and no induced by formalin. The analysis of the data found in table IV exhibited that formalin induced a significant decrease ($p<0.05$) of SOD and GPX in kidney and a significant increase ($p<0.05$) of MDA and no in kidney as compared to the control group. Animals treated with formalin and *A.nilotica* and *R.raetam* significantly counteract the decrease SOD and GPX in kidney and the increase of MDA and no induced by formalin in kidney. The structure of the control liver showed normal hepatocytes, vascular sinusoids and centrolobular vein (figure 1-a). Treatment with daily doses of formalin (7.2 mg/kg b.w.) For 2 weeks caused a hoop of oedema in the periportal area, which compressed the surrounding hepatocytes. The hepatocytes plate pattern of *A.nilotica* and *R.raetam* methanol extracts in formalin treated rats was found to be within a normal limit as in the normal liver (fig 1-c&d). The kidneys of the control rats reveal normal structure of the renal corpuscles and the renal tubules (figure 2-a). The kidney of rats treated with formalin showed renal corpuscles calcification in some rats and hyper-cellular and narrow urinary space in others. The proximal convoluted tubules exhibited necrosis in some cells that line it. In some cases, the cells of renal tubules showed glassy-appearance cytoplasm. Some renal tubules showed proliferated epithelial cells (figure 2– b). In rats treated with formalin then given methanol extracts of *A.nilotica* and *R.raetam*, the sections of the glomeruli and the renal tubules were normal(fig. 2- c & d).

DISCUSSION

Formalin induces blood toxicity by increasing the number of RBCs and hemoglobin content and decreasing the number of WBCs. Treatment with methanol extracts of *A.nilotica* and *R.raetam* directs blood parameters to the normal levels because of chelating activity of flavonoids constituents. They bind to iron molecules of hemoglobin, thus decrease hemoglobin content and consequently red blood cells. Flavonoids-containing chemicals have immuno-reactive activity, where they induce proliferation of lymphocytes as well as the antibody production**.** The proliferation of spleen lymphocyte cells was observed after flavonoids-containing plants administration {25, 26}. Al-Mustafa and Dafallah {27} observed a significant decrease in the levels of hemoglobin in animals fed on a diet of 8% *Acacia nilotica* for up to 4 weeks.

Hayet *et al*.{28} found that the ethyl acetate extract of *R.raetam* showed the best activity against Gram positive organisms with minimum inhibitory concentration (MICs) of 128 to 256 µg/ml against methicillin resistant *staphylococcus aureus* but low activity against the different *Candida* species. The ethanol extracts of *A.nilotica* showed antimicrobial activity (range from 9.75-313 µg/ml) against multi-drug resistant (MDR) strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans* {29}.

Formalin causes hyperglycemia, but methanol extracts of *A.nilotica* and *R.raetma* restore the serum glucose to the normal values. Such findings were in agreement with Maghrani *et al.*{11} who found that the aqueous extract of *R.raetam* at a dose of 20mg/kg significantly reduces the blood glucose in normal rats in 6 hours after a single oral administration ($P<0.05$). The aqueous extract of *R.raetam* had no effect on basal plasma insulin levels indicating that the underlying mechanism of RR activity is extra-pancreatic. In addition, Maghrani *et al*. {14} reported that the aqueous extract of *R.raetam* at a dose of 10 mg/kg/h produced a significant decrease in blood glucose levels in normal rats and diabetic rats. An aqueous extract of *R.raetam* caused a potent inhibition of renal glucose re-absorption. This renal effect explains the observed hypoglycemic activity of this plant in normal and diabetic rats.

Formalin induces hepato-toxicity by increasing serum liver enzymes and decreasing serum proteins. However, methyl alcohol extracts of both *A.nilotica* and *R.raetam* have the ability to re-direct all parameters to the normal levels. Such observations were made by Hussein *et al*. {30} who found that methanol extract of *A.nilotica* was the most active extract (≥90% inhibition at 100 µg/ml) with regard to its inhibitory effect on hepatitis C virus (HCV) , protease (PR) using in vitro assay methods. Nephro-toxicity produced by Formalin is due to the increase of serum urea and creatinine. However, after the administration of methyl alcohol extracts of *A.nilotica* and *R.raetam* an insignificant

increase in serum urea and creatinine were observed as compared to the control group.

