Anti-hyperglycaemic Effect of *Aegle marmelos (L.)* in Animal Experiments-PRISMA based Systematic Review and Meta-analysis

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Abstract

Aegle marmelos is a folklore medicine which is widely consumed as an anti-hyperglycaemic drug, its properties are known since prehistoric times. Plenty of cell line as well as animal research have reported the anti-hyperglycaemic activity of the drug. Even though quality researches are necessary to fulfil further human studies and marketing, no quality assessments for these studies have been undertaken till date. The aim was to critically analyse the quality standards and evaluate the anti-hyperglycaemic potential of *A. marmeloes* in rodent model researches. The search strategy was done using the key words '*Aegle marmelos*', along with 'Diabetes or Hyperglycaemia,' in different databases like PubMed (n = 45), Google Scholar (n = 43) and Science Direct (n = 46). However, the filter used during the search was 'Animal experimentation'. All animal experimentations collected with the keyword search was analysed for the inclusion criteria like serum glucose estimation as primary outcome, timeline of publication 1997 to 2017 in English and treatments containing any part and any form of *A. marmelos* in single or in combination. Based on the inclusion criteria, 11 articles were selected for the review. Data extraction underwent a blind procedure independently by two reviewers. If full texts were not available, communicating the authors was also tried and all the four authors responded. All the studies selected after screening were given unique codes, to ensure blind review. Result observed indicated higher reduction of serum glucose levels in hyperglycaemia-induced rodent models. After administering the drug, significant changes were observed in total cholesterol as well as triglycerides. Rest of the LDL, HDL, SOD and serum insulin were an in-significant find. Sample size calculation was not mentioned in any of the studies. Only 9% of the studies reported on blinding and 18% reported on randomisation. This might have affected the internal validity of the studies. It can be concluded that *A. marmelos* is effective in lowering the serum glucose levels in experimental hyperglycaemia. Further research has to be conducted to explore its target specific mode of action. This may lead to the development of an effective anti-hyperglycaemic drug with minimal side effects.

Keywords: Ayurveda, Diabetes, Prameha, Vilwa

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Abbreviations

LDL - Low density lipoprotein

HDL - High density lipoprotein

SOD - Super Oxide Dismutase

WHO - World Health Organisation

ROB - Risk of Bias

STZ - Streptozotocin

AM - *Aegle marmelos*

ALT - Alanine Transaminase

AST - Aspartate Transaminase

HFN - Human Fibronectin

SGPT - Alanine Transaminase

SGOT - Serum glutamic oxaloacetic transaminase

VLDL - Serum glutamic pyruvic transaminase

GSH - Glutathione

MDA - Malondialdehyde

LPO - Lipid Peroxidation

CAT - Catalase

FBS - Fasting Blood Sugar

TBARS - Thiobarbituric Acid Reactive Substances

HP - Hydrogen Peroxide

GP_X - Glutathione Peroxidase

SD - Standard Deviation

1. Introduction

Pre-senile diabetes mellitus is the primary cause of a majority of the deaths in India. Diabetes will be the seventh pre-eminent cause of death in 2030 as per WHO. Its prevalence rate has augmented from 108 million to 421 million in 30 years. The occurrence of diabetes among adults above 18 yrs has also risen to about 3.7% in 30 years. The dispersion of diabetes is high in developing countries[1]. Medicinal herbs are the plausible source to aid treating diabetes in health care system all over the world. Ayurveda, the ancient Indian system of medicine elaborates the use of plants in various ailments. Chemical constituents in plants vary depending on the species, the plant part used, the storage measures use, the harvesting time, as well as the tropo-geographical area etc. The finished products may also contain constituents in varied amounts in different manufacturing batches. This variability accounts for the difference in pharmacological action of the herbal medicine[2]. It is the time to expound and conduct a pragmatic herbal research as there is enormous and emergent use of the natural products. It is worth procuring an unswerving data for the health care system[3].

Aegle marmelos is extensively consumed as an anti-hyperglycaemic drug and its properties are known since prehistoric times. Bark juice and leaves of *Aegle marmelos* are said to hold anti-hyperglycaemic property as mentioned in classical texts of Ayurveda[4]. Effect and quality of these experimental studies have to be analysed for the maintenance of uniform standards. This meta-analysis is the first of its kind conducted in Ayurveda. Various pharmacological activities of the herb were researched pre-clinically. Analysis of these studies in terms of risk of bias and heterogeneity is essential for further clinical and marketing researches. Aim of this review was to analyse the effects and quality assessment in terms of risk of bias and calculation of heterogeneity of pre-clinical anti-hyperglycaemic activity of *A. marmelos*.

