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RESEARCH ARTICLE

ASSESSMENT OF PROTECTIVE EFFECTS OF ALMOND SEED AND VITAMIN E SUPPLEMENTATION ON KIDNEY AND LIVER OF RATS EXPOSED TO PARACETAMOL TOXICITY

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Abstract

Paracetamol, a widely available analgesic and antipyretic drug has been reported to cause toxicity when taken in overdose. This study investigated the protective effects of almond seeds and vitamin E on liver and kidney of rats exposed to paracetamol toxicity. Fifty- one (51) male albino rats grouped into nine (9) groups (A-I) were used for this study. Groups A, B and C served as normal control, post-treatment control and pre-treatment control respectively. Groups D, E and F are the post-treatment test groups while groups G, H and I are the pre-treatment test groups. The various test groups in both the post-treatment and the pre-treatment groups were given almond seeds, vitamin E and combined treatment by oral gavage for 14 days. Three kilogram (3g/kg) body weight (b.w) of paracetamol were used to induce hepatorenal toxicity in the paracetamol control and test groups of the post-treatment groups on day 1 before commencement of treatment while paracetamol control and test groups of the pre-treatment groups were induced on day 14 after treatment. The animals were sacrificed on day 15. Renal and liver indices investigated were Laboratory (Urea and Creatinine) and (AST, ALT and ALP). The result showed that oral administration of 3g/kg b.w paracetamol caused a significant increase in renal indices and liver enzymes in rats. Post-treatment result showed that almond group (group D) significantly reduced LDL and increased SOD level. Vitamin E (group E) showed no significant difference in all parameters. The combined treatment (group F) significantly reduced ALT levels. Pre-treatment results revealed that almond (group F) significantly reduced Urea and ALP. Vitamin E (group H) significantly reduced Urea, Creatinine, AST, ALT and ALP. Combined treatment (group I) significantly reduced Urea, Creatinine, AST, ALT and ALP. Hence from the results, it is concluded that pre-treatment with almond, vitamin E and their combination have potentials in preventing hepatic and nephrotoxic damage induced by paracetamol overdose.

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Introduction:-

The incidence of drug induced toxicity has risen in recent times. Drug induced hepatotoxicity accounts for approximately 10% of all cases of community and hospital acquired episodes of acute liver injuries while drug

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induced nephrotoxicity accounts for 20% of all cases of community and hospital acquired episodes of acute kidney injuries (Nash et al., 2002; Zimmerman et al., 2009). Hepatotoxicity is a term applied to liver damage respectively due to toxins. It has become an increasingly important issue with the increase in prescription medication and herbal supplement use. Age, pre-existing disease conditions, multiple medications, exposure to more diagnostic and therapeutic procedures are also contributory factors to high prevalence. Drug induced toxicity is the most common form of acute liver failure in the United States (Larson et al., 2005) and hence one of the reasons for withdrawal of drugs from the market. Treatment can be costly and may require multiple interventions, including hospitalization (Gandhi et al., 2000).

The liver plays a vital role in regulating various physiochemical functions of the body and is always the first target organ for the metabolism of drugs and toxic chemicals (Sahreen et al., 2015; Salama et al., 2015). The liver has the ability to convert xenobiotics to compounds with low toxicity and also excrete them due to the high concentration of toxin-metabolizing enzymes they possess. Although, sometimes hepatic damage can be induced due to conversion of toxic substances to active metabolites during metabolism (Cullen, 2005). Mitochondrial dysfunction has been reported as one of the major mechanisms of drug induced hepatotoxicity. Severe mitochondrial dysfunction in the liver as a result of drugs can induce hepatic necrosis, cytolytic hepatitis and eventually liver failure (Gopiet al., 2010). Damage to the liver causes accumulation of toxins in the body faster than it can process.