Table I: The chemical constituents of methyl extracts of *Acacia nilotica* (AN) and *Retama raetam* (RR)

Methyl Extract of	Flavonoids		Alkaloids		Glyco-sides	Unsat. St.		Sap.	Anth.	Cou.	Tann.
	Quer-cetin	Catechin	1,2,3	4		LB	H_2SO_4				
***Acacia nilotica* (AN)**	+++	+++	+++	+	+	++	++	+	++	++	++
***Retama raetam* (RR)**	+++	++	++	+	+++	+	+	+	+	++	+++

LB= Liebermann Burchardt Test Unsat. st.= Unsaturated sterol Sap.= Saponin Anth.= Anthraquinones Cou.= Coumarines Tann.= Tannins

Table II: The concentration of formalin occurred in different body organs

Organ	Concentration
Testis	($\leq 6.8 \times 10^{-6}$ mg/kg)
Ovary	($\leq 7.5 \times 10^{-6}$ mg/kg)
Spleen	($\leq 8.1 \times 10^{-6}$ mg/kg)
Stomach	($\leq 7.8 \times 10^{-6}$ mg/kg)
Liver	($\leq 9.2 \times 10^{-6}$ mg/kg)
Kidney	($\leq 9.0 \times 10^{-6}$ mg/kg)

Table III: Blood, liver and kidney toxicity of rats treated with formalin and followed by the treatment with methyl extracts of *Acacia nilotica* and *Retama raetam*

Parameters	***Group I***	***Group II***	***Group III***	***Group IV***
RBC ($\times 10^6/MM^3$)	8.5±0.7	12.0±0.8**	10.0±0.6[a]	9.5±0.9[a]
HB (MG/DL)	14.7±0.9	19.0±0.8**	17.1±0.5[a]	16.0±0.6[b]
WBC ($\times 10^3/MM^3$)	13.5±0.6	10.4±0.8**	11.6±0.8	12.0±0.7
GLUCOSE (MG/DL)	73.5±3.8	90.6±2.9**	80.3±3.2[a]	75.4±2.9[b]
AST (U/L)	123 ±3.9	137 ±2.7**	127 ±3.8[a]	125 ±3.9[a]
ALT (U/L)	62.5±2.5	70.8±2.0**	59.7±2.9[b]	63.0±2.9[a]
TOTAL BILIRUBIN (MG/DL)	0.56±0.08	0.84±0.05**	0.67±0.07[a]	0.60±0.08[b]
ALP (U/L)	211 ±6.8	233 ±7.1*	224 ±6.6	217±7.9
TOTAL PROTEIN (G/DL)	7.64±0.7	5.89±0.4*	8.09±0.9[a]	7.58±0.7[a]
ALBUMIN (G/DL)	3.76±0.5	2.65±0.2*	4.03±0.5[b]	3.81±0.5[a]
GLOBULIN (G/DL)	3.88±0.3	3.24±0.2*	4.03±0.2[b]	3.77±0.2[a]
ALB/GLOB.RATIO	0.97±0.05	0.82±0.03**	0.99±0.04[b]	1.01±0.07[a]
UREA (MG/DL)	26.5±2.9	36.1±2.6*	31.4±3.0	29.0±3.4
CREATININE (MG/DL)	0.75±0.08	1.00±0.09*	0.83±0.07	0.90±0.09
CHOLESTEROL (MG/DL)	98.2 ± 3.5	115.1 ± 2.9**	104.0 ± 4.1[a]	101.2 ± 3.9[b]
LDL (MG/DL)	40.7 ± 2.0	49.3 ± 1.9**	44.5 ± 2.0	42.8 ± 1.9[a]
TRIGLYCERIDES (MG/DL)	47.1 ± 2.0	55.0 ± 1.9**	51.8 ± 2.1	49.0 ± 1.7[a]
HDL (MG/DL)	48.1 ± 1.9	54.8 ± 2.0**	49.1 ± 1.9[a]	48.6 ± 1.7[a]

Data presented as mean ± SE * Significant change (P<0.05) * * Highly Significant change (P<0.01)
a Significant change compared to formalin group (P<0.05)
b Highly Significant change compared to formalin group (P<0.01)

Table IV: Blood, liver and kidney oxidative stress induced by formalin and followed by the treatment with methyl extracts of *Acacia nilotica* and *Retama raetam*