2. Materials and Methods

Animal experiments commenting on the anti-hyperglycaemic action of *A. marmelos* published from 1997 October – 2017 October, were searched using the key words '*Aegle marmelos*' and 'Hyperglycaemia', '*Aegle marmelos*' with 'diabetes' in databases like PubMed, Science direct and Google scholar. Experimental studies that involved rodent animal models were considered for this review. The filter used while searching was 'animal experiments'. Published researches in English language were included in the review. Those studies that contained serum glucose estimation as outcome measures were considered. The title may or may not contain the word 'hyperglycaemia' or 'diabetes'. Among the selected studies, those studies that did not include serum glucose estimation as outcome measure or sub group analysis were excluded. Available animal publications of *A. marmelos* were searched irrespective of parts, dose, route, and duration of administration[5].

3. Data Analysis

In the current study, if the parameter concerned was described in three or more studies, meta-analysis of that activity was performed. Serum glucose was taken as a primary parameter and serum insulin, total cholesterol, triglyceride, LDL, HDL and SOD were used as secondary parameters. All the study characteristics like inducing agent, control drug, number of groups described were taken into consideration[6]. Random statistic model was used in the meta-analysis. Heterogeneity was assessed using I^2. Sources of heterogeneity were compared between studies. The data was analysed using MICROSOFT EXCEL-2015. Statistical significance was defined in all studies where the P-value is < 0.05 [7].

4. Results

4.1 Article Selection

Based on the inclusion criteria, 11 articles were selected for the review. The search scheme and portrayals of the studies are briefed in Figure 1 and Table 1 respectively.

4.2 ROB Evaluation

The ROB and quality of the included studies were evaluated individually. ROB was analysed with SYRCLE's Tool[8]. Possible sources of risk of bias were not reported in
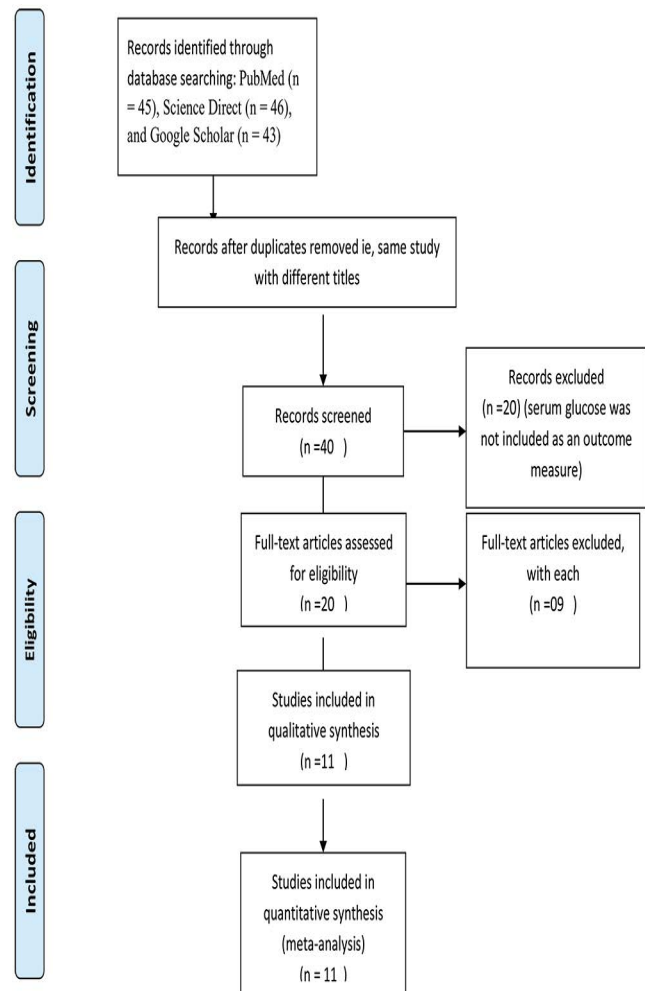

Figure 1. Search strategy.