The kidney is also a sensitive and dynamic organ responsible for homeostasis and regulation of the extracellular environment. It is also involved in detoxification and excretion of toxic metabolites and drugs (Inui et al., 2010; Parazella and Mockel, 2010). Drugs can exert their toxic effects by inducing nephrotoxicity by one or more common pathogenic mechanisms. Most people suffer from drug induced nephrotoxicity because of the presence of dangerous factors that increase their susceptibility to the damage caused by drugs (Tionget al., 2014). Drug-induced nephrotoxicity is an important cause of renal injury (Padmini & Kumar, 2012) and has been found to cause approximately 20% of community and hospital acquired episodes of AKI (Nash et al., 2002; Bellomo, 2006).

Paracetamol (PCM), is an analgesic and antipyretic drug (Aghababian, 2010). At recommended doses, it is generally safe even when taken for a long time. However, paracetamol can be very toxic and fatal when taken at overdose (FDA, 2008). Acute overdose of paracetamol is dose-dependent and causes potentially fatal hepatic necrosis which may be associated with renal tubular necrosis (Ogunbayode et al., 2010). At therapeutic doses, acetaminophen is metabolized via glucuronidation and sulfation reactions occurring primarily in the liver which result in the water-soluble metabolites that are excreted via the kidney. Paracetamol toxicity is dose dependent and increased overdose causes massive depletion of glutathione stores and increased production of NAPQI metabolites which are unbound and highly reactive. These electrophilic intermediates form adducts with cellular proteins, which disrupts homeostasis, activates caspases and lysosomal enzymes that initiate apoptosis (Bessemers and Vermeulen, 2001; Gopiet al., 2010).

Vitamin E is a group of lipid-soluble compounds which includes tocopherols and tocotrienols (Traber and Atkinson 2007). It carries out its function by acting as peroxyl radical scavenger, thus preventing the propagation of free radicals in tissues (Traber and Stevens, 2011). It also facilitates membrane repair by preventing the formation of oxidized phospholipids that theoretically might interfere with the membrane fusion events. Vitamin E has been shown to protect cell membrane from acetaminophen induced toxicity by inhibiting lipid peroxidation (Herrera and Barbas, 2001; Traber and Atkinson, 2007). Vitamin E also protects against some forms of xenobiotics that cause damage (Khaster, 2015).

Almonds are natural antioxidants owing to its phytochemical constituents. Some epidemiological studies have shown that the consumption of foods rich in natural antioxidants increases plasma antioxidant capacity and reduces the risk of diseases (Frisson-Norrie and Sporns, 2002; Philips et al., 2005). Several studies have revealed that antioxidant activity may be ascribed more to compounds such as flavonoids, isoflavones, flavones and so on. The almond is an effective health building food, both for the body and the mind. It is also a valuable food remedy for several common ailments. The seeds of *Prunus amygdalus* are found to possess various pharmacological properties, such as anti-stress (Bansal et al., 2009), anti-oxidant (Pinelo et al., 2004), immunostimulant (Puriet al., 2000), lipid lowering and laxative (Spiller et al., 1998). Despite the considerable advancements in medicine, synthetic drugs still have many side effects and exacerbate the disease. It has become necessary to use natural and safe alternatives from medicinal plants to replace chemical drugs using various experimental models (Muriel and Rivera-Espinoza 2008, Patwardhan et al. 2004). In this context, more attention has been paid to the protective effects of natural antioxidants

of herbal medicine and isolated bioactive constituents, which are considered as the most effective and safe treatments for hepatotoxicity/nephrotoxicity (Grajales and Muriel, 2015).

The high incidence of liver and kidney damage as a result of drug induced toxicity and its impact on human health have motivated studies on medicinal plants which have been claimed to possess protective characteristics against drug toxicity. Presently, the use of herbal medicines for prevention and control of diseases is in the focus of attention for the physicians, pharmaceutical manufacturers and patients. The reasons for such shift toward the use of herbals include the expensive cost of conventional drugs, inefficacy of drugs and adverse drug reactions (Aghalet al., 2007)

It is therefore of scientific and economic interest to find out if consumption of vitamin E and/or almond seeds would ameliorate and/or prevent hepatotoxicity caused by drugs in albino rats.