Parameters	***Group I***	***Group II***	***Group III***	***Group IV***
BLOOD SOD (U/ML)	260 ± 8.4	235 ± 7.9*	255 ± 6.6[a]	259 ± 7.9[a]
BLOOD GPX (U/L)	6250 ± 83	5975 ± 99*	6250 ± 87[a]	6350 ± 100[b]
SERUM MDA (µ MOL/L)	3.5 ± 0.5	5.0± 0.5*	3.9 ± 0.3[a]	3.7 ± 0.4[a]
PLASMA NO (µ MOL/L)	34.5 ± 1.8	41.3 ± 2.3*	37.5 ± 1.7	35.0 ± 1.6[a]
LIVER SOD (U/G)	48.5±4.3	36.3± 3.4*	45.5±2.9[a]	52.3± 3.7[b]
LIVER GPX (U/G)	14.2±1.4	10.7± 0.9*	13.2±0.5[a]	15.4± 0.6[b]
LIVER MDA (µ MOL/G)	29.6 ± 1.9	35.4 ± 2.0*	28.9± 1.7[a]	29.1± 1.5[a]
LIVER NO (µ MOL/G)	9.7± 1.8	21.5 ± 2.3	16.8± 4.4[a]	14.8± 2.9[b]
KIDNEY SOD (MG/G)	36.0±3.5	25.4± 4.1*	37.3±4.2[a]	35.6± 3.1[a]
KIDNEY GPX (MG/G)	17.2±1.6	12.4± 1.2*	15.2±0.8[a]	19.1± 1.3[b]
KIDNEY MDA (µ MOL/G)	41.2 ± 1.9	47.9 ± 2.4*	40.9± 1.9[a]	42.0± 2.1[a]
KIDNEY NO (µ MOL/G)	16.5± 2.3	23.0 ± 2.1*	17.4± 1.9[a]	15.3± 2.5[a]

Data presented as mean ± SE * Significant change (P<0.05) ** Highly Significant change (P<0.01)
a Significant change compared to formalin group (P<0.05)
b Highly Significant change compared to formalin group (P<0.01)

Fig 1: Liver of Control group (A) with preserved hepatic architecture and formalin treated group (B) with a hoop of oedema in the periportal area, which compressed the surrounding hepatocytes. the intra-cytoplasm vaculation was found. Formalin + *Acacia nilotica* (C) with large preserved hepatic lobular architecture. Formalin + *Retama raetam* (D) with preserved hepatic lobular architecture (H&E stain, X200)...B

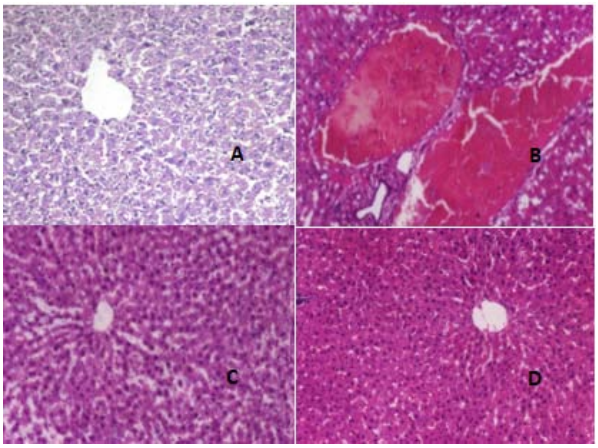
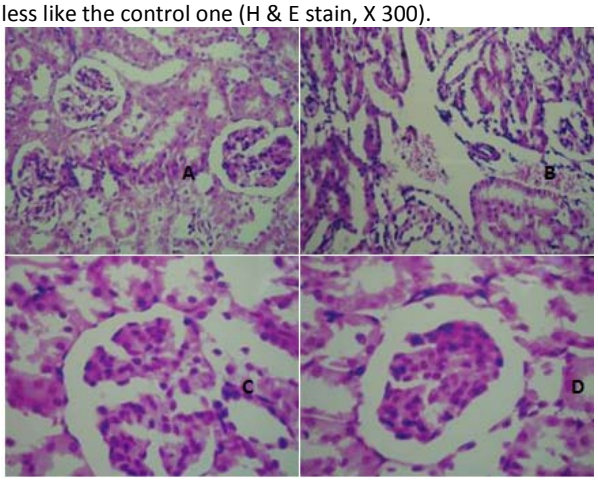