Table 1. Study characteristics

Authors	Description of *Aegle marmelos*	Dose-mg/kg,route of administration	Study description	Parameters used
Rahman *et al* [9]	Ripe fruit purchased from a local market and authenticated from herbarium of botany department of karachi university	500, 1000 and 2000 mg/kg body wt.(oral)	Wistar rats of both sexes(n=30),STZ induced diabetes, total 5 groups, control (n=6),AM (n=6),duration-42 days	Serum glucose, serum insulin, triglyceride, cholesterol, creatinine, ALT, AST, western blot
Mudi *et al* [10]	Fruit and leaves collected from a district of Bangladesh and authenticated by pharmacognosist	450 mg/kg(oral)	Long evans rats of both sexes, STZ induced diabetes, total 3 groups, control (n=6), AM(n=7),duration-21 days	Serum glucose, serum insulin, insulin resistance, insulin sensitivity, triglyceride, total cholesterol, HDL
Ansari *et al* [11]	Drug is authenticated by Bangladesh national herbarium and extracted(part is not mentioned by author)	250mg/kg,500mg/kg	Long evans rats(sex not mentioned) (n=20),STZ induced diabetes, total 2 groups, control (n=10), AM (n=10), duration=28 days	Serum glucose, serum insulin, sucrose by intestinal perfusion technique), alpha-amylase

Saravanan *et al* [12]	Drug is authenticated by taxonomist from layola college and voucher specimen stored in herbarium of entomology research institute	20 mg/kg and 50 mg/kg of halfordinol(HFN) isolated from leaves of *A. marmelos*	Male mice (n=30), fructose fed obese mice, total 5 groups, control (n=6), HFN treated (n=6). duration 28 days	Serum glucose, triglyceride, cholesterol, creatinine, glycogen
Swati manik *et al* [13]	Drug is authenticated by taxonomist and voucher specimen stored in herbarium of Guru Nanak dev university, Punjab	500mg/kg,750 mg/kg	Wistar rats of both sexes(n=30),STZ induced diabetes, total 5 groups, control (n=6), PHF contain *A. marmelos* (n=6), duration-21 days	Serum glucose, triglyceride, cholesterol, HDL,LDL,SGPT,SGOT
Kumar *et al* [14]	Drug is authenticated by taxonomist and voucher specimen stored in herbarium of Siddhartha institute of pharmacy, Dehradun	10,20,40 mg/kg of umbelliferone extracted from stem bark of *A. marmelos*	Swiss albino wistar rats of both sexes(n=42),STZ induced diabetes, total 7 groups, control (n=6), umbelliferone (n=6),duration-28 days	Serum glucose, serum insulin, triglyceride, cholesterol, HDL,LDL, haemoglobin, VLDL
Rajbirbhatt *et al* [15]	Drug is authenticated by taxonomist and voucher specimen stored in herbarium of Guru Nanak dev university	25,50,100,200 mg/kg of *A. marmelos* extract	Wistar albino rats of both sexes(n=48),STZ induced diabetes, total 8 groups, control (n=6), *marmelos* extract (n=6), duration-14 days	Serum glucose, serum insulin, triglyceride, cholesterol, creatinine, urea
Ramesh b *et al* [16]	Umbelliferone procured from Karlsruhe, Germany	30mg/kg of umbelliferone	Wistar albino male rats (n=30),STZ induced diabetes, total 5 groups, control (n=6), umbelliferone (n=6),duration-45 days	Serum glucose, serum insulin, collagen, glycation extent
Sharmila *et al* [17]	Not mentioned	500 mg/kg	Albino male rats (n=26),STZ induced diabetes, total 5 groups, control (n=9), extract (n=9),duration-28 days	Serum glucose, urea, GSH, MDA, plama GST
Aravindkar *et al* [18]	Plant extracts obtained from amsar pvt ltd, Indore	0.1 ml of plant extract	Swiss albino male mice (n=77),STZ induced diabetes, total 11 groups, control (n=7), umbelliferone (n=7),duration-15days	Serum glucose, LPO,SOD,CAT
Kamalakannan *et al* [19]	*A. marmelos* fruit extract obtained from chemiloids, Vijayawada	125,250 mg/kg	Wistar albino female rats (n=30),STZ induced diabetes, total 5 groups, control (n=6), umbelliferone (n=6),duration-30 days	FBS,TBARS,HP,GSH,SOD,GPX