Materials And Methods:-

Chemicals:

All drugs and chemicals were analytical grade. Vitamin E in gelatin capsule and powdered paracetamol was purchased from standard vendors

Pilot Study:

A total of 4 adult male albino rats weighing 150 - 170g, were used for the study. After 2 weeks of acclimatization, the rats were weighed and randomly put into individual cages. Two were designated PCM group and the other two, control group. All the rats were fed on standardized rat chow and clean tap water. PCM group were given single dose of paracetamol 3g/kg body weight. Blood samples were collected by cardiac puncture after 24 hours for analysis of liver variables and oxidative profile.

Study Design and Population:

A total of 70 rats were used for this study. Four (4) rats were used for pilot study; fifteen (15) rats were used for acute toxicity study while fifty- one (51) male albino rats were sampled for the main study.

Acquisition and Acclimatization of Animals:

Seventy (70) male albino rats weighing between 130 - 150 g were acquired from a known animal. The rats were kept in the animal house throughout the study at an average temperature of 30°C, relative humidity of 80% and a 12- hour light/dark cycle. The animals were put on standardized rat feed and water for 2 weeks to acclimatize. Fifty- one albino rats were randomly grouped into 9 groups with five animals each in groups A, B, C and six animals each in groups D, E, F, G, H and I. Procedures involving the care and use of the animals were done in compliance with standard guidelines for the use of animals in biomedical research.

Preparation of Paracetamol (Acetaminophen):

Powdered paracetamol was suspended in distilled water and administered orally at a dose of 3g/kg b.w. This dosage is known to cause hepato-renal toxicity in rats according to the pilot study.

Extraction of Almond Seeds:

Bligh and Dyer method for lipid extraction was used (Bligh and Dyer, 1959). Seven hundred and fifty grams (750g) of almond seeds were weighed and blended using an electric grinder. The extraction was done by adding 1.5 litre of methanol to the grinded almond seeds, stirring and allowing it to stand for 24 hours at room temperature (26 - 28°C). After 24 hours, the mixture was filtered using Whatman No. 1 filter paper. The methanol filtrate was then concentrated by drying with a rotary evaporator at 40-60°C. The essence of drying in a rotary evaporator is to gently remove the solvent used (methanol). The percentage yield after extraction was 10 percent. The extract obtained was then further dried under the sun and stored in an air-tight plastic container in the refrigerator (4°C) and used for the study.

Induction of Hepato-nephrotoxicity:

Having established from the pilot study that a single dose of 3g/kg b.w of paracetamol was adequate to induce liver dysfunction in the rats, nephrotoxicity was induced by weighing each animal on a weighing scale (Hana brand) and calculating the corresponding dose of paracetamol required for the induction. Powdered paracetamol was suspended in distilled water and administered orally at a dose of 3g/kg b.w. The animals were starved for 24 hours before the commencement of the experiment but had free access to drinking water.

Extract and Drug Administration:

Based on the pre-determined LD50 values of almond extract obtained by using Lorke's method, the dose 1000mg/kg b.w was used for this study. The extract was diluted in distilled water which acted as a vehicle and administered orally through gastric gavage. Vitamin E 100mg/kg b.w was administered also by gastric gavage. A tiny drop of tween 80 was added to increase Vitamin E solubility in distilled water before administration. Single dose of paracetamol 3g/kg b.w was administered orally by gastric gavage to induce nephrotoxicity and hepatotoxicity.

Calculation of Dosage:

Dose of paracetamol and Vitamin E corresponding to the average weight of the rats were calculated based on:

$$\text{Average weight} = \text{total weight of rats} / \text{total number of rats}$$

Experimental Design:**Table 3.1:-** Treatment Schedule of the various groups.