Fig 2: Kidney of control rat (**A**), Kidney of rat injected with doses of formalin (**B**), the glomerulus shows hyper-cellular and narrow urinary space (arrowhead). Notice necrosis of some cells of the proximal convoluted tubules. Some renal tubules showed proliferated epithelial cells and Kidney of rats treated with formalin then *Acacia nilotica*(**C**) and *Retama raetam* (**D**) methyl extracts, respectively showing the renal corpuscles and renal tubules that appear more or less like the control one (H & E stain, X 300).



These results were in agreement with that of Agunu *et al*. {31} who found that *A.nilotica* (3.0 mg/ml) caused initial relaxation quickly followed by contraction of the urinary tract. In the castor oil-induced diarrhea, 100% protections were shown by extracts of *A.nilotica* (100 and 200 mg/kg). Maghrani et al. {14} found that intravenous administration of the aqueous *R.raetam* (RR) extract produced a significant increment on diuresis from the second hour ($P<0.01$) to the fourth hour ($P<0.001$).

Formalin induces hypercholesterolemic, while methyl alcohol extracts of *A.nilotica* and *R.raetam* decrease serum cholesterol levels. These effects were in agreement with that of Maghrani et al. {32}, who reported that the aqueous extract of *R.raetam* induced a significant decrease of the plasma triglycerides concentrations in normal and diabetic rats in one week after repeated oral administration. They concluded that the aqueous extract of *R.raetam* exhibits lipid-lowering activity in both normal and severe hyperglycemic rats after repeated oral administration of *R. raetam* aqueous extract at a dose of 20 mg/kg.

Formalin induces oxidative stress in blood, liver, and kidney, while methyl alcohol extracts of *A.nilotica* and *R.raetam* increase blood, liver, and kidney antioxidant activities as reported by Singh et al. {15}. They found that pre-treatment of *A.nilotica* (75 and 150 mg/kg b.wt.) for 6 days caused a significant increase in the levels of catalase and SOD enzymes and a decrease in the level of MDA content in liver, lungs, kidneys and blood as compared to CCl_4-intoxicated rats. The treatment with *A.nilotica* flower and leaf aqueous extracts by oral gavages for 15 days resulted in a significant decrease in the lipid peroxidation and an increase in glutathione levels in the liver as observed by Meena et al., {33}. Moreover, Conforti et al. {12} found that the methanol extracts of *R.raetam* leaves and seeds show significant antioxidant effect. Singh et al. {34} showed that *A.nilotica* bark extract (ANBE) also increases the activities of antioxidant enzymes in the liver after a single intra-peritoneal injection of N-nitrosodiethylamine (NDEA, 200mg/kg) followed by weekly subcutaneous injections of carbon tetrachloride (CCl_4, 3ml/kg) for 6 weeks.

Histological examinations of this study show that formalin intoxicated groups reveal a loss of hepatic lobular architecture. This coincides with the observations of Keller et al. {4}, who observed the formaldehyde dehydrogenase (FDH) activity in tissues and found a toxic response. Shimizu et al. {1} found that ingestion of formaldehyde causes harmful effects to major organ such as kidney and liver. The administration of methanol extracts of *A.nilotica* and *R.raetam* to formalin treated rats showed that the medicinal plants restored liver and kidney injury. Such findings coincides with that of Singh et al. {34}**,** who found that treatment with ANBE correlate with histological injury of liver tissues against single intra-peritoneal injection of NDEA (200mg/kg) followed by (CCl_4 3ml/kg) for 6 weeks.

This paper provides remarkable evidence of the protective effects of two new medicinal plants that are rich in flavonoids, alkaloids, saponin, and tannins against formalin induced blood, liver, and kidney toxicity and associated oxidative stress. Koriem et al., {13} also studied the protective effects of plants "*Acacia nilotica and Retama raetam"* with cadmium chloride toxicity. The toxicity induced by formalin is better encountered by *A.nilotica* and *R.raetam*. The effects of formalin in the rats are analogous in many aspects to those of environmental pollutants in humans. Therefore, these plants are recommended in treatment of environmental toxicity for humans; however, further clinical investigations are required.

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