majority of the studies. While assessing the quality of the studies, the process of blinding and sample size calculation were not explained in most of the studies (80%). There were 60% of unclear risk of bias as essential parameters were not mentioned, this includes reporting of withdrawal symptoms, which constitutes attrition bias. The results of the **ROB** evaluation are given in Figure 2 , 3 and Table 2.
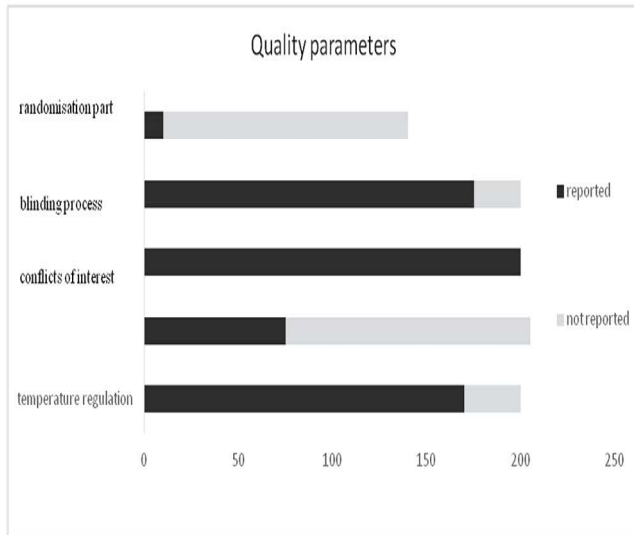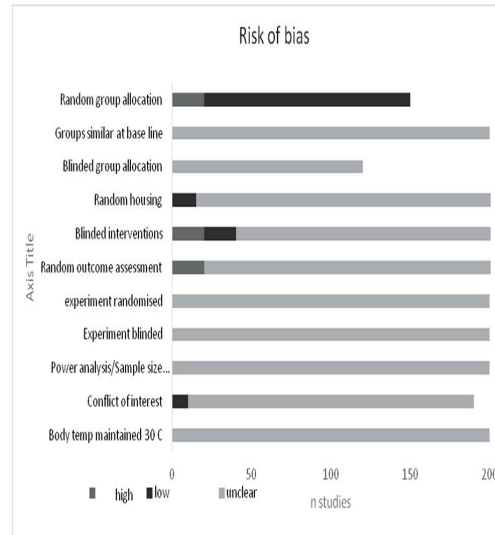

Figure 2. Assessment of quality parameters.

Figure 3. Assessment of risk of bias.

Table 2. ROB assessment with Syrcle's tool

studies	Group allocation random isation	Simila rity of groups	blinding	Random housing	Blinded inter ventions	Group allocation random isation	out come	Drop outs repo	Selective outcom esorting	Other biases	experi ment rando mised	Experiment blinded	Power analysi calculation	Interest conflict	Body temp
Rahman *et al*	#	Y	N	Y			L	H		L	Y	N	N	Y	N
Mudi *et al*	#	Y	N	N						H	Y	N	N	N	N
Ansari *et al*	#	Y	N	N			H			L	Y	N	N	N	N
Saravanan *et al*	#	?	N	Y						L	N	N	N	Y	N
Swati manik *et al*				N						L	N	N	N	N	N
Kumar *et al*				N						L	Y	Y	N	N	N
Rajbirbhatt *et al*				N						L	N	Y	N	N	N
Ramesh *et al*				N						L	Y	Y	N	N	N
Sharmila *et al*				Y						L	Y	Y	N		N
Aravindkar *et al*				N						H	Y	N	N	N	N
Kamala kannan *et al*				Y				H		L	Y	Y	N	N	N

Key to abbreviation-#=unclear risk of bias, L-low risk bias, H=high risk of bias, y=yes, N=no

4.3 Data Blend

In meta-analysis, mean differences in each studies were used in all calculations. Individual and overall effect sizes were calculated with random effect model of Cohen's D. Cohens' D is a simple and effective measure for estimation of effect sizes individually and as a whole. Heterogeneity I^2 were also determined. Subgroup analysis was accomplished. Chi-square values were assessed with degrees of freedom, k-1 where k denotes the number of total studies included. For each outcome, inverse variance, significance levels, Q value and 95% CI were also analysed.

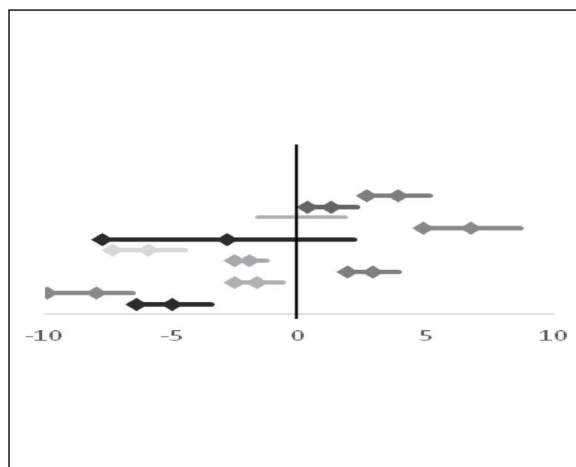### 4.4 Synthesis of the Result

Primary outcome: Serum glucose levels

Table 3. Effect of *A. marmelos* on serum glucose

For analysis, 11 comparisons were included. Administration of *A. marmelos* significantly reduced the serum glucose levels, (Effect size, Cohens d =1.28; inverse variance (IV); 0.05, 95% Confidence interval (CI), 1.71,0.85, I2=86.68%, P <0.01)