GROUPS	TREATMENT
A (Neg C)	Distilled Water (day 1-14)
B (post-C)	PCM (day 1) + distilled water (day 2-14)
C (pre-C)	Distilled water (day 1-13) + PCM (day 14)
POST TREATMENT GROUPS	
D	PCM (day 1) + Almond (day 2-14)
E	PCM (day 1) + Vit E (day 2-14)
F	PCM (day 1) + Almond + Vit E (day 2-14)
PRE TREATMENT GROUPS	
G	Almond (day 1-13) + PCM (day 14)
H	Vit E (day 1-13) + PCM (day 14)
I	Almond + Vit E (day 1-13) + PCM (day14)

PCM is paracetamol, Post-C is post-treatment control group, Pre-C is pre-treatment control group, vit. E is vitamin E. PCM=3g/kg, vit E=100mg/kg, Almond=1g/kg.

This experimental setup lasted for a period of fourteen (14) days. On the 15th day, all the rats were sacrificed and blood collected for biochemical analysis.

Collection of Blood Samples for Biochemical Analysis:

Blood samples were taken by cardiac puncture (after anaesthetizing the animals with diethyl ether inhalation) at the commencement (as baseline measures) of the study, before and after interventions (sacrifice of the animals). This was done on day 15. About 4ml of whole blood was collected from each animal into plain sample bottle, labeled properly for biochemical analysis (urea, creatinine, AST, ALT and ALP).

Statistical Analysis:

Data were analyzed using Graph Pad Prism 5.1. The data were presented as mean and standard deviations. Statistical comparisons were considered significant at $p < 0.05$.

Results:-**Renal Indices and Liver Enzymes Levels of the Various Group:**

Details of the renal indices and liver enzymes of various groups are shown in table 4.1 and 4.1b

Comparison of the means of the various groups using analysis of variance (ANOVA) showed significant difference in urea levels ($p = 0.0001$). The Tukey multiple comparison test of the means showed significant variations in urea levels amongst group A vs B, group A vs C, group A vs G, group A vs H, group B vs C, group C vs D, group C vs E, group C vs F, group C vs G, group C vs H, group C vs I, group D vs G, group D vs H, group E vs G, group F vs G, group F vs H and group G vs I at ($P = 0.0001$, $F = 22.98$). There were no significant variations observed in the rest of the groups.

Comparison using ANOVA showed significant difference in creatinine levels ($P=0.0001$). The Tukey's multiple comparison test of the means showed significant variations in creatinine levels amongst group A vs B, group A vs C,

group A vs G, A vs H, A vs I, B vs C, C vs D, C vs E, C vs F, C vs H, C vs I, D vs G, F vs G at ($P = 0.0001$, $F = 12.44$). However, there were no significant variations observed in the rest of the groups.

Comparison using AVOVA showed significant difference in AST level ($p = 0.0001$). Tukey's multiple comparison test of the means showed significant variations amongst group A vs C, group B vs C, group C vs D, group C vs E, group C vs F, group C vs H and group C vs I at ($p = 0.0001$, $F = 6.301$). There were no significant differences amongst the rest of the groups.

Comparison using ANOVA shows significant difference in ALT level ($P=0.0001$). The Tukey's multiple comparison test of the means shows significant variation in the ALT levels amongst group A vs B, group A vs C, group A vs G, group A vs H, group B vs C, group C vs D, group C vs E, group C vs F, group C vs H, group C vs I and group F vs G at ($p = 0.0001$, $F = 9.074$). The rest of the groups showed no significant variations.

Comparison using ANOVA showed significant difference ($p = 0.0002$). Tukey's multiple comparison of the means showed significant variation in ALP levels amongst group A vs C, group C vs D, group C vs E, group C vs F, group C vs G, group C vs H and group C vs I at ($p = 0.0002$, $F = 5.093$). The rest of the groups showed no significant variations.

Table 3.1:- Renal indices and liver enzymes levels of the various groups.

	Urea (mmol/L)	Creatinine ($\mu\text{mol/L}$)	AST(U/L)	ALT(U/L)	ALP(U/L)
Group A	2.74 ± 0.34	95.4 ± 7.44	53.4 ± 9.21	21 ± 3.16	61 ± 9.43
Group B	3.98 ± 0.70	126.75 ± 9.25	80.5 ± 7.19	36.75 ± 3.5	73.25 ± 6.5
Group C	6.37 ± 0.71	164.5 ± 19.12	118.75 ± 21.70	51.75 ± 5.12	95.5 ± 8.66
Group D	3.06 ± 0.38	114.16 ± 14.74	64.16 ± 14.78	28.67 ± 5.68	64 ± 8.71
Group E	3.4 ± 0.59	120 ± 10.88	77.2 ± 12.43	31.8 ± 4.33	67.4 ± 8.96
Group F	3.21 ± 0.38	116.16 ± 7.67	61.5 ± 17.21	25.5 ± 3.39	62 ± 7.01
Group G	4.74 ± 0.35	144.4 ± 6.80	85.6 ± 22.67	38.6 ± 10.45	72.2 ± 14.82
Group H	4.18 ± 0.39	130.33 ± 14.76	76.67 ± 20.60	33.67 ± 10.26	66.83 ± 13.11
Group I	3.7 ± 0.54	126.5 ± 11.31	69.16 ± 7.73	31.66 ± 3.14	62.66 ± 7.71
p-values	0.0001	0.0001	0.0001	0.0001	0.0002
F-values	22.98	12.44	6.301	9.074	5.093

Group A- normal control, Group B- post-treatment control, Group C – pre treatment control, Group D- almond post treatment group, Group E - vitamin E post treatment group, Group F – (almond+vitE) post treatment group, group G –almond pre treatment group, Group H- vitamin E pre treatment group, Group I – (almond+vit E) pre treatment group.

Table 3.1b:- Tukey's Multiple Comparison Test for table 4.1.

Tukey's Multiple Comparison Test	Summary	Summary	Summary	Summary	Summary
Group A vs Group B	*	**	ns	*	ns
Group A vs Group C	***	***	***	***	***
Group A vs Group D	ns	ns	ns	ns	ns
Group A vs Group E	ns	ns	ns	ns	ns
Group A vs Group F	ns	ns	ns	ns	ns
Group A vs Group G	***	***	ns	**	ns
Group A vs Group H	***	***	ns	*	ns
Group A vs Group I	ns	**	ns	ns	ns
Group B vs Group C	***	**	*	*	ns
Group B vs Group D	ns	ns	ns	ns	ns
Group B vs Group E	ns	ns	ns	ns	ns
Group B vs Group F	ns	ns	ns	ns	ns
Group B vs Group G	ns	ns	ns	ns	ns
Group B vs Group H	ns	ns	ns	ns	ns
Group B vs Group I	ns	ns	ns	ns	ns
Group C vs Group D	***	***	***	***	***

Group C vs Group E	***	***	*	***	**
Group C vs Group F	***	***	***	***	***
Group C vs Group G	***	ns	ns	ns	*
Group C vs Group H	***	**	**	**	**
Group C vs Group I	***	***	***	***	***
Group D vs Group E	ns	ns	ns	ns	ns
Group D vs Group F	ns	ns	ns	ns	ns
Group D vs Group G	***	**	ns	ns	ns
Group D vs Group H	**	ns	ns	ns	ns
Group D vs Group I	ns	ns	ns	ns	ns
Group E vs Group F	ns	ns	ns	ns	ns
Group E vs Group G	**	ns	ns	ns	ns
Group E vs Group H	ns	ns	ns	ns	ns
Group E vs Group I	ns	ns	ns	ns	ns
Group F vs Group G	***	**	ns	*	ns
Group F vs Group H	*	ns	ns	ns	ns
Group F vs Group I	ns	ns	ns	ns	ns
Group G vs Group H	ns	ns	ns	ns	ns
Group G vs Group I	*	ns	ns	ns	ns
Group H vs Group I	ns	ns	ns	ns	ns

Renal Indices and Liver Enzymes Levels of the Post-Treatment Groups and Control:

The details of the renal indices and liver enzymes levels of the post-treatment groups and control are shown in table 3.2.