4.5 Outcome of *A. marmelos* on Fasting Serum Glucose

While analysing 11 studies, serum glucose was found to be the primary parameter for evaluation. Subsequently, after administration of *A. marmelos* in diabetic rats, we found significant decrease in the serum glucose levels (Effect size, Cohens d =1.28; inverse variance (IV);.05, 95% Confidence interval (CI),1.71,0.85, I2=86.68%, P <0.01) (Table 3, Figure 4).

Study	n	d	w	wd	v	CI95%
Rahman *et al* [9]	30	3.85	1.19	4.1	0.84	4.593.11
Mudi *et al* [10]	20	0.95	3.22	3.05	0.31	1.920.14
Ansari *et al* [11]	30	0.06	3.03	0.18	0.33	0.92-0.5
Saravanan *et al* [12]	30	8.16	0.32	2.61	3.10	7.496.01
Swati manik *et al* [13]	30	0.56	0.41	0.22	2.39	0.72-0.71
Kumar *et al* [14]	42	3	1.42	4.26	0.70	3.82.57
Rajbirbhatt *et al* [15]	56	1.09	2.70	2.94	0.37	1.0-0.04
Ramesh b *et al* [16]	30	-7.36	0.38	-2.79	2.58	3.592.11
Sharmila *et al* [17]	26	0.23	3.03	.696	0.33	1.02-0.52
Aravindkar *et al* [18]	77	4.87	0.90	4.38	1.10	5.394.95
Kamalakannan *et al* [19]	30	2.19	1.92	4.20	.52	1.630.19

Figure 4. Effect of *A. marmelos* on serum glucose.

4.6 Outcome of *A. marmelos* on Serum Insulin

In five studies, serum insulin was being used as an outcome measure. There were no significant changes in the serum insulin after administration of the drug. (Effect size, Cohens d =.53; inverse variance (IV) = .07;95% Confidence interval (CI),1.71,0.85, I2=0 %, P > 0.05) (Table 4, Figure 5) [9, 10, 12, 14, 16].

4.7 Outcome of *A. marmelos* on Total cholesterol

Subgroup analysis of effect of *A.marmelos* on cholesterol in 3 studies showed significant reduction (Effect size, Cohens d =0.96; inverse variance (IV) =.045; 95% Confidence interval (CI)=1.38,0.54; I2=86.05%, P <0.05) (Table 5, Figure 6) [9, 13, 14].

4.8 Outcome of *A. marmelos* on Triglyceride

Triglycerides increase the risk of heart diseases. Administration of *A.marmelos* significantly reduces the increased triglyceride in diabetic rats (Effect size, Cohens d =2.29; Inverse variance (IV)=3.56; 95% Confidence interval (CI)= 3.32-1.26, I2=68%, P <0.05) (Table 6, Figure 7)[9, 13, 14].

Table 4. Effect of *A. marmelos* on serum insulin

Study	n	d	w	wd	v	CI 95%
Rahman *et al* [9]	30	1.54	2.38	3.66	0.42	2.57 -0.01
Mudi *et al* [10]	20	1.38	2.63	3.63	0.34	2.44 0.12
Saravanan *et al* [12]	30	0.013	3.03	0.039	0.33	2.42 0.14
Kumar *et al* [14]	42	0.418	3.03	0.140	0.33	2.42 0.14
Ramesh b *et al* [16]	30	-0.58	3.03	0.12	0.33	2.42 0.14