The table depicted significant differences in urea levels amongst group A vs B, group A vs E, and group A vs F at ($p = 0.013$, $F = 4.104$). There were no significant variations among the rest of the groups.

The table also shows a significant variation in creatinine levels amongst group A vs B at ($p = 0.0026$, $F = 5.823$), but there were no significant variations among the rest groups.

There was also a significant variation in the AST level between group A vs B at ($p = 0.0267$, $F 3.413$). Although no significant differences exist among the rest of the groups.

The table also portrays significant variations in ALT levels amongst group A vs B, group A vs D, group A vs E and group B vs F at ($p = 0.0002$, $F = 9.321$).

The results for ALP shows no significant variation amongst all the groups at ($p = 0.2066$, $F = 1.619$).

Table 3.2:- Renal indices and liver enzymes levels of the post-treatment groups and control.

	Urea (mmol/L)	Creatinine(μ mol/L)	AST(U/L)	ALT(U/L)	ALP(U/L)
Group A	2.74 \pm 0.34	95.4 \pm 7.44	53.4 \pm 9.21	21 \pm 3.16	61 \pm 9.43
Group B	3.98 \pm 0.70	126.75 \pm 9.25	80.5 \pm 7.19	36.75 \pm 3.5	73.25 \pm 6.5
Group D	3.06 \pm 0.38	114.16 \pm 14.74	64.16 \pm 14.78	28.67 \pm 5.68	64 \pm 8.71
Group E	3.4 \pm 0.59	120 \pm 10.88	77.2 \pm 12.43	31.8 \pm 4.33	67.4 \pm 8.96
Group F	3.21 \pm 0.38	116.16 \pm 7.67	61.5 \pm 17.21	25.5 \pm 3.39	62 \pm 7.01
p-values	0.013	0.0026	0.0267	0.0002	0.2066
F-values	4.104	5.823	3.413	9.321	1.619
Tukey's Multiple Comparison Test	Summary	Summary	Summary	Summary	Summary

Group A vs Group B	**	*	*	***	ns
Group A vs Group D	ns	ns	ns	*	ns
Group A vs Group E	*	ns	ns	**	ns
Group A vs Group F	*	ns	ns	ns	ns
Group B vs Group D	ns	ns	ns	ns	ns
Group B vs Group E	ns	ns	ns	ns	ns
Group B vs Group F	ns	ns	ns	**	ns
Group D vs Group E	ns	ns	ns	ns	ns
Group D vs Group F	ns	ns	ns	ns	ns
Pre-Vi.E vs Group F	ns	ns	ns	ns	ns

Group A- normal control, Group B – post treatment control, Group D- almond post treatment group, Group E- vitamin e post treatment group, Group F – (almond+vit e) post treatment group.

Renal Indices and Liver Enzymes Levels of the Pre-treatment Groups and Control:

The details of the results of the renal indices and liver enzymes levels of the pre-treatment groups and control are shown in table 3.3.

The table depicted significant variations in the urea levels amongst group A vs C, group A vs G, group A vs H, group A vs I, group C vs G, group C vs H, group C vs I and group G vs I at (P = 0.0001, F = 36.31). The rest groups showed no significant differences amongst them. There were also significant differences observed in creatinine levels amongst group A vs C, group A vs G, group A vs H and group A vs I at (P = 0.0001, F = 14.18). There are no significant differences amongst the rest of the groups.

Results for ALT levels also shows significant differences amongst group A vs C and group A vs G at (p = 0.0308, F = 3.28). The rest groups show significant differences amongst them. Results for ALT level shows significant differences between group A vs C and group A vs G at (p = 0.009, F = 4.474). There were no significant differences amongst the rest groups.

Results for ALP levels shows no significant variations amongst all the groups at (p = 0.334, F = 1.215).

Table 3.3:- Renal indices and liver enzymes levels of the pre-treatment groups and control.