Table 5. Effect size of *A. marmelos* on total cholesterol

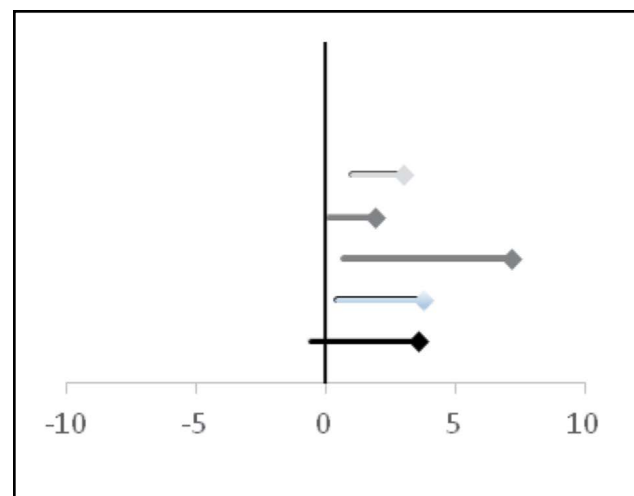| Study	n	d	w	wd	v	CI 95%
Rahman *et al* [9]	30	1.14	16.6	18.92	0.06	1.38 0.54
Swati manik *et al* [13]	30	-1.27	2.56	-3.25	0.39	-2.51 -0.03
Kumar *et al* [14]	42	0.314	3.03	0.0951	0.33	1.45 -0.834

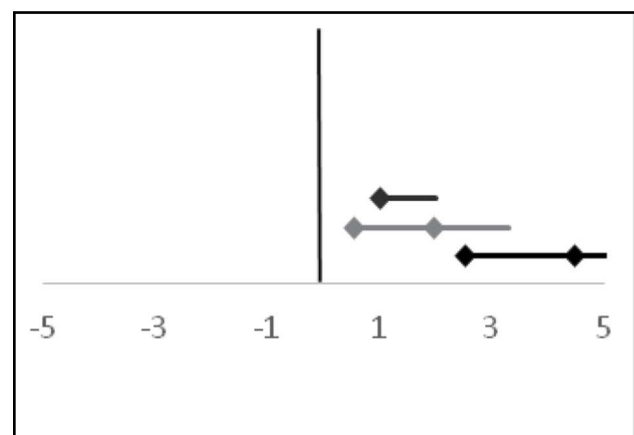**Figure 5.** Effect of *A. marmelos* on serum insulin.

Figure 6. Effect size of *A. marmelos* on total cholesterol.

Table 6.   Effect size of *A. marmelos* on triglyceride

Study	n	d	w	wd	v	CI 95%
Rahman *et al*[9]	30	0.54	0.66	0.35	1.5	2.98 -1.86
Swati manik *et al*[13]	30	1.96	2.04	3.99	0.49	2.68 1.24
Kumar *et al*[14]	42	4.46	0.86	3.83	1.15	6.7 2.5

Figure 7.   Effect size of *A. marmelos* on triglyceride.

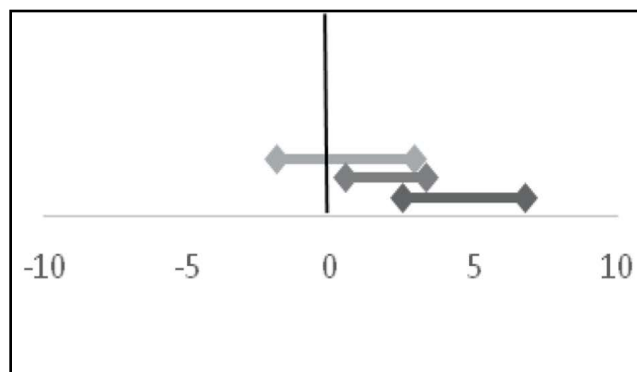## 4.9 Outcome of *A. marmelos* on LDL

Three studies were evaluated for effect of the drug on LDL. The change after administration of the drug was not significant at all. (Effect size, Cohens d =1.52; inverse variance (IV); 0.15, 95% Confidence interval (CI) =2.27,.77; I2=53.8 %, P < 0.05) (Table 7, Figure 8)[9, 13, 14].

4.10 Outcome of *A. marmelos* on HDL

In 3 studies HDL was used as a parameter for anti-obesity assessment. The result derived after analysis was not found to be significant. (Effect size, Cohens d =1.11; inverse variance (IV)=0.13; 95% Confidence interval (CI)=1.81,.40; I2=0; P < 0.05) (Table 8, Figure 9)[9, 13, 14].

Table 7.   Effect size of *A. marmelos* on LDL

Study	n	d	w	wd	v	CI 95%
Rahman *et al* [9]	30	2.90	1.4	4.06	0.68	3.13-0.09
Swati manik *et al* [13]	30	0.76	2.85	2.16	0.35	3.03.01
Kumar *et al* [14]	42	1.64	2.27	3.72	2.27	4.45-1.43

Figure 8.   Effect size of *A. marmelos* on LDL.