	Urea(mmol/L)	Creatinine(μ mol/L)	AST(U/L)	ALT(U/L)	ALP(U/L)
Group A	2.74 \pm 0.34	95.4 \pm 7.44	53.4 \pm 9.21	21 \pm 3.16	61 \pm 9.43
Group C	6.37 \pm 0.71	164.5 \pm 19.12	118.75 \pm 21.70	51.75 \pm 5.12	95.5 \pm 8.66
Group G	4.74 \pm 0.35	144.4 \pm 6.80	85.6 \pm 22.67	38.6 \pm 10.45	72.2 \pm 14.82
Group H	4.18 \pm 0.39	130.33 \pm 14.76	76.67 \pm 20.60	33.67 \pm 10.26	66.83 \pm 13.11
Group I	3.7 \pm 0.54	126.5 \pm 11.31	69.16 \pm 7.73	31.66 \pm 3.14	62.66 \pm 7.71
p-values	< 0.0001	< 0.0001	0.0308	0.009	0.334
F-values	36.31	14.18	3.28	4.474	1.215
Tukey's Multiple Test	Summary	Summary	Summary	Summary	Summary
Group A vs Group C	***	**	ns	*	ns
Group A vs Group G	***	***	*	**	ns
Group A vs Group H	***	***	ns	ns	ns
Group A vs Group I	*	***	ns	ns	ns
Group C vs Group G	***	ns	ns	ns	ns
Group C vs Group H	***	ns	ns	ns	ns
Group C vs Group I	***	ns	ns	ns	ns
Group G vs Group H	ns	ns	ns	ns	ns
Group G vs Group I	*	ns	ns	ns	ns
Group H vs Group I	ns	ns	ns	ns	ns

Group A- normal control, Group C – pre- treatment control group, group G – almond pre- treatment group, Group H- vitamin E pre- treatment group, Group I – (almond+vit E) pre- treatment group.

Discussion:-

This research work assessed the effect of vitamin E and almond seeds on paracetamol induced nephrotoxicity and hepatotoxicity. Preventive (pre-treatment) and curative (post-treatment) studies were carried out. For the preventive (pre-treatment) groups, the rats were given almond extract, vitamin E and a combination of both before induction of hepatorenal toxicity on day fourteen (14) while for the curative (post-treatment) groups, the rats were first induced on day one (1) before treatment with almond extract, vitamin E and combination of both. Both preventive and curative groups had their controls and also a negative control (normal).

In this study, oral administration of 3g/kg of paracetamol produced a significant ($p < 0.05$) increase in urea levels in the paracetamol control groups (group B and group C) when compared with the value for rats that were not induced (group A). Creatinine values were also significantly increased ($p < 0.05$) in the paracetamol induced controls (group B and group C) when compared with values for the rats that were not induced (group A). The rise in both urea and creatinine levels is suggestive of acute kidney injury caused by the oral administration of 3g/kg of paracetamol. It has been previously reported that an acute paracetamol overdose can lead to potentially lethal kidney failure in humans and experimental animals (Schismitta et al., 2015; Blakely and McDonald, 1995; Emeighet et al., 1996; Eguia and Materson, 1997). Tubular cell loss has been reported to be a characteristic feature of both acute renal failure and chronic renal disease (Ortiz et al., 2000) and is observed when cell death predominates over mitosis. It has been extensively studied and well understood that Paracetamol causes liver damage by promoting hepatocyte apoptosis (Rayet et al., 1993; Ray and Jena, 2000; Boulares et al., 2002). However, the mode of renal cell death during paracetamol nephrotoxicity and the mechanisms involved are obscure. Indeed, there is evidence that the molecular basis of nephrotoxicity may differ from those of hepatotoxicity, as N-acetyl-cysteine protects from the latter, but has been shown not to protect from nephrotoxicity, (Blakely and McDonald, 1995). Corina and colleagues however reported that induction of apoptosis may underlie the nephrotoxic potential of paracetamol and they identified renal endoplasmic reticulum stress as a therapeutic target in nephrotoxicity, (Corina et al., 2004). They further reported that upon treatment with paracetamol, primary cultures of murine tubular epithelial cells and the murine proximal tubular cell line MCT showed morphologic changes associated with apoptosis, such as chromatin condensation and internucleosomal DNA fragmentation. Moreover, the loss of membrane asymmetry observed after paracetamol treatment as detected by annexin-V staining without loss of membrane integrity suggests that apoptosis is the primary mode of cell death in tubular cells treated with paracetamol, (Corina et al., 2004).