Table 8.   Effect size of *A. marmelos* on HDL

Study	n	d	w	wd	v	CI95%
Rahman *et al* [9]	30	0.73	2.85	2.08	0.35	2.26-.39
Swati manik *et al* [13]	30	0.93	2.65	2.46	0.36	2.28-.59
Kumar *et al* [14]	42	1.9	2.0	3.8	0.48	2.46-0.24
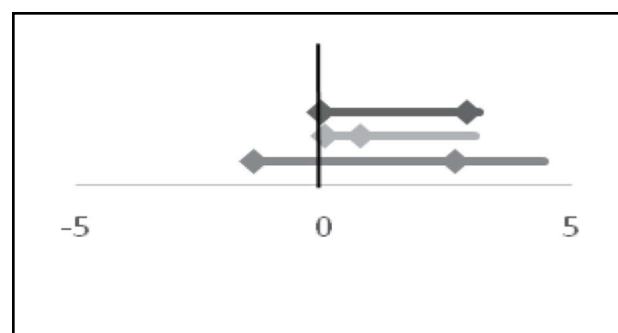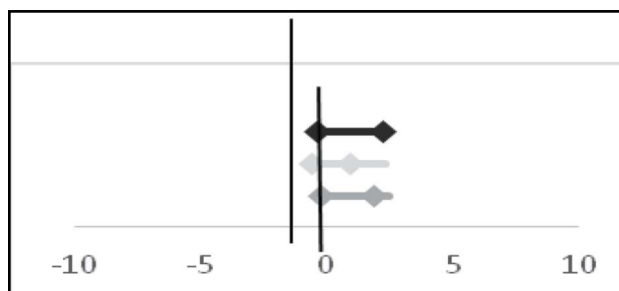
Figure 9.   Effect size of *A. marmelos* on HDL.

4.11 Outcome of *A. marmelos* on SOD

Rodents might develop stress induced cell damage as they are exposed to different chemicals, foods, care takers and environment. In order to distinguish the cause of the cell damage in the context, SOD evaluation is important. Administration of *A. marmelos* showed non-significant results of decreased SOD activity (Effect size, Cohens d = .40; inverse variance (IV) =50, 95% Confidence interval (CI)=0.13-0.67; I2=51.6%, P < 0.05 level even though it was significant in individual studies (Table 10, Figure 10)[15, 18, 19].
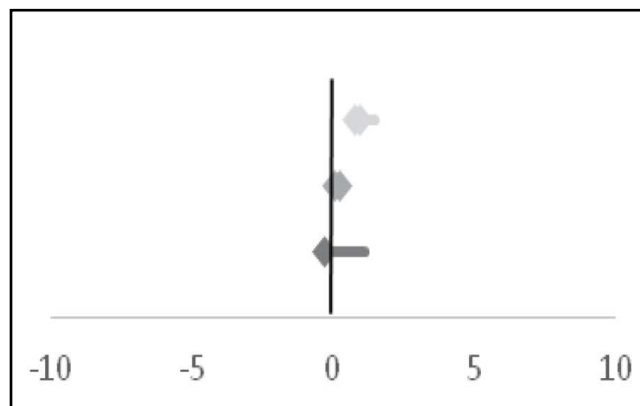
Table 9. Effect size of *A. marmelos* on SOD

Study	n	d	w	wd	v	CI95%
Rajbirbhatt *et al* [15]	56	0.96	3.15	12.62	0.076	1.51.76
Aravindkar *et al* [18]	77	0.25	20	5	0.05	.47.03
Kamala kannan *et al* [19]	30	0.41	7.69	3.15	0.13	1.13-.31

Figure 10. Effect size of *A. marmelos* on SOD.

4.12 Active constituents of *A. marmelos*

Different parts of the *A. marmelos* possess different chemical constituents.

4.13 Fruits

A. marmelos is an herb that is described to hold a variety of sterols, alkaloids, coumarins and essential oils. Alkaloids present were aegeline and marmeline, which were identified as N-2- hydroxy-2-ethyl cinnamide and beta sitosterol identified as N-2-hydroxy-2-ethyl cinnamide. Coumarins present were imperatorin, xanthotoxol and alloimperatorin[20].

4.14 Root and Stem Bark

Roots and stem bark consist of coumarins named aegelinol, psoralen, xanthotoxin, 6,7-dimethoxycoumarin and skimmianine[21].

4.15 Leaves

Leaves hold 11 alkaloids including halfordinol, N-2-ethoxy-2 ethyl cinnamide, Rutin, mermesinin, etc. A chain of phenylethylcinnamides were isolated from Aegle marmelos' leaves which comprised of aegelinosides A and B and anhydromarmeline, as alpha- glucosidase inhibitors. Anhydroaegeline is considered as the most active inhibitory effect against alpha- glucosidase[22].