Pre-treatment before inducing nephrotoxicity has significantly decreased ($P < 0.005$) serum urea levels of almond (group G), vitamin E (group H) and combined treatment (group I) compared to the paracetamol group (group C). The decrease was lowest in the combined group (group I). This suggests reno-protective potentials of the treatments. Few studies have reported the renal protective effect of almond. Lorenzo and colleagues reported amelioration of acute kidney injury by almond, (Lorenzo et al., 2017). They however attributed the therapeutic effect to the phytochemical constituents of almond. Almonds are among the richest food sources of vitamin E, as RRR- α -tocopherol. Almonds also contain a variety of phenolic compounds which are localized principally in their skin, including flavonols (isorhamnetin, kaempferol, quercetin, catechin and epicatechin), flavanones (naringenin), anthocyanins (cyanidins and delphinidin), procyanidins, and phenolic acids, (Frison-Norrie and Sporns, 2002). Previous studies have also demonstrated renoprotective effect of vitamin E (Naziroglu et al., 2004; Waribo et al., 2017). Vitamin E is a powerful peroxy radical scavenger that returns to its reduced state by reacting with vitamin C or other hydrogen donors, (Traber and Stevens, 2011). Moreover, it reduces the mitochondrial generation of hydrogen peroxide and it regulates the expression of genes implicated in inflammation and fibrosis which occurs in CKD patients (Yoshikawa et al., 1998). For the combined treatment, no known studies have been reported on almonds and vitamin E. Although, it is possible that vitamin E must have synergistically reacted with the phytochemicals, polyphenols and minerals contained in almond.

Post treatment after inducing nephrotoxicity shows non-significant ($P > 0.05$) decrease in urea levels of almond group (group D), Vitamin E (group E) and combined treatment group (group F) compared with the paracetamol group (group B). For almonds, there is no reported study of effect on diseased kidney. However, in human study conducted by Yngbar et al, it was rather reported that renal failure occurred due to excessive intake of almond, (Yngbar et al, 2015).

Comparing the urea levels of the pre-treatment group and post treatment groups showed significant ($P < 0.05$) variation in almond groups ($P < 0.0001$, $T = 7.55$). The vitamin E and combined treatment groups showed no significant variation.

Creatinine levels of the pre-treated groups showed a non-significant decrease in the almond group (group G) compared to paracetamol control (group C). However, vitamin E (group H) and combined treatment group (group I) showed significant ($p < 0.05$) decrease in creatinine levels. Creatinine levels are lowest for the combined treatment group (group D). Previous studies have also demonstrated renoprotective effect of vitamin E (Nazirogluet al., 2004; Wariboet al., 2017). Vitamin E is a powerful peroxyredical scavenger that returns to its reduced state by reacting with vitamin C (or other hydrogen donors), (Traber and Stevens, 2011). Moreover, it reduces the mitochondrial generation of hydrogen peroxide and it regulates the expression of genes implicated in inflammation and fibrosis which occurs in CKD patients (Yoshikawa et al., 1998). For the combined treatment, no known studies have been reported on almonds and vitamin E.

Creatinine levels of the post-treated groups showed a non-significant increase in almond group (group D), vitamin E (group E) and combined treatment group (group F) when compared to the normal control (group A) whereas comparing with paracetamol control group (group B) showed non-significant decrease in creatinine levels.

Comparing the creatinine levels of the pre-treatment and post treatment groups showed significant ($p < 0.05$) variation in almond groups ($p = 0.0028$, $T = 4.428$). However, there were no significant variation in vitamin E groups and combined treatment groups.

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