An uncommon alkaloid, shahidine, four new ethyl cinnamide derivative alkaloids, marmenol, trans-cinnamic acid, valencic acid, 4-methoxy benzoic acid, betulinic acid, montanine and rutaretinare are also present.

4.16 Seeds

In addition to this, a few new compounds like plumbagin, imperatorin, b-sitosterol, b-sitosterol glucoside, stigmasterol, vanillin and salicin were identified from seeds of *Aegle marmelos Correa*[23].

5. Limitations

Sample size calculation was not mentioned by any of the studies. Only 9% of the studies reported on blinding and 18% reported on randomisation. This might have affected the internal validity of the studies. External validity is mainly through biological differences of study subjects, pathological differences occurring during induction, animal species and sexual variation. It was not possible to rule out the risk of publication bias such as overestimation of the result based on available data.

6. Discussion

This meta-analysis using 11 animal research studies found that anti-hyperglycemic potential of *A. marmelos*, was through reduction in serum glucose, serum insulin; anti-obesity effect through change in total cholesterol, LDL, HDL and oxidation potential through enzyme SOD are analysed. Values were taken from the group containing drugs in 2:2:1, in which *A. marmelos* constitutes 2 parts. This group showed significant reduction of serum glucose compared to others. Serum insulin was a parameter included in five of the selected studies. Active principles present in *A. marmelos* might be accountable for its favourable actions such as Aegeline, Marmeline, Halfordinol, Quercetin, Rutin and Umbelliferone. In addition, the same has showed significant anti-hyperglycaemic action in animals. Quercetin and rutin are two active ingredients found in *A. marmelos*, which were found to be possessing anti-hyperglycaemic action of *A. marmelos*. Other compounds having effect on insulin secretion were ferulic acid, ellagic acid, and eugenol. Umbelliferone could reverse plasma glucose and some action on serum insulin. Halfordinol from leaves have potent effect on hyperglycaemia. It augments insulin secretion by inhibition of alpha amylase enzymes. All the animal studies reported showed significant improvement except in glucose insulin, cholesterol and SOD. The variation might be, owing to not considering the outcomes of the study in terms of the effective size individually. This meta-analysis revealed that *A. marmelos* significantly reduces the serum glucose levels and total cholesterol levels in experimental rats. Nevertheless, high variability was observed in the estimation of serum glucose (I^2=86%). In this meta-analysis, the number of sub-group analysis was limited, so the expected outcome measure was not available. Total of 11 studies were included in the analysis which used serum glucose as one of the parameter.

Two different chemicals, streptozotocin and alloxan were found in the studies as inducing agents. Glibenclamide, metformin and glipizide were the drugs included as diabetic controls. Allocated study groups were in a range of 2-8 groups, which contained test drug in different concentrations like- non-diabetic control and diabetic control in all the studies. Number of animals present in each group varied from 6-8. If more than one concentration of test drug was included

in the study, the mean differences and standard deviations of maximum concentration of the test drug was taken for analysis. This might be the reason behind validity issues and unsolved crises in the study. After analysing the sub-groups, it was found that *A. marmelos* possess superior effect than the control drug, glibenclamide in lowering the serum glucose levels. The authenticity of the outcome was lowered due to the fewer number of subjects in each group and other differences in experimental designs.

Some of the detected advantageous effects of *A. marmelos* were accountable to its anti-oxidant effect. The induced stress might have produced profuse amounts of reactive oxygen species in animals. However, SOD findings in the present meta-analysis has showed non-significant results. This result might be due to the variations in external validity. High variability observed in some of the parameters might be due to the differences in sample size, designs, inducing agents, dose, number of groups present and the part used. The same study can be repeated with sophisticated and stringent study protocols.

7. Conclusion

Antihyperglycaemic potential of *A. marmelos* might be accountable for the additive or synergistic mechanisms of active constituents. Umbelliferone can reverse plasma glucose and halfordinol has potent effect on hyperglycaemia. It augments insulin secretion by inhibition of alpha amylase enzymes. Total cholesterol and triglycerides were significantly lowered from the administration of *A. marmelos*, while LDL and HDL values were found not to be significant. To develop valid clinical data, pre-clinical animal researches are very essential, provided experiments are screened for checking its adherence to rules and regulations.

8. Declarations

Conflict of Interest

The authors state that they have no conflicts of interest to reveal. The authors alone are answerable for the content and writing of the paper.

9. Funding

10. References